SYNOPSIS

Synopsis of work done by Mayuri Gogoi (SR No. 10-09-00-10-31-11-1-08534) for the award of Ph.D. degree in the Faculty of Science, Indian Institute of Science, Bangalore, India.

Thesis title: *Salmonella* pathogenesis in dendritic cells: stealthy approach against adaptive immune response

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Chapter 1

Introduction

Salmonella is an enteric pathogen and can cause pathogenesis in a wide array of hosts ranging from cold-blooded animal to humans. In humans, it causes a various diseases ranging from mild gastroenteritis to systemic illness like typhoid fever. Despite the current medical advancements, *Salmonella* still remains as one of the major cause of morbidity and mortality in developing countries. The host-pathogen relationship of *Salmonella* pathogenesis is multifaceted.Being a successful pathogen *Salmonella* engages a range of evasion strategy to escape host immune response. An inoculum of 10^3 to 10^6 is sufficient for causing the disease. Once ingested, *Salmonella* crosses the host intestinal barrier by infecting "M cells" (Microfold cells) and dendritic cells present in the Peyer's patches. Additionally, it can induce bacterial uptake by intestinal epithelial cells via injection of virulence factor into the host cell. These virulence factors are encoded by various pathogenicity island present in *Salmonella* genome and are critical for entry and intracellular survival. After the initial invasion step, various white blood cells disseminate

the bacteria to distal organs. Due to the scarcity of animal model for *Salmonella* Typhi infection most of the insights into this disease is attainted from *Salmonella* Typhimurium infection of the mouse. *Salmonella* Typhimurium causes typhoid like systemic disease in mice. Both during mice and human infection of *Salmonella*, DCs serve as a nexus which is essential for bacterial invasion, dissemination and clearance. The efficient clearance of bacteria requires the well-coordinated function of both innate and adaptive immune response. The interplay among host innate, adaptive immune system and *Salmonella* is crucial for understanding the disease. These comprehensiveknowledge of the pathogenesis process can assist in designing state of the art therapeutic strategy and unique drug regime against these diseases in future.

Chapter 2

Salmonella downregulates MHC II in dendritic cells by endosomal proteolysis

Induction of both $CD4^+$ and $CD8^+$ T cells response is vital for successful clearance of *Salmonella* Typhimurium infection. In particular, $CD4^+$ T cells appear to play a decisive role in mitigating *Salmonella* infection as mice compromised in $CD4^+$ T cell responses show higher organ burden and succumb to the infection faster. Activation of $CD4^+$ T cells require two signal, firstly, T cell receptor (TCR) recognition of antigenic peptide-MHC II complexes and the second being, co-stimulatory signal. MHC II pathway generally presents antigens of exogenous origin and are generally expressed only by three professional antigen presenting cells (APCs) such as dendritic cells (DCs), macrophages and B cells. *Salmonella* has previously been reported to suppress antigen presentation by DCs. This reduction of antigen presentation is attributed to reduced MHC II presentation.

In addition, studies on human DCs have shown that *Salmonella* reduces the cell surface MHC II levels. However, the mechanism by which *Salmonella* Typhimurium impairs MHC II expression is yet unknown. In this study, we have elucidated the mechanism of *Salmonella* mediated downregulation of the total cellular MHC II pool in dendritic cells. In DCs, *Salmonella* infection upregulates E3 ubiquitin ligase, MARCH1 expression. This results in increased poly-ubiquitination of MHC II which is accompanied by enhanced internalization of surface MHC II. Our immunoprecipitation experiments indicate that *Salmonella* enhances lysine 63 linked polyubiquitination. Subsequently, ubiquitin- tagged MHC II molecules are degraded by endosomal proteases as the inhibitor against endosomal protease can rescue the phenotype. Further, perturbation of endosomal pH by endosomal acidification inhibitor also prevents *Salmonella* mediated downregulation of MHC II in DCs.

