Chapter 1

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Shigellosis or Bacillary dysentery is an acute diarrheal disease caused by the gram negative bacillus *Shigella* which belongs to the family *Enterobacteriaceae*. *Shigella* was named after its discoverer Kiyoshi Shiga who identified the bacilli as the etiology agent in 1898 during an outbreak in Japan in 1897 (Kotloff et al., 1999; Niyogi, 2005). The genus *Shigella* includes four species viz., *Shigella dysenteriae* (13 serotypes), *Shigella flexneri* (15 serotypes), *Shigella boydii* (18 serotypes) and *Shigella sonnei* (1 serotype). *Shigella* spp. invades, replicates intracellularly and spread intercellularly within the colonic epithelium which results in the destruction of the host intestinal epithelium. This leads to the symptoms ranging from mild watery diarrhea to severe inflammatory dysentery characterized by strong abdominal cramps, fever and stools containing blood and mucus (Sansonetti, 2001; Torres, 2004).

World-wide it continues to be a major health problem causing one million deaths and over 163 million cases annually, particularly children among less than five years of age (Kotloff et al., 1999). It is transmitted through fecal-oral route. Only 10-100 organisms are sufficient to cause the infection. It is manifested by the passage of loose stools mixed with mucous and blood, accompanied by fever, abdominal cramps and tenesmus (Sur et al., 2004). The other symptoms includes fever, malaise, abdominal cramping and convulsions. The onset of symptoms usually occurs within 24 to 48 hours of ingestion of the etiologic agent. Possible other complications of shigellosis include bacteremia, septicemia, hypoglycemia, dehydration, hemolytic-uremic syndrome, reactive arthritis, toxic mega colon and other neurological problems.
(Phalipon and Sansonetti, 2007). The persons infected with *S. flexneri* subsequently develop pain in their joints, irritation of the eyes and painful urination. This condition is called Reiter’s syndrome. It is the late complication of *S. flexneri* infection and lasts for months which lead to chronic arthritis. Shiga toxin of *S. dysenteriae* type I is responsible for the Hemolytic-uremic syndrome. Life-threatening are often seen in malnourished infants and young children less than 5 years of age, also in elderly people who have weak immune system (Kotloff *et al.*, 1999; Ashkenazi, 2004; Seidlein *et al.*, 2006).

Malnutrition and lack of medical intervention leads to the severity of the disease by increasing the mortality rate especially in children. Oral rehydration therapy is not adequate in treating Shigellosis. The emerging antibiotic resistance limits the number of drugs available for the treatment of Shigellosis (Taylor, 2003). Shigellosis is a highly invasive disease that induces considerable immune pathology in the intestine. The ability of vaccines to invade is the major requirement for the induction of effective immunity. Currently there is no *Shigella* vaccine available and most of them including live attenuated, conjugate, broad spectrum and proteosome based vaccines are under development at different clinical trial phases. Owing to the wide range of *Shigella* serotypes and subtypes, there is a need for a multivalent vaccine representing prevalent species and serotypes. The lack of an appropriate animal model which mimics the human bacillary dysentery is also the drawback for the development of a successful *Shigella* vaccine. The licensed *Shigella* vaccine used in China is a live, oral, noninvasive, bivalent vaccine expressing O-antigens of *S. sonnei* and *S. flexneri* 2a, was designed to provide protection against both serotypes and based on passive surveillance studies reportedly provides ~60% protection in
adults (WHO, 2006; Kweon, 2008). But mice, guinea pigs, rabbits and macaques have been considered as possible bacillary dysentery models for clinical experiments (mallet et al., 1995; Shim et al., 2007; Rabbani et al., 1995; Takeuchi et al., 1968).

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Shigella spp. contains a large 220-Kb virulence plasmid for invasion. The virulence plasmid encodes 30 Kb Mxi-Spa type III secretion system (TTSS). The TTSS is used by Shigella to invade the epithelial cells of the human colon. It acts as a molecular syringe and comprises of effector proteins designed to penetrate and manipulate the targeted host cell. These primarily consist of the invasion plasmid antigens (IpaA, IpaB, IpaC, IpaD) required for the invasion of epithelial cells. These Ipa genes which are primarily effector/ translocator proteins are directly responsible

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![Figure 1.1: Clinical progression of Shigellosis](image-url)
for the release of the bacteria into the host cell cytosol by inducing cytoskeletal rearrangements, membrane ruffling and pathogen uptake (Menard et al., 1993; Sansonetti, 2001; Jennison et al., 2004). Ipa proteins were reported as the dominant antigens inducing humoral immune response during infection (Oaks et al., 1986). Macrophages are the primary cells that S. flexneri meets after transeptosis through M cells from the lumen to the sub epithelial pocket of the intestine. Then the phagocytes engulf Shigella, which in turn after its uptake rapidly disrupts the phagosomal membrane which leads to apoptosis (Clerc et al., 1987). This macrophage death is mediated by the Mxi-Spa secreted effector protein IpaB, which is required to induce phagocyte apoptosis in host macrophages and dentritic cells leading to inflammation (Thirumalai et al., 1997; Hilbi et al., 1998; Sansonetti, 2001; Guichon et al., 2001). IpaB protein has been shown to be the major immunogen in the convalescent sera from the infected monkeys or humans (Oaks et al., 1986). Since Ipas are antigenically similar in different Shigella species, therefore this led to speculation about stimulating cross-protective immunity directed against these proteins. Recent studies clearly indicate the immunogenicity and protective efficacy of Invasin complex (IpaB, IpaC and LPS) conferring significant protection against Shigella infections in mouse lung model (Turbyfill et al., 2008; Martinez Becerra et al., 2012). The immunodominant domain regions of IpaB necessary for the process of invasion have been predicted in the earlier studies (Mills et al., 1988; Barzu et al., 1993; Shen et al., 2010). Based on those studies, here we have selected the IpaB domain regions. Due to the above pathogenic and immunogenic properties, in this study, IpaB was selected as a protective antigen, cloned and expressed its domain region.
HSPs (Heat shock proteins) are the phylogenetically conserved molecules throughout evolution in all forms of life (Kaufmann, 1990; Zugel and Kaufmann, 1999; Feder and Hofmann, 1999). These proteins are expressed under various stressful conditions including pathological, environmental and physiological insults (Jaattela, 1999; Wu et al., 2006). Based on their molecular weight these proteins are classified into six families viz., Hsp 10, Hsp 40, Hsp 60, Hsp 70, Hsp 90 and Hsp 100. The representatives of HSP60 subfamily are found in bacteria, plant chloroplasts, and mitochondria of animal cells.

