SYNOPSIS

Innate immunity refers to the first line of defence system of the host that comes into play immediately or within hours of appearance of invading pathogens like bacteria, viruses and fungi. Cells of the innate immune system such as macrophages and dendritic cells are equipped with several cell surface pattern recognition receptors (PRRs) like TLR1, TLR2, TLR4, TLR5, TLR6, TLR11, DECTIN 1, DECTIN 2, DECTIN3, MINCLE while TLR3, TLR7, TLR8, and TLR9 are expressed in endosome. Another cytosolic class of PRR include NOD2 and RIGI. These PRRs recognize pathogen associated molecular patterns (PAMs) carried by the foreign organisms but not present in the host. The type of PRRs involved upon appearance of foreign bodies depend on the type of PAMs displayed by the invading micro-organisms. Activation of PRRs by invading pathogens are essential to turn on the host innate immune system for early containment of the pathogen. For example, upon triggering of innate immune system various effectors molecules required to control the infection such as TNF-α, IL-1β, IL-12, IL-6, IL-18, chemokines, IFNβ etc. are upregulated.

Tuberculosis; an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) is one of the leading cause of death worldwide. According to World Health Organization, about one third of the world's population is latently infected by Mtb. Mtb is inhaled as droplet from the atmosphere. Alveolar macrophages phagocytosed the Mtb and induces localized pro-inflammatory responses that lead to the recruitment of mononuclear cells; the building block of granuloma from neighboring blood vessels. Granuloma consists of a kernel of infected macrophages, surrounded by foamy giant cells and macrophages with a mantle of lymphocytes delineating the periphery of the structure. At this stage Mtb is contained and the individuals do not show overt signs of the disease. However, the containment of Mtb fails after a change in the immune status of the host, which is usually a consequence of old age, malnutrition, or HIV-coinfection. Under such circumstances, the centre of the granuloma undergoes caseation and

spills viable, infectious bacilli into the airways. This leads to development of a productive cough that facilitates aerosol spread of infectious bacilli.

The host innate immune and adaptive immune system plays crucial role in containment of the Mtb. The predominant effector molecules that are crucial for controlling Mtb such as IFN-γ, TNF-α, IL-12, IL-6, IL-1β, chemokines, prostaglandins, reactive oxygen and nitrite species are being produced during infection. These effector molecules trigger series of events that are essential for the containment of the infection. Despite the robust immune responses elicited by Mtb is highly successful pathogen and still survive within the host. The success of Mtb to survive within the host amidst robust immune response can be attributed in its ability to modulate host defensive operations. For example, as a successful pathogen Mtb can induce production of anti-inflammatory effector molecules, downregulate MHC class II expression, inhibit fusion of phagosome with lysosome and inhibit apoptosis. Mtb is also known to modulate of epigenetic factors and micro RNAs of the host for its benefit. Increasing number of studies has shown that modulation of host cellular signaling cascades is one of the strategies employed by Mtb to significantly reprogram the innate immune cells for its benefit. Although, certain signaling cascades of host are also essential for controlling Mtb infection. In this context, understanding the modulation of host signaling cascades during mycobacterial infection of primary responder of innate immune system like macrophages and dendritic cells is crucial to get deeper insight into the pathogenesis of Mtb. In this regard, the role for PRRs in orchestrating host innate immune responses attain prime importance because the PRRs play significant role in shaping the immune response to mycobacterial infection.

TLR2 is the major receptor that recognizes the Mtb. Recognition of Mtb by TLR2 leads to the activation of downstream signalling cascades which eventually lead to the containment or elimination of the pathogen. Mtb contains complex lipid rich cell which can serve as ligands for PRRs. TLR2 ligands of Mtb includes phosphatidyl Inositol Mannosides (PIM), 19-kDa

antigen, Lipoarabinomannan, ESTAT6 etc. Studies have also shown that some of these TLR2 ligands such as LpqH (Rv3763) and ESTAT6 can inhibit TLR2 driven responses. However, during mycobacterial infection besides, TLR2 mediate signaling networks several other signaling cascades takes place as an effector cascade of TLR to execute specific functions in divergent context. Recent studies from our laboratory has reported that several developmental specific signaling pathways like Wnt, Notch and Sonic-hedgehog are reprogrammed during mycobacterial infection to regulate specific functions in a TLR2 dependent manner. One such developmental specific pathway garnering importance is Hippo pathway. The current study primarily focuses on the role of Hippo signaling in regulating specific functions of the host during infection with Mtb and also during TLR4 stimulation by its agonist lipopolysaccharide (LPS).

