## Summary

Tuberculosis is one of the oldest infectious disease that remain a threat for public health around the world. *Mycobacterium tuberculosis,* the etiologic agent for this disease infect one third of the world population and is responsible for 1-2 million deaths every year (WHO, 2012). This bacterium is known to induce strong immune responses, yet successfully survive within its host macrophages. Although, macrophages are equipped to phagocytose and then kill most of the pathogens they encounter, but virulent *M. tuberculosis* is only phagocytosed but not killed by these cells. Recent studies have demonstrated that induction of autophagy leads to the delivery of mycobacteria into lysosomes thereby killing of the intracellular bacteria (Mnz, 2009; Deretic, 2008).

Autophagy is a catabolic process for the degradation of a cell's own components through the lysosomal machinery. It is a highly regulated process that plays a normal part in cell growth, development and homeostasis, helping to maintain a balance between the synthesis, degradation, and subsequent recycling of cellular products. Autophagy has been characterized as an innate defense mechanism for elimination of intracellular pathogens (Harris et al., 2009). During this process regions of the cytoplasm as well as organelles are first engulfed by double or multiple membrane structures called autophagosomes and subsequently the trapped material is delivered to autolysosomes which are formed by the fusion of autophagosomes with lysosomes (Levin and Klionsky, 2004). The trapped cytoplasmic material is eventually degraded by lysosomal hydrolytic enzymes.

IFN-γ, a predominant activator of autophagy and the microbicidal function of macrophages. Induction of autophagy by IFN-γ is associated with protective immunity against tuberculosis. It has been shown that lymphocytes isolated from the lung of pulmonary tuberculosis patients typically have a Th1 phenotype secreting IFN-γ. However, despite the local production of IFN-γ, the immune response generated is not sufficient to eradicate tuberculosis infection in human (Jo et al., 2003; Raja, 2004). It is well documented now that *M. tuberculosis* prevents macrophages from responding to IFN-γ. Earlier, it was shown that IL-6 produced by mycobacteria infected macrophages selectively inhibits macrophage response to IFN-γ (Nagabhushanam et al., 2003) but no report exists to clarify whether IL-6 interferes with the autophagic process induced by

IFN-γ. Thus the present study was designed to see the effect of IL-6 on IFN-γ induced autophagosome maturation process in virulent mycobacteria infected macrophages.

First, we checked the effect of IL-6 on autophagy induced by different autophagy inducers like IFN-γ, starvation and rapamycin. IL-6 clearly inhibited starvation, rapamycin and IFN-γ induced autophagosome formation as revealed by MDC and acridine orange staining. The inhibitory effect of IL-6 was further confirmed by GFP-LC3 puncta formation where IL-6 treatment lowered the LC3 puncta formation induced by IFN-γ or starvation or rapamycin treatment. The inhibitory effect of IL-6 is not cell type specific as IL-6 inhibited autophagy in HEK293 and HeLa cells. Autophagy can be inhibited in two ways either by inhibiting autophagosome formation or by accelerating autophagosome degradation. IL-6 was found to inhibit autophagy by inhibiting the formation of autophagosome as IL-6 treatment lowered the IFN-γ or starvation induced GFP-LC3 puncta even in presence of lysosomal protease inhibitors E64D and pepstatin A. Effect of IL-6 on fusion of autophagosome with lysosome was also studied by examining the co-localization of GFP-LC3 labeled autophagosome with LysoTracker stained lysosomes. IL-6 treatment showed decreased LC3 puncta under starvation or IFN-γ and which did not co-localize with LysoTracker, whereas IFN-γ or HBSS treated cells showed increased LC3 puncta and most of them fused with lysosomes indicating that IL-6 inhibits autophagy by inhibiting the formation of autophagosome rather than enhancing its degradation.

Next, we studied the role of IL-6 on autophagy formation in mycobacterial infection by examining the co-localization of *M. tuberculosis* with different autophagy as well as acidification markers. We found that IL-6 inhibits both IFN-γ and starvation induced autophagy as well as acidification of mycobacteria containing phagosome. IL-6 treatment significantly lowered the IFN-γ or starvation induced colocalization of GFP-*M. tuberculosis* H37Rv containing phagosomes with LC3 or Beclin 1 positive autophagosomes. Decreased colocalization of GFP-*M. tuberculosis* H37Rv with LysoTracker or CD63 was also observed in THP-1 cells treated with IFN-γ or HBSS and IL-6 together compared to only IFN-γ or HBSS treated cells.

Further, we studied the effect of IL-6 on intracellular survival of mycobacteria inside macrophage. We found that exogenous addition of IL-6 inhibited the IFN-γ mediated killing of intracellular mycobacteria. Next, we studied the role of endogenous IL-6 on

urvival of mtb in macrophage as we observed time dependent increase in IL-6 level fter internalization of the bacilli in THP-1 cells. We found that neutralization of ndogenous IL-6 by anti-IL-6 antibody enhances the IFN-γ mediated killing of *M. iberculosis* H37Rv in THP-1 cells. Neutralization of IL-6 also resulted in enhanced utophagy in *M. tuberculosis* H37Rv infection at both 24 h and 48 h post infection time oints.

.fter establishing the role of IL-6 in autophagy we looked for the molecular iechanisms behind IL-6 mediated autophagy inhibition. We found that IL-6 ownregulate ATG5-ATG12 complex. This complex is formed during elongation step of ıtophagy and essential for completion of elongation step of autophagosome formation. y inhibiting the complex in a time dependent manner IL-6 inhibits the formation of ıtophagosome. We also found the involvement of p38 and JNK MAP kinase pathways ı IFN-γ mediated autophagy in THP-1 cells. IFN-γ upregulates whereas IL-6 ɔwnregulates the expression p38 and JNK MAP kinases. We found that IL-6 actually ıimic the role of p38 and JNK inhibitors in exerting its effect on IFN-γ induced ıtophagy in mycobacteria infected cells since both p38 and JNK inhibitors like IL-6 ock IFN-γ induced autophagosome formation and thereafter, the acidification of ycobacteria containing phagosomes. One of the best known pathways for autophagy gulation is mTOR pathway. Class I PI3K pathway through Akt and mTOR act as gative regulator of autophagy (Petiot et al., 2000). We have found that IFN-γ inhibits hereas IL-6 induces the phosphorylation of mTOR and thus IL-6 counteracts the effect IFN-γ. IL-6 signals through JAK/STAT pathway. We have shown that inhibition of AK or knockdown of STAT3 by siRNA abrogate IL-6 mediated autophagy inhibition *M. tuberculosis* H37Rv infection. Thus our finding indicate that STAT3 is involved in .-6 mediated autophagy inhibition. Collectively, our results support the existence of ultiple pathways by which IL-6 inhibit autophagy.

ırther, we targeted IL-6 by various means and studied its effect on mycobacterial rvival and autophagy. Targeting of IL-6 by siRNA or antisence IL-6 plasmid enhanced e mycobactericidal effect of IFN-γ and mycobacterial phagosome maturation. Next, e targeted IL-6 by chemical compound NAC. NAC lowered the IL-6 production in ycobacterial infection. Treatment of NAC also enhanced the IFN-γ induced ıagosomal acidification.

Collectively, these results clearly indicate that virulent mycobacteria strategically upregulate IL-6 production to combat innate immunity. Considerable advancement has been made in the field of tuberculosis research; still the mortality rate all over the world is very high. In order to develop more effective therapy for TB, proper understanding of the immunopathogenesis of this infection is required. Further research to identify the mycobacteria induced mediators that orchestrate the expression of survival signals in host macrophages would prove extremely beneficial to combat this disease.