Abstract

Salmonella comprises a genus of rod-shaped, Gram-negative, non-sporulating and primarily motile enterobacteria. They have remained a major cause of morbidity and mortality worldwide, claiming several hundreds of lives per year. Infection by these facultative anaerobes culminates in a broad spectrum of illnesses ranging from the self-limiting gastroenteritis to the occasionally fatal typhoid fever. Salmonella infection occurs via the faeco-oral route following ingestion of a dose sufficient to cause infection (105-108). Salmonella overcomes the acidic pH of the stomach and adheres to the mucosa in the small intestine. It then usually utilizes the M-cells as a gateway to enter the reticuloendothelial system.

Intracellular pathogens face a major challenge in avoiding fusion with lysosomes. A variety of intracellular pathogens have devised various means of evading lysosomal degradation. Listeria and Shigella escape into the cytosol and multiply. Mycobacterium in contrast prevents maturation of its phagosome thereby escaping fusion with lysosomes. In case of Salmonella, numerous mechanisms have been proposed and there have been contradictory reports.

During the intracellular life of Salmonella, the fusion targets for lysosomes are the SCVs. Therefore, the number of SCVs present in a host cell at a particular infection load is crucial. If each vacuole houses multiple bacteria then relatively lesser number of lysosomes would efficiently bring about the killing of the pathogens. In contrast, a single bacterium per vacuole would necessitate an enormous increase in the required lysosome number to combat similar bacterial loads.

In literature, SCVs have sometimes been represented as a large vacuole containing multiple bacteria. However, electron micrographs of SCVs in certain studies show the presence of a single bacterium per vacuole.

A previous study from our lab has clearly indicated the presence of single bacterium per SCV which, as mentioned above, brings about a huge lysosome number requirement. It had also been observed by our group that there is a reduction in total acidic compartments measured by the pH probe Lysotracker

green, upon infection with S.Typhimurium. In this study, we tried to understand the mechanism of division of the SCV and unravel the mechanism of modulation of lysosome dynamics by Salmonella.

The thesis is divided into four chapters. In Chapter 1, we have introduced our pathogen of interest, Salmonella. A brief overview of the pathogen properties and disease manifestations along with drug resistance is discussed in this chapter. The subsequent sections describe the virulence factors, pathogenesis and host defense mechanisms associated with the pathogen. Towards the end, the objectives of the present study have been discussed. The Salmonella containing vacuole (SCV) comprises two membranes: an inner membrane of bacterial origin and an outer membrane of host cell origin.

In Chapter 2 we have discussed our study in which we aimed at understanding how the SCV maintains its single bacterium per vacuole status through late time points of infection. The SCV requires membrane acquisition at the initial stages of infection followed by continuous membrane contacts with endosomes some of which is mediated by the Rab7 GTPase at various stages of SCV maturation. We have screened a plethora of mutants to unveil possible genes which might play important roles in SCV division. We have isolated a bacterial mutant which resides as multiple bacteria per vacuole and hence provides a tool to study the membrane dynamics of the SCV. Our results also indicate the requirement of the endoplasmic reticulum for dividing the bacterial vacuole. Interestingly, S.Typhimurium had been found to colocalize with dynamin related protein (Drp1). Both laser scanning confocal microscopy and immunogold electron microscopy on isolated vacuoles containing S.Typhimurium indicate the presence of Drp1.Moreover cells expressing dominant negative forms of Drp1 showed significantly reduced bacterial proliferation. This chapter concludes with a discussion on the proposed mechanism of division of the SCV and how the pathogen intelligently utilizes components of the host cell to successfully proliferate within.

It was observed in a previous study from our lab that there is a decrement in total volume of acidic compartments as measured by the fluorescence of the pH probe Lysotracker green. Conceivably, this decrement in the total volume of acidic compartments indicated by a reduction in the pH-probe

LTG fluorescence could result from two possibilities: an actual decrease in lysosome number or a mere change in pH of the lysosomal compartments. In Chapter 3, we describe our dissection of these possibilities. We generated a calibration curve utilizing FITC dextran to determine the exact lysosomalpH. The pH of lysosomes in infected cells was identical to those in uninfected cells. In trying to investigate the mechanism by which this reduction in acidic compartments is brought about, we observed Salmonella Typhimurium's interactions with a master regulator of lysosome biogenesis, namely, transcription factor EB. We report in this chapter that S. Typhimurium recruits this factor onto the SCV. We have tried to tease apart the roles of the major pathogenicity islands of S. Typhimurium in this phenomenon as well as the role for other genes. The various changes in the host cell upon infection which might be important in the interplay between the pathogen of interest and the above transcription factor have been explored. This chapter concludes with a discussion on the complex sequel of events that would ensue upon the interaction of the pathogen with a master transcription factor.

In contrast to the intracellular dynamics of the pathogen, we also tried to look at a relatively unexplored arena in Salmonella systemic infection; namely, its capacity to invade the host's brain. Neurological abnormalities have sometimes been reported in Typhoid patients and it would be intriguing to know whether the bacterium is capable of invading the host brain to bring about these abnormalities. We report our observations on this topic in Chapter 4.

We investigated the colonization of various brain parts by Salmonella in mice. We report that the bacterium is frequently able to invade various brain parts in mice. We show that the primary pathogenicity islands SPI-1 and 2 are not essential in brain invasion. Associated behavioral abnormalities were quantitated and the effect of antibiotic treatment on the pathogen load in brain was determined. This chapter concludes with a discussion on this under-investigated arena of Salmonella infection and the far-reaching impact of the pathogen's brain invasion capacities.