Abstract

Pathogenic mycobacteria are among the most dreadful pathogens known to mankind as one third of the world's population is latently infected with *M. tuberculosis*, the causative agent of pulmonary tuberculosis. Host immune responses to mycobacterial infections is characterized by increased cell-mediated immunity constituted by CD4+, CD8+, γδT cells, macrophages, and dendritic cells (DCs). The effector mechanisms of these cells are chiefly mediated by the molecules such as IFN-γ, TNF-α, IL-12, IL-23, lymphotoxins, prostaglandins, reactive oxygen species (ROS) and reactive nitrogenous species (RNI) which are secreted during infection. These effector molecules trigger series of events that culminate into containment of the infection. Despite such robust immune responses elicited, mycobacteria still survive within the host. The success of mycobacteria for immune evasion lies in its ability to regulate the functions of sentinels of the immune system like macrophages and DCs. Modulation of the host cellular signaling cascades is one of the strategies that are employed by mycobacteria to significantly reprogram the innate immune cells for their benefit. Therefore, the key to control mycobacterial growth and limit pathogenesis lies in the understanding of the initial events of the mycobacterial infection including interaction between *Mycobacterium* and primary responders like macrophages and DCs. In this context, the role of pattern recognition receptors (PRRs) in orchestrating the host mediated immune responses attains central importance. The host cell surface/ intracellularly expressed PRRs play a vital role in shaping the immune response to the mycobacterial infections.

Of these PRRs, Toll-Like Receptors (TLRs) are the predominant interacting partners on macrophages for mycobacteria that activate the initial signaling cascades which eventually lead to containment or elimination of the pathogen. Of the TLRs, TLR2 constitute the key immune surveillance mechanism for recognition as well as control of mycobacterial infection. *Mycobacteria* contain complex lipid rich cell wall that is constituted by several natural ligands which can trigger various PRRs on the host immune cells. Lipo-arabinomannan (LAM), Lipomannan (LM), Phosphotidyl Inositol Mannosides (PIM) and 19-kDa antigens of *Mycobacteria* are known to interact with TLR2 and induce pro-inflammatory responses. Recent reports have shown that, PE_PGRS antigens of *M. tuberculosis* interact with TLR2 to regulate DC maturation and functions as well as to regulate DC mediated activation of T cells. Rv0754, Rv0978c, Rv0980c that belong to PE family of genes of mycobacteria have been implicated in modulation of human DCs. In another study, it has been reported that antigen processing and presentation by host immune cells can be inhibited by 19-kDa protein, LpqH (Rv3763) and LprG (Rv1411c) by utilizing TLR2. Surprisingly, recognition as well as amplification of the pathogen specific signaling events play important role not only in mounting immune response to infection but also to regulate explicit immune responses. In this scenario, integration of key signaling networks which modulate host immunity to pathogenic mycobacterial infections remains to be explored.

In accordance with these above observations, signal transducing pathways that act downstream of TLRs play crucial role in modulation of host cell gene expression in terms of immune modulatory cytokines and chemokines which bring in the primary responders like neutrophils, NK cells, T cells, etc including macrophages to the site of infection. This emphasizes the decisive role of TLRs in programming host immune cell function. However, during the process of ensuing immunity against invading pathogens, beside TLR-mediated signaling, many other signaling cascades takes place as an effector cascade of TLR or in parallel to TLR signaling pathway to execute specific functions in divergent cellular contexts. Recent studies from our laboratory have shown the role for TLR2 dependent activation of Notch1 pathway in regulating the expression of an immune-modulator, SOCS-3 in macrophages during mycobacterial infection. Very recently, it has been also shown that activation of Sonic Hedgehog signaling pathway plays a crucial role in fine-tuning the TLR2-mediated gene expression upon mycobacterial infection in macrophages. The current study primarily focuses on the role for Wnt signaling pathway in TLR2-mediated regulation of macrophage gene expression and its functional significance.

The current investigation demonstrates the differential activation of Wnt-β-Catenin and Notch1 signaling in response to pathogen-specific activation of TLR2 during infection with *M. bovis* BCG, *S. typhimurium* and *S. aureus*. Among these pathogens, *M. bovis* BCG triggered robust expression of Wnt5a, FzD4, LRP5 including a heightened stabilization and nuclear translocation of β-Catenin. Thus, effectuating the transcriptional activation of Jagged1 forming functional overlap between Wnt-β-Catenin and Notch1 signaling. Bringing correlation with the clinical manifestations of *M. tuberculosis* infection *in vivo*, we could detect augmented expression of signaling cohorts of Wnt-β-Catenin-Notch1 cascade as well as SOCS-3, a Notch1 responsive gene, in PBMC of pulmonary tuberculosis patients or brain samples derived from TBM patients. Induced expression of SOCS-3 acts as a significant factor in influencing the initiation and strength of the mounted innate immune response. SOCS-3, a negative regulator of multiple cytokines and TLR-induced signaling, is often associated with down modulation of proinflammatory responses during infection with pathogenic microbes. During intensive interplay between signaling pathways, nitric oxide (NO) serves as a pathological link that modulates direct cooperation of TLR2 with Notch1 signaling to regulate specific components of TLR2 responses. Significantly, NO was shown to regulate Wnt-mediated responses in colitis. In the view of these observations, we explored whether TLR2 triggered activation of Wnt-β-Catenin signaling could include the capacity of inducible nitric oxide synthase (iNOS)/NO to regulate Notch1 responses. We show that stabilization of β-Catenin in wild type macrophages but not in iNOS-/- could trigger the activation of Notch1 signaling as evidenced by activation of γ-secretase complex as well as expression of Notch1 ligand, Jagged1 and Notch1 target gene product SOCS-3. Our study identified Wnt-β-Catenin as critical regulator of pathogen-specific TLR2 responses which in conjunction with Notch1 controls the macrophage gene expression.

