Synopsis

Infectious diseases account for a large proportion of morbidity and mortality worldwide. The major global efforts lie in effectively enhancing the health span of infected individuals and more importantly, curbing infection onset and spread. A multitude of host- and pathogen-derived factors contribute towards coining the outcome of infections. In this regard, the virulence of major successful infectious agents is believed to be determined by their prowess to swivel host immune system to their own benefit. The pathogens effectuate such immune subversions by modulating various host signalling pathways and reprogramming host cellular homeostatic processes. Thus, understanding the transactions occurring at the host-pathogen interface becomes an asset for the development of rational diagnostic, preventative and therapeutic interventions for infectious diseases.

The current study focuses on the implications of Epidermal Growth Factor Receptor (EGFR) signalling during selected bacterial infections. Conventionally associated with cancers of various origins; accumulating evidences indicate aberrant activation of EGFR (Receptor Tyrosine Kinase, RTK) pathway during infection scenarios and Pattern Recognition Receptor (PRR) engagements. For instance, EGFR has been reported to be deregulated during infections with *Helicobacter pylori*, *Shigella flexneri*, *Mycobacterium tuberculosis* and *Toxoplasma sp.*; however, there is a dearth of information relating to the molecular mechanisms governed by EGFR during such infections. In the purview of the stated literature, the specific contributions of EGFR in the pathogenesis of *Mycobacterium tuberculosis* (Mtb) and *Shigella flexneri* (*S. flexneri*) was conceived. Infection with both Mtb and *S. flexneri* activated signalling through EGFR pathway. During Mtb infection, EGFR was intricately involved in regulating key processes that allow pathogen survival. EGFR was found to employ a specific reader of epigenetic marks, Bromodomain-containing protein 4 (BRD4), to alter gene expression and assist Mtb pathogenesis by regulating the phenomenon of angiogenesis, i.e. blood vessel formation (Chapter 3). Further, the receptor-epigenetic reader pair was also found to orchestrate Mtb-induced accumulation of lipid droplets and inhibition of autophagy (Chapter 4). In studies with the gram-negative bacterium *S. felxneri*, it was found that EGFR regulated the expression of anti-inflammatory indoleamine 2, 3 dioxygenase (IDO), which contributed significantly to host immune homeostasis during *S. flexneri* infection (Chapter 5).

Mtb afflicts over one-fourth of the world population and poses a global health concern. The successful co-evolution of the pathogen with its host, along with the emergence of its drug resistant variants presses on the need for unravelling critical host factors that aid in its persistence. Within the host, alveolar macrophages form the primary site of infection for the pathogen and are extensively modulated by Mtb to generate a secure and nutrient-rich niche. In this scheme of events, infection drives the formation of a highly organized immune structure - the granuloma, consisting of concentric whorls of distinct immune cells, with infected macrophages residing in the core, surrounded by neutrophils, T lymphocytes and B lymphocytes. With such an organization, it has been perceived that one of the major constraints to effective antibiotic therapy for tuberculosis (TB) would stem from the lack of vascular perfusion to the bacteria-populated core of the granuloma. Therefore, granuloma-associated host angiogenic modulation stands critical for Mtb survival, dissemination and evasion from antibiotics as well as host immune effectors. Existing literature suggests the upregulation of angiogenic markers in mycobacteria-

infected host cells. Macrophages contained in the hypoxic microenvironment of the granuloma are reported to form the major source of the potent angiogenic factor, VEGF-A. Interestingly, it has been observed that the extent of expression of VEGF-A is strongly correlated with the virulence of Mtb. Apart from the mice and zebrafish model for the study of Mtb-associated angiogenesis, another study has recently reported the potential efficacy of anti-VEGF therapy as an adjunct for TB control in rabbit model. Further, the presence of angiogenic properties in the serum of active TB patients and the abundance of VEGF-A during TB infection necessitates the exploration of the consequences and the molecular mechanisms governing the process. In this regard, we found that EGFR signalling is a significant regulator of Mtb-induced angiogenic effects. EGFR was found to mediate this differential regulation through BRD4, in conjunction with KLF5, a transcription factor belonging to the Kruppel-like family. Interestingly, both these members have earlier been associated with VEGF-A expression. Also literature evidences suggest the contribution of distinct bromodomain proteins during bacterial infections and LPS treatment. Finally, through the use of pharmacological inhibitors of EGFR and BRD4, in an established model of TB, we demonstrate the ability of these small molecule inhibitors in potentiating anti-TB effects.

The different phenomena exploited by Mtb to accentuate its survival within the host includes alteration of nutrient sources such as lipids; skewing of immune responses such as T cell functions and MHC recognition; and perturbation of bacterial elimination processes such as autophagy and apoptosis. EGFR was earlier reported as a potential therapeutic target for TB infection. Here, we additionally find the implication of EGFR signalling dependent BRD4 in differentially modulating two key cellular events during Mtb infection; i.e. lipid accumulation and autophagy. Mtb-induced lipid droplets (LDs) are suggested to act as source of nutrients and a secure niche for the bacterium; and autophagy (as a bacterial clearance mechanism) is suppressed by the pathogen. We determine the interplay of these phenomena and underscore their relevance in Mtb pathogenesis. The intricate regulatory circuits of these Mtb survival events form essential targets for effective TB therapy. Several studies propose key signalling intermediates/ transcription factors in the regulation of Mtb-driven immune evasion. In the current study, we uncover that inhibition of EGFR signalling dependent BRD4 compromises the ability of Mtb to induce lipid droplet formation. We also observe the induction of host autophagic process in Mtb-infected cells treated with the concerned pharmacological inhibitors. Corroborating these observations, *in vitro* CFU enumeration revealed compromised mycobacterial survival upon perturbation of EGFR signalling dependent BRD4; validating their possible implication in Mtb pathogenesis.

In another facet, we determined the implication of EGFR in *S. flexneri* infection. Recognition of *S. flexneri* by PRRs triggers a gamut of immune responses, which majorly culminate in hyper-inflammation. Resolution of such overt inflammation is crucial for host survival. Several anti-inflammatory factors such as RUNX3, NRF2 and ERBIN have been reported to provide homeostasis in a context-dependent manner. Using *in vitro* and *in vivo* models of *S. flexneri* infection, we find that *S. flexneri* leads to the expression of the immune homeostat indoleamine 2, 3 dioxygenase 1 (IDO1). We observed a critical role of EGFR-driven signalling cascade in governing IDO1 production. Further insights into the functional significance of IDO1 revealed that the loss of IDO1 function *in vivo* leads to exacerbated Shigellosis. We delineated that the observed effect

is correlated with the skewed inflammatory mediators, with excessive pro-inflammatory cytokines (IL-1β, IL-12, IL-17) and compromised expression of the anti-inflammatory arm (IL-10, Arginase1, IL-4, TGF-β). Interestingly, administration of recombinant EGF in IDO1 inhibited mice restored the cytokine balance and consequently ameliorated exacerbated Shigellosis and bacterial growth. This study focused on the homeostatic effect of IDO1 and uncovered the possible contribution of EGFR in conserving host inflammatory milieu. Together, we unravel the potential implications of EGFR in discrete bacterial infections. We find that pathogen-specific activation of EGFR pathway may have diverse manifestations during distinct infections. Here, we observed that whereas on one hand EGFR assists mycobacterial pathogenesis; it protects the host from exacerbated Shigellosis. These mechanisms shed light on the conundrum of events that determine infection outcome and also project the relevance of calibrated host responses to specific immune insults.