Chapter 7

Summary
Shigellosis or bacillary dysentery is a highly contagious diarrheal disease worldwide causing a major public health problem. Malnutrition and the lack of appropriate medical intervention contribute to the high mortality rate, especially for young children. Despite these, the emergence of multi-drug resistant *Shigella* strains urged WHO to put the development of *Shigella* vaccine at the top of its priority list of awaited vaccine against enteric infections. Considering these global facts, the aim of the study was to develop a common protective recombinant vaccine candidate molecule against all *Shigella* species and its serotypes.

As HSPs share a high degree of sequence homology between various pathogens, it was proposed in the present study to investigate the cross-protective efficacy of recombinant GroEL of *S.*Typhi against various bacterial pathogens including *Shigella* spp. *In vitro* serum bactericidal assay (SBA) with GroEL antisera showed 50-55% inhibition of cells of *Shigella* Spp., 65-75% of *Esherichia coli*, 60-65% of *Klebsiella pneumoniae*, 45-50% of *Pseudomonas aeruginosa*. In *in vivo* experiments, mice immunized with GroEL protein of *S.*Typhi showed 60-65% protection against *S.*flexneri, *S.* dysenteriae type I, *S.*boydii. Similarly 75-80% protection was observed against enteropathogenic *E.*coli, 70-80% against *K. Pneumoniae*. 50% of mice survived the lethal infection against *P. aeruginosa*. Organ burden and histopathological studies also revealed significant reduction of bacterial infection suggesting the cross-protective efficacy of recombinant GroEL of *S.*Typhi.
The selected vaccine antigen is domain region of Invasion plasmid antigen B (IpaB) of *Shigella flexneri*. The IpaB domain (800 bp) was cloned in prSET A expression vector, expressed and purified the 37 Kda recombinant protein from the *E. coli* BL21 cells. Further, the immunogenicity and protective efficacy of Shigella recombinant IpaB domain, S. Typhi GroEL as adjuvant, IpaB+GroEL when co-administered and when fused (IpaB-GroEL fusion protein) was assessed in mice immunized with these proteins against *Shigella* infection (*S. flexneri*, *S. boydii* and *S. sonnei*). Immunization (i.n.) of mice with rIpaB Domain protein, GroEL, IpaB+GroEL IpaB-GroEL fusion protein resulted in the increase of both serum IgG and IgA and BALF IgA titres as compared to the control. But the titres were highest in fusion protein group followed by co-immunized group, IpaB/GroEL alone. Antibody isotyping revealed elevated levels of both IgG1 and IgG2a antibodies indicating induction of both Th1 and Th2 type of immune responses. The ratio of IgG1 and IgG2a was also high in all the immunized groups but showed decreasing trend towards fusion protein group, suggesting shift towards Th1 immune response.

High lymphocyte proliferation in fusion protein group as compared to other immunized groups was observed. Cytokine estimation showed higher production of IFN-γ indicating a strong Th1 immune response in mice. 60-70% protection was observed in the IpaB immunized mice, challenged with *S. flexneri, S. boydii and S. sonnei* (i.n). Interestingly when GroEL was administered along with IpaB the survival percentage of mice increased to 80% indicating the adjuvant effect of GroEL which was further increased to 90% in fusion protein group against the lethal infection of *Shigella* spp. However, passive immunization studies showed 50-60% protection in mice immunized either with anti-IpaB or anti-GroEL+anti IpaB or anti IpaB anti-
GroEL immune sera, demonstrating that antibodies alone are not sufficient for protection against Shigellosis. Organ burden studies revealed significant reduction in the number of colonies of *S. flexneri*, *S. boydii* and *S. sonnei* in the lung tissues of fusion group as compared to co-immunized mice and IpaB/ GroEL alone. Histopathological studies of lung tissue also showed improved tissue morphology in immunized mice challenged with *Shigella* spp. as compared to control.

In conclusion, these findings provides the platform for the development of safe and effective recombinant vaccine against Shigellosis. This study also shows the potential of IpaB domain as an effective vaccine candidate molecule and GroEL of *S. Typhi* as an effective adjuvant for the development of broadly protective vaccine against all *Shigella* species and its serotypes.