As mentioned, among several PRRs, TLRs especially TLR2 has been reported to interact with *Mycobacterium* and regulate gene expression in macrophages. For efficient activation or fine tuning of the TLR mediated immune responses, TLR may be complemented or even may relay on PRRs that belong to divergent families. Interestingly, it has been reported that, mycobacterial infections lead to induced expression of IL-12, a key cytokine that mount efficient inflammatory response and drives infected DCs to draining lymphnode for antigen presentation independent of TLR2. In this regard, C-type lectin receptors (CLR) like Dectin-1 has been implicated in both opsonic and nonopsonic binding and internalization of pathogens and is therefore thought to be important player in mouthing effective immune responses to infections. Dectin-1 being the important PRR that recognize fungi and fungal cell wall components like β 1- 3 glucan linked carbohydrates can directly induce respiratory burst in macrophages and IL-12, IL-10 as well as phagocytosis in DCs. Despite the growing literature on the biology of Dectin-1 in fungal immunity, there remains scarcity of information on the function of Dectin-1 in host recognition of other pathogens like mycobacteria. In this study, we assessed the possible involvement of Dectin-1 in *Mycobacterium* mediated immune responses in murine macrophages.

Though mycobacterium is shown to interact with Dectin-1, the components of mycobacteria that induce the signal are yet to be identified. We utilized Curdlan, a specific ligand to Dectin-1 to address the downstream signaling pathways which are dependent or independent of TLR2 and their role in fine tuning TLR mediated inflammatory responses. Signaling perturbation studies as well as the existing literature clearly suggest that there is activation of SyK kinase immediate downstream of Dectin-1 receptor that induces respiratory burst. The current investigation demonstrated that activation of Dectin-1 receptor stabilized cytosolic β-Catenin and induced expression of Wnt5a in macrophages. Pharmacological signaling intervention as well as genetic knock out studies clearly implicated the critical role of ROS in stabilization of β-Catenin and Wnt5a expression. Activation of Ca+2/CAMKII-mediated Wnt pathway by Wnt5a and its interaction with several signaling cohorts eventually led to induction of members of two cytokine regulatory families PIAS-1 and SOCS-1 respectively. Surprisingly, activation of TLRs and Dectin-1 lead to counteraction of signaling cascades and down regulated the TLR-induced inflammatory gene expression. Competitive ligand mediated inhibition or dominant negative approach or pharmacological inhibitor studies that block Dectin- 1 signaling relieved the Dectin-1 inhibitory effect over TLR signaling. Ectopic expression of Dectin-1-induced genes such as PIAS-1 and SOCS-1 led to modulation of the protein levels of MyD88, IRAK1 and IRAK4, which are the key adaptor molecules for generating TLR responses. Thus, the present study highlights the decisive role for Wnt signaling pathway activated by Dectin-1 in fine-tuning the TLR mediated inflammatory responses.

In perspective of these studies, sequential and coordinated activation of TLR2-driven signal transduction pathways influence the overall strength of innate immune responses. As described, TLR2 exhibits dominant role in sensing various agonists and pathogen associated molecular patterns of microbes at the cell surface and considered to be a major effectuator of proinflammatory responses. Mycobacterial cell wall antigens that belong to PE and PPE family such as Rv0978c, Rv0980c and Rv0754 are shown to induce maturation of human DCs and drive the T cell mediated immune responses towards Th1 or Th2. In addition to this, our laboratory data has shown that DC maturation was rescued upon treatment with TLR2-agoninsts like Rv0754 in the presence of immunosuppressive conditions exhibited by CTLA-4 and TGF-β in a coordinated fashion along with other PRR family receptors NOD1 and NOD2. TLR2 and NOD dependent activation of Notch/PI3K kinase pathway is implicated in maturation of human DCs under immunosuppressive milieu. In the current investigation, we demonstrated that TLR2 receptor triggering by mycobacterial cell wall antigens like Rv0754 and Rv1917c induce maturation of human DCs and activation of T cells under Programmed Death-1 (PD-1) induced immune suppressive condition. During this process, Wnt signaling pathway plays a decisive role and matures the human DCs in coordination with other host cell signaling cascades. Altogether, our findings establish the understanding of the conceptual framework of regulation of TLR responses by Wnt signaling pathway. Integration of PRRs signaling by Wnt signaling led to fine tuning of TLR-mediated inflammatory gene expression. This study underscores the role for Wnt responsive molecular regulators like PIAS-1 and SOCS-1 in deciding the macrophage cell-fate during immune responses elicited by multiple pathogenic infections. Importantly, our results also suggest the critical role of Wnt in rescuing the human DC maturation under immunosuppressive conditions induced by PD-1.