Chapter I
Intramacrophage pathogen *Leishmani donovani* escapes host immune response by subverting Toll-like receptor (TLR) signaling, which is critically regulated by protein ubiquitination. In the present study, we identified tumor necrosis factor receptor associated factor (TRAF) 3, degradative ubiquitination of which is essential for TLR4 activation, as a target for *Leishmania* to deactivate LPS-mediated TLR4 signaling. We used LPS-treated RAW 264.7 cells and compared the TLR4-mediated immune response in these cells with *L. donovani* and *L. donovani*+ LPS costimulated macrophages. TRAF3, which was ubiquitinated (2.1-fold over control) at lys 48 position and subsequently degraded following LPS treatment, persisted in *L. donovani* and *L. donovani* + LPS costimulated cells due to defective lys 48 ubiquitination. Lys 63-linked ubiquitination of upstream proteins in the cascade (cIAP1/2 and TRAF6), mandatory for TRAF3 degradation, was also reduced postinfection. This may be attributed to reduced association between ubiquitin-conjugating enzyme Ubc13 and TRAF6 during infection. Inhibition of TRAF3 before infection by shRNA in Balb/c mice showed enhanced IL-12 and TNF-α (10.8- and 8.1-fold over infected control) and decreased spleen parasite burden (61.3% suppression, P<0.001), thereby marking reduction in disease progression. Our findings identified TRAF3 as a novel molecular regulator exploited by *Leishmania* for successful infection.

Chapter II
Apoptosis is one of the mechanisms used by host cells to get rid of unwanted intracellular organisms, and often found to be subverted by pathogens through use of host anti-apoptotic proteins. In the present study, with the help of in vitro and in vivo approaches, we documented that the macrophage anti-apoptotic protein myeloid cell leukemia1 (MCL-1) is exploited by the intra-macrophage parasite *Leishmania* donovani to protect their “home” from Actinomycin D-induced mitochondria-dependent apoptosis. Amongst all the anti apoptotic BCL-2 family members, infection preferentially up-regulated expression of MCL-1 at both the mRNA and protein levels, and compared to infected control, MCL-1 silenced infected macrophages documented enhanced caspase activity and increased apoptosis when subjected to actinomycin D treatment. Phosphorylation kinetics and ChIP assay demonstrated that infection induced MCL-1 expression was regulated by the transcription factor CREB and silencing of CREB resulted in reduced expression of MCL-1 and increased apoptosis. During infection, MCL-1 was found to be
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localized in mitochondria and this was significantly reduced in Tom70 silenced macrophages, suggesting the active role of TOM70 in MCL-1 transport. In the mitochondria, MCL-1 interacts with the major pro-apoptotic protein BAK and prevents BAK-BAK homo-oligomer formation thereby preventing cytochrome c release mediated mitochondrial dysfunction. Silencing of MCL-1 in the spleen of infected mice showed decreased parasite burden and increased induction of splenocyte apoptosis. Collectively our results showed that *L. donovani* exploited macrophage anti-apoptotic protein MCL-1 to prevent BAK mediated mitochondria dependent apoptosis, thereby protecting its niche essential for disease progression.

**Chapter III**

*Leishmania* being an intracellular parasite subverts host apoptotic machinery thereby preventing rapid eradication of parasite from macrophages. In our earlier observations, we documented that *L. donovani* exploits MCL-1, a BCL-2 family protein, to inhibit mitochondria dependent macrophage apoptosis, as observed up to 24 h post infection. However, in order to establish successful infection, *Leishmania* needs to reside inside macrophages for almost 72 h. Therefore, in the present study we tried to elucidate whether inhibition of macrophage apoptosis at late hour of infection is also facilitated by MCL-1, known to be a very labile protein. Although the transcript level of MCL-1 showed a sharp decline at 36 h post-infection, translational level of MCL-1 found to be maintained a steady level up to 48 h post-infection. Moreover, upon Actinomycin D treatment, MCL-1 silenced infected cells demonstrated enhanced caspase activity and increased apoptosis compared with wild type infected cells, thus validating importance of MCL-1 in late hour of infection. When IL-6 or GM-CSF, strong inducers of MCL-1, was withdrawn from macrophages, the protein level expression of MCL-1 was significantly reduced but again restored upon infection with *Leishmania*. This may be attributed to induced stability of MCL-1 during infection, and we tried to find out the mechanism behind it. Since degradation of MCL-1 is mediated through proteasomal pathway, we studied ubiquitination pattern of MCL-1, which is a requirement for proteasome mediated degradation. Increased ubiquitination of MCL-1 in IL-6 or GM-CSF withdrawn cells found to be substantially reduced upon *Leishmania* infection further supported our infection induced stability of MCL-1 hypothesis. However, MCL-1 specific E3 ligase MULE, responsible for attaching ubiquitin moieties with MCL-1, was strongly interacted with MCL-1 during infection. Since the only other
possible explanation of MCL-1 for not getting ubiquitinated would be the blockage of ubiquitin binding site on MCL-1 by some other protein, we screened different well known binding partners of MCL-1 using co-immunoprecipitation study. Amongst different binding partners of MCL-1 such as BIM, BID, PUMA NOXA and TCTP (translationally controlled tumour protein); infection induced strong interaction of MCL-1 with only TCTP. Therefore from our observations we may propose that during infection ubiquitin moieties attachment site on MCL-1 is blocked by TCTP, thus preventing rapid degradation of MCL-1. Though it requires further analysis to find out the exact mechanism of TCTP mediated MCL-1 stabilization during infection, our study up to this, suggested that *L. donovani* may exploit TCTP to prevent rapid proteasomal mediated degradation of MCL-1 for prolonging its survival within host macrophages.