7. Summary

This thesis describes the function of NRs in *M. tuberculosis* infection and inflammation. We have also determined the role of heterologous cysteine protease SBM in *M. tuberculosis* infection.

M. tuberculosis the causative agent of TB, has remarkable ability to avert host defenses in order to survive and cause infection. The bacteria successfully circumvent the bacteriocidial environment inside macrophages to habitat them as their primary niche. Introduction of multidrug chemotherapy proved successful against *M. tuberculosis* and has yielded immense public health benefits. But poor compliance to treatment regimen leaded to the emergence of various resistant *M. tuberculosis* strains including TDR bacilli that are non-sensitive to all available first-and second-line drugs currently available for TB treatment. The quest for new drug targets and new drug molecules that can be effective against the resistant bacteria is on. The emphasis has also been to develop host-based therapies that swing host immune response against *M. tuberculosis* and might also prove effective against untreatable drug-resistant *M. tuberculosis* strains. We took two very diverse approaches to target the intracellular persistence of TB.

In first approach described in chapter 3, we looked for the dependence of *M. tuberculosis* on those host LSNRs that effects the intracellular survival of the pathogen. We performed loss of function experiments by silencing these LSNRs and concluded that two among them namely; PPARγ and TR4 are used by *M. tuberculosis* for its survival, whereas LXR is used by host to control *M. tuberculosis* infection. In order to convincingly implicate these receptors in TB susceptibility, we determined relative mRNA expression of these receptors in PBMCs obtained from TB patients, close contacts and healthy individuals. The study revealed that

PPARγ expression was significantly higher for TB patients and that of LXRα lower when compared to healthy controls, while no significant change in expression was observed for TR4. Further, an elevated expression of PPARγ and/or TR4 was observed in few close contacts (Mahajan et al., 2012b). We also observed that *M. tuberculosis* keto-MA induces *in vitro* granuloma formation which was abrogated in the absence of TR4, suggestive of a functional cross-talk between a host protein and *M. tuberculosis* lipid (Dkhar et al., 2014). These observations propose a model for pharmacological modulation of LSNRs in *M. tuberculosis* infection.

In second approach described in chapter 6, we studied the anti-*M. tuberculosis* effect of SBM, a pharmacologically active member of the sulfhydryl proteolytic enzyme family, obtained from *A. comosus*. We found that SBM induces apoptosis in macrophages and augments the production of pro-inflammtory cytokines, co-stimulatory molecules, and ROS thereby overturning the *M. tuberculosis* ability to circumvent the bacteriocidal activities of macrophages. SBM also leads to decrease in lipid accumulation in un-infected and *M. tuberculosis* infected macrophages (Mahajan et al., 2012a). Thus SBM ability to clear intracellular *M. tuberculosis* infection depends upon its ability to activate the macrophages and lower the foam cell formation.

Inflammation is a double-edged sword. It is required to provide protection from invading pathogens while an uncontrolled chronic inflammation is a key component of autoimmune, metabolic and neurodegenerative disorders. In the thesis we have attempted to determine the role of NRs in inflammation and CIA.

In chapter 4, we have pursued the role of Nr4a2 in inflammation and macrophage polarization. Recent studies highlight the role of macrophage

polarization and plasticity in various physiological processes. In inflammation differently polarized M1 and M2 macrophage sub-types regulate its different stages. M2 macrophages are present in the resolution phase of inflammation and they possess anti-inflammatory properties. Eventhough, the characteristics of M2 macrophages are well defined but the transcriptional programme that directs the induction of M2 phenotype remains poorly understood. We observed that exposure to ligands for TLRs robustly induces Nr4a2 expression in macrophages and is uniformly distributed both in the nucleus and the cytoplasm. The elevated expression of Nr4a2 by LPS as a stimulus is tightly regulated by the PI3K-Akt-mTOR signaling axis. We further observed that overexpression of Nr4a2 leads to alternative activation of macrophages with increased expression of genes that are prototypical M2 markers. Interestingly, Nr4a2 transcriptionally activates arginase-1 expression by directly binding to its promoter. Adoptive transfer experiments revealed that Nr4a2 provides protection in endotoxin-induced septic animals by controlling unchecked release of pro-inflammatory cytokines. Thus our data identifies a previously unknown role for Nr4a2 in the regulation of macrophage polarization.

In chapter 5, we have attempted to develop an atlas for CIA. Gene expression profiling analysis had some success in the past in RA patients, especially in identifying new targets for therapy and successfully anticipating the response to any given therapy. The last decade highlighted the unprecedented role NRs play in RA and using various animal models it has been proved that ligands for various NRs reduces disease progression. This leads to the need for studying expression dynamics of NR superfamily during the entire course of disease progression. Using customized array plates we monitored the expression of NRs in $CD4^+$ T cells isolated from the spleen of control and

CIA mice during initial days of disease progression and found that few members of this superfamily are particularly regulated in the diseased mice. This study might prove helpful in predicting new targets for RA-therapies.