Summary and Conclusions
Summary

The host-pathogen interactions during typhoid fever, caused by *Salmonella typhi*, remain poorly understood primarily due to the non-availability of a suitable animal model. *S. typhi* produces this systemic infection only in humans. On the other hand, non-typhoidal *Salmonella* serovars such as *S. typhimurium* produces only self-limiting gastroenteritis in humans. This, in spite of the fact, that except for a small number of genes, *S. typhimurium* shares a high degree of homology with *S. typhi* in its genetic constitution. Remarkably, however, *S. typhimurium* causes a systemic infection in mice that is analogous to human typhoid; *S. typhi* does not cause any pathogenesis in mice. The reasons for different clinical outcomes produced by these two closely related *Salmonella* species are not understood. Clearly, one or more molecules not conserved between these two pathogens must be playing a role in determining host-specific manifestations and it is important to identify these interactions in order to understand *S. typhi* pathogenesis. Both humoral and cell mediated immune responses are known to be important in protection against *S. typhi*, with T cells playing a more crucial role in clearance of bacteria and long term immunity. It is therefore imperative for *S. typhi* to counter the immune mechanisms, both innate and adaptive types, in order to establish infection.

The results presented in this thesis describe two *S. typhi*-specific interactions that are capable of modulating T cell responses. The first interaction involves the virulence polysaccharide, Vi, which forms a capsule around *S. typhi*, but is absent in *S. typhimurium*. This polysaccharide has been previously shown to protect *S. typhi* from various defense mechanisms of the host. The results described here show that Vi can interact with T lymphocytes through a membrane recognition complex comprising of putative tumor suppressor molecule prohibitin and its closely related homologue, B cell receptor...
associated protein (BAP-37). Prohibitin is a highly conserved molecule that is involved in the regulation of cell cycle and stabilization of mitochondrial respiratory chain complexes. More recently it has been shown to regulate the MAP-kinase pathway of intracellular signaling in epithelial cells.

Our study shows that activation of model human T cell leukemia line, Jurkat, through the TCR in the presence of Vi leads to reduced tyrosine phosphorylation. The polysaccharide targeted and suppressed activation of src-kinase p56^{ck}, considered to be the master kinase involved in initiating signaling through the TCR. Significantly, this suppression was associated with a modest increase in the activity of another src-kinase p59^{syn} that has been recently shown to negatively regulate intracellular signaling in T cells. By differently modulating the activities of these two tyrosine kinases, Vi inhibited tyrosine phosphorylation of key intracellular targets including the critically important tyrosine kinase ZAP-70.

Treatment of T cells with Vi led to significant inhibition in the activation of MAP-kinases, ERK and p38 MAP-kinase, upon activation through the TCR. It also modulated Raf-1 kinase and activation of NF-κB.

Engagement of TCR in the presence of Vi brought about considerable changes in the cytoskeletal rearrangements that are produced following activation of T cells. Vi induced exit of prohibitin and actin from the lipid raft and also affected recruitment of Vav, a guanine nucleotide exchange factor for Rho GTPases, to these membrane microdomains. These changes in the cytoskeletal rearrangements were associated with dramatic reorganization of GM1 in the membrane as revealed by binding to cholera toxin B. The polysaccharide also inhibited activation-induced TCR downregulation in T cells.
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The modulations caused by Vi in various intracellular signaling events led to suppression of IL-2 secretion from activated T cells, which can have significant implications on the functioning of T cell immunity in vivo. S.typhi could therefore employ this virulence factor to dampen adaptive immunity. Our findings have unveiled another face of Vi, which is otherwise known to defend the pathogen against anti-LPS antibodies and many innate defense mechanisms of the host.

The second host-pathogen interaction that was identified in this study shows that S.typhi possesses an effector / effectors that induce cell death of activated T lymphocytes. The cytotoxicity mediated by cell-free extract of S.typhi was highly specific to T cells and did not kill macrophages or B-lymphocytes. This activity is therefore different from previously identified toxic effectors such as SipB, which can kill macrophages after intracellular delivery. Significantly, this cytotoxicity was not observed with the closely related Salmonella serovar, S.typhimurium, which does not cause typhoid in humans. Cell death caused by this S.typhi-cytotoxin was caspase-independent and most likely involved Fas-FasL interaction. The toxin killed both CD4^+ and CD8^+ activated T cells although CD8^+ T cells seem to be preferred targets. In vivo, this type of a toxin could promote establishment of infection with S.typhi by modulating cell-mediated immunity against this pathogen.

In conclusion, our study has identified two S.typhi–specific interactions having the ability to downregulate T cell–mediated immune responses during infection with this pathogen. These modulations could play a crucial role in the establishment of systemic infection with S.typhi in humans.