Figure 1.2: Diagram shows the role of heat-shock proteins and a chaperonin in protein folding. As the ribosome moves along the molecule of messenger RNA, a chain of amino acids is built up to form a new protein molecule. The chain is protected against unwanted interactions with other cytoplasmic molecules by heat-shock proteins and a chaperonin molecule until it has successfully completed its folding.

HSPs function as molecular chaperones in numerous processes such as folding and unfolding of proteins, assembly and disassembly of protein complexes and antigen processing under physiologic and stress conditions (Zugel and Kaufmann, 1999). Various families of HSPs especially Hsp 60, Hsp 70 and Hsp 90 are reported
to elicit innate and adaptive immune responses (Basu and Srivastava, 2000; Srivastava et al., 2001; Tsan and Gao, a, b, 2004).

HSPs shares a high degree of sequence homology between bacteria and mammals (Zugel and Kaufmann, 1999). This strong conservation results in the presence of cross-reactive epitopes on different Hsps. During microbial infections, the host cells rapidly degrade the foreign Hsps and these Hsp derived determinants form a major group of antigens inducing both humoral and cell-mediated immune responses in mammals (Kaufmann, 1990; Zugel and Kaufmann, 1999). For the host, frequent interaction with microbes results in the generation of an immunological memory for these cross-reactive determinants. As a result, an immune response to the conserved determinants shared by HSP is developed which prevents further colonization of host by the microbes. It was reported that this cross-reactivity to shared epitopes might elicit cross-protection against different pathogens (Zugel and Kaufmann, 1999).

HSPs are reported to be a potential therapeutic agents and their role in priming multiple host defence pathways are being exploited in vaccine development in cancer and infectious diseases (Graham Pockley, 2001; Segal et al., 2006). Recently, we reported significant protection (70-90%) using recombinant DnaJ (Hsp40), GroEL (Hsp60), DnaK (Hsp70) of S.Typhi as candidate vaccine molecules against lethal challenge by both Salmonella enterica serovar Typhi and S. Typhimurium (Sagi et al., 2006; Paliwal et al., 2008, Bansal et al., 2010; Paliwal et al., 2011). Immune responses to HSPs have been observed in infectious diseases caused by bacteria, protozoa, fungi, nematodes, as well as in various experimental infection models. Several other researchers have also reported the protective efficacy
of microbial HSPs (Hsp60, Hsp70, Hsp90) against respective pathogens viz., Porphyromonas gingivalis (Lee et al., 2006), Helicobacter pylori (Ferrero et al., 1995), Histoplasma capsulatum (Gomez et al., 1995), Piscirickettsia salmonis (Wilhelm et al., 2005), Yersinia enterocolitica (Noll et al., 1996), Mycobacterium tuberculosis (Lowrie et al., 1997), Candida albicans (Matthews et al., 1992), inducing both arms of immunity, thus fulfilling the requirement of traditional vaccine. We have earlier also reported the adjuvant property of S. Typhi GroEL in mice (Bansal et al., 2010).

With this background, the present study was planned to investigate the cross-protective efficacy of recombinant GroEL of S. Typhi against various bacterial pathogens the immunogenicity and protective efficacy of recombinant Shigella IpaB domain against Shigella spp. Since we have already reported earlier that recombinant GroEL of S. Typhi is protective without adjuvant, in this study we also examined the adjuvanticity of GroEL when co-administered or fused with IpaB of Shigella. Since IpaB and GroEL are conserved in all the serotypes of Shigella spp. it will be a potent cross-protective antigen for the development of a safe and effective vaccine candidate against all Shigella serotypes.

Considering the importance for the development of an effective vaccine candidate molecule against Shigella infection, the following was the aim and objective of the present study.

**AIM**

To develop an effective recombinant vaccine candidate molecule against Shigella infections.
OBJECTIVES

1) To study the cross-protective efficacy of recombinant GroEL (Hsp60) of S. Typhi (developed earlier in the lab) against multiple pathogens including *Shigella* species.

2) Cloning, expression, isolation and purification of the *Shigella* IpaB domain.

3) Immunological and protective efficacy studies with purified IpaB protein against lethal infection by *S. flexneri, S. boydii* and *S. sonnei* in mice.

4) Immunogenicity and protective efficacy of recombinant IpaB co-administered with *S. Typhi* GroEL against *S. flexneri, S. boydii* and *S. sonnei* in mice.

5) Development of fusion gene construct of IpaB with GroEL.

6) Cloning, expression, isolation and purification of the fusion protein (IpaB-GroEL).

7) To study immunological parameters and protective efficacy of fusion protein against *S. flexneri, S. boydii* and *S. sonnei* in mice.