Chapter 3

Role of endosomal acidification in MHC II downregulation during *Salmonella* infection in dendritic cells

Endosomal proteolysis plays a crucial part in both MHC II trafficking and antigen presentation. Although, the role of endosomal protease and endosomal pH is well explored with respect to MHC II synthesis, processing and antigen processing, however, their role in MHC II degradation remains largely unexplored. As previously mentioned in chapter 2, endosomal acidification inhibitor and protease inhibitor treatment rescues MHC II downregulation in infected DCs. These results altogether implicate the involvement of

endosomal acidification and endosomal protease in *Salmonella* mediated MHC II

degradation. In principle, endosomal protease function can be regulated at multiple levels such as transcription, translation, synthesis as pro-enzyme and pH of endosomes. Although, transcriptional regulation of endosomal protease has been previously reported, still the most prevalent regulation strategy is via synthesis of pro-enzyme and pH. Generally, conversion of the pro-enzyme is either dependent on the action of another active protease or exposure to the acidic environment. Both these events result in the release of active site from pro-domain rendering the protease active. As results discussed in chapter 2 suggests an involvement of both endosomal protease and pH in MHC II degradation hence, in this chapter, we investigate the effect of endosomal acidification on *Salmonella* mediated MHC II degradation.

Our results unravel a novel link between endosomal proteolysis of MHC II and delayed NOX2 recruitment to phagosomes in infected BMDCs. In this study, we have demonstrated how *Salmonella* evades MHC II mediated adaptive immune response in dendritic cells through enhanced endosomal proteolysis. Lysotracker green and pH rodo dye based experiments demonstrate that endosomes present in infected cells have a lower pH as compared to that of control DCs. This difference is pH is transient and disappears at a later time point. In addition, confocal imaging suggests that *Salmonella* delays NOX2 recruitment to the phagosome, an innate immune response element in infected cells. This prohibits phagosomal alkalinization. The role of NOX2 recruitment in MHC II degradation is further validated by experiments performed using DCs isolated from Gp91 phox$^{-/-}$ mice. In these DCs, paraformaldehyde fixed *Salmonella* treatment show reduction of MHC II as compared to wild type DCs. Notably, endosomal acidification inhibitor like chloroquinetreatment of live *Salmonella* infected Gp91 phox$^{-/-}$ DCs show rescue of MHC

II levels. In totality, these results confirm the role of NOX2 regulated endosomal acidification and its effects in*Salmonella* mediated MHC II degradation in DCs. The current study has revealed phagosomal acidification as a mode of regulation for protease activity in DCs.

Chapter 4

Salmonella escapes adaptive immune response via SIRT2 mediated modulation of innate immune response in dendritic cells

Salmonella being a successful pathogen employs a plethora of immune evasion mechanism. This contributes to pathogenesis, persistence and also limits the efficacy of available treatment. All these contributing factors impinge upon the need for new drug targets against *Salmonella*. Although, various previous studies have focused on the role of host epigenetic modification during *Salmonell*a pathogenesis, however, till date there has been no study on the role of SIRT2 in *Salmonella* infection. For the first time, we have demonstrated that *Salmonella* upregulates sirtuin2 (SIRT2), a NAD+ dependent deacetylase in dendritic cells (DC) both in transcript and protein level. Inhibition of SIRT2 *ex-vivo*, increases intracellular survival of the pathogen and enhances antigen presentation. SIRT2 upregulation brings about degradation of IκB and translocation of NFκB p65 to the nucleus. Co-immunoprecipitation experiment establishes physical interaction between SIRT2 and NFκB p65. Nuclear translocation of NFκB p65 upregulates iNOS transcription and subsequently nitric oxide (NO) production. Being an antimicrobial agent as well as a suppressor of T cell proliferation, NO regulation can affect *Salmonella* infection in positive and negative ways. Our results establish the trade-off made by *Salmonella* where infection

mediated upregulation of SIRT2 enhances antimicrobial response, however, simultaneously this process inhibits $CD8^{+}$T cell response resulting in successful pathogenesis. Inhibition of SIRT2 *in-vivo* shows lower bacterial organ burden and reduced tissue damage. iNOS mediated effect of SIRT2 is further validated by the absence of the effect of SIRT2 inhibition in $iNOS^{-/-}$ mice. SIRT2 $^{-/-}$mice also demonstrate reduced bacterial organ burden as compared to wild type mice. Collectively, our results prove the role of SIRT2 in *Salmonella* pathogenesis and the mechanism of action. It also highlights how pathogen modulates host innate immune system to evade more specialized adaptive immune response. This finding can aid in designing of novel host targeted anti-*Salmonella* therapeutics.

Salmonella enterica is the cause of infectious diseases which ranges from self-limiting diarrhea to fatal systemic illness like typhoid. The success of *Salmonella* as a pathogen lies in its ability to escape host immune response. During its pathogenesis, *Salmonella* not only survives inside DCs but also suppressed antigen presentation. This results in compromised immune response to the pathogen. Here we show, *Salmonella* regulates SIRT2 expression in DCs, which in turn upregulates nitric oxide production.