Hippo signaling was originally identified in Drosophila as a significant regulator of apoptosis and cell proliferation. However, various studies had shown that Hippo signaling pathway also regulates wide ranges of biological processes including T cell development, autophagy etc. Mice lacking Hippo pathway component MST1 were reported to have reduced $CD4^+$, $CD8^+$ T cells and neutropenia. Very recent studies had also shown the implication of Hippo signaling in regulating reactive oxygen species production and also in regulating viral infection. However, the role of Hippo signaling in modulating host responses during mycobacterial infection is not known. The current investigation demonstrates that during mycobacterial infection Hippo signaling pathway is activated *in vitro* as well as *in vivo* to regulate host immune responses. The activation of Hippo pathway was dependent on TLR2 pathway. Core component of TLR2 signaling such as MyD88, IRAK1 and IRAK4 were essential for activation of Hippo signaling during mycobacterial infection of host. We identified IRAK1 and IRAK4 as novel interacting partners of core component of Hippo pathway MST1/2. Further, mechanistic insights led us to the identification of transcription factor

interferon regulatory factor (IRF-3) to be activated by MST1/2 to orchestrate the production of chemokines like CXCL1 and CXCL2. Altogether, this study highlights the involvement of TLR2-IRAK1/4-MST1/2-IRF3 axis in Mtb-triggered modulation of chemokines CXCL1 and CXCL2 production and identifies MST1/2 as novel regulators of host-Mtb interactions.

As mentioned, Mtb driven MST1/2 orchestrated the expression of CXCL1 and CXCL2. Conventionally, these chemokines are well known for their crucial role in directing the recruitment of immune cells to the site of immunity breach. However, mounting evidences suggest the involvement of chemokines in regulating cellular processes like cytokine production, T cell activation and reactive oxygen species. One of the prime pathway that regulates inflammation is the inflammasome pathway. Inflammasome is a multiprotein complex activated in response to invading foreign organisms and also by endogenous signals like ATP, Ca^{2+}, MSU, LTB4, ROS etc to generate active inflammatory cytokine IL-1β. Because chemokines CXCL1 and CXCL2 also modulate inflammatory responses the modulation of inflammasome by CXCL1 and CXCL2 was intriguing. In this context, for the first time we have unravelled interesting and novel role for CXCL1 and CXCL2 where the concerned chemokines driven by MST1/2 during mycobacterial infection activated selective signaling cascades involving PKCμ-ILK axis leading to the activation of one of the prime innate immune defence pathway called the “Inflammasome”. Activation of inflammasome is crucial for generation of bioactive IL-1β and is very much required by the host to defend against infections. Activation of inflammasome by CXCL1 and CXCL2 was dependent on their cognate receptor CXCR2; a G-protein coupled receptor (GPCR). We found that CXCR2 is expressed on macrophages. Interestingly GPCRs ligand such as LTB4 and extracellular Ca^{2+} were being shown to regulate inflammaosme. The mechanistic study further, identified CXCR2 dependent activation of PKCμ and ILK in regulating the co-ordinated assembly of component of inflammasome in response to CXCL1. Altogether, the current study demonstrates for the

first time that Mtb infection driven CXCL1/2-CXCR2-PKCμ-ILK-NLRP3-CASPASE1 axis orchestrate the generation of mature pro- inflammatory IL-1β.

The results obtained so far demonstrated that Mtb specific TLR2 drives the Hippo pathway activation to enhance the production of inflammatory chemokines CXCL1 and CXCL2. Further, these CXCL1 and CXCL2 go on to evoke one of the prime inflammatory pathway "NLRP3 INFLAMMASOME" to regulate production of active IL-1β. With these perspectives, we further went on to elucidate the role of TLR4 in regulating the Hippo pathway. In this context, we found significant induction in the activation of Hippo upon stimulation of TLR with LPS. Epigenetic modifiers like histone methyl transferases, acetyl transferases, lysine demethylases are significant regulator of gene expression in various scenarios and also work from our laboratory have significantly emphasized the crucial role of various epigenetic factors like ASHL2, JMJD3, EZH2, SIRTUINS etc. in regulating host responses during mycobacterial infection. With these perspectives, our screening experiments has led to the identification of one of epigenetic modulator known as Lysine demethylase 1 (LSD1) as novel regulator of inflammation upon stimulation of macrophages or mice with LPS in *vitro* as well as *in vivo*. We observed significant upregulation of LSD1 during inflammation and the upregulation of LSD1 was significantly dependent on Hippo pathway component MST1/2. LSD1 is known to remove gene repressive marks such as H3K9 or activating marks H3K4. Depending on the context, LSD1 is known to act as activator of gene expression or repressor of gene expression. Activation of TLR4 leads to the enhanced production of both pro-inflammatory as well as anti-inflammatory cytokines. Although, pro-inflammatory cytokines are crucial for mounting an effective immune response to restrict infection, the overt and sustained productions of these cytokines are harmful to the host and hence, fine tuning of expression of pro-inflammatory cytokines are essential to maintain an immunological homeostasis. In this context, we observed that LSD1 surprisingly, inhibited the excessive

production of pro-inflammatory cytokines like TNF-α and IL-12 during LPS induced inflammation. LSD1 was found to execute this phenomenon by inhibiting hyper- activation of MAPK pathway components including ERK1/2, JNK1/2 and P38 signaling. Further, deep into mechanistic details revealed that the enhanced expression of the dual specificity phosphatases DUSP4 and DUSP10 are positively regulated by LSD1 during LPS induced inflammation. DUSP4 and DUSP10 are known to dephosphorylate MAPK pathway components and thus, prevent their hyperactivation. Altogether, this study identified a previously unknown function of LSD1 as a feedback inhibitor of LPS induced pro-inflammatory cytokine production.