Mutation of *ompA* gene of *Shigella flexneri*: towards development of an oral live attenuated *Shigella* vaccine

by

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Abstract

Shigellosis is one of the major infectious diseases in the world. Each year in developing country, *Shigella* spp. cause illness in over 150 million individuals and death in over one million (Kotloff *et al.*, 1999). Children under the age of 5 are most severely affected. In developing countries, *S. flexneri* and *S. sonnei* are the most prevalent species, causing about 60% and 15% of episodes, respectively.

The enormous global burden of *Shigella*, augmented by the growing rate of anti-microbial resistance, makes development of an effective vaccine essential. At present there is no vaccine against *Shigella*, although a variety of live and subunit vaccines which elicit an anti-LPS mucosal response have been shown to confer protection in experimental models of shigellosis (Levine *et al.*, 1976; Sanchez *et al.*, 1994; Jennison *et al.*, 2004).

Live, attenuated *Shigella* strains have been the dominant approach to *Shigella* vaccine development since a 1966 report (Formal *et al.*, 1966) showing that monkeys were protected against subsequent disease if previously administered attenuated organisms.

S. flexneri poses a structural gene, *ompA* which encodes for outer membrane protein A. Outer membrane protein A adds to the stability of the cell envelope by linking the outer membrane to the peptidoglycan. Therefore, in this study the gene of interest is *ompA* whereby the gene will be mutated by insertional mutation. The mutants are assumed to induce protective immunity against shigellosis. In this study, *ompA* has been mutated by using kanamycin resistance gene (*aphA*). The use of this antibiotic resistance gene is to facilitate the process of manipulating the genes as it serves as a selective marker. The creation of the construct *ompA::aphA* is to facilitate the approach into the development of a potential live attenuated *Shigella* vaccine.

Abstrak

Shigellosis merupakan salah satu penyakit berjangkit utama di dunia. Setiap tahun, *Shigella* spp. menyebabkan penyakit ke atas lebih daripada 150 juta orang dan kematian lebih daripada 1 juta orang dalam negara yang sedang membangun (Kotloff *et al.*, 1999). Kumpulan yang paling serius dijangkiti adalah kanak-kanak di bawah umur 5 tahun. Di negara yang sedang membangun, *S. flexneri* dan *S. sonnei* merupakan spesies utama yang menyebabkan jangkitan, masing-masing menyumbang kepada 60% dan 15% kes.

Bebanan global *Shigella* yang besar telah diperkembangkan dengan kadar rintangan antibiotik yang kian meningkat. Akibatnya, penghasilan vaksin yang efektif adalah diperlukan. Walaupun pelbagai jenis vaksin hidup dan vaksin subunit yang dapat merangsangkan gerak balas mukosa dan memberikan perlindungan kepada model eksperimen telah dihasilkan, tetapi masih tidak terdapat vaksin *Shigella* di pasaran (Levine *et al.*, 1976; Sanchez *et al.*, 1994; Jennison *et al.*, 2004)

Pada tahun 1966, satu laporan telah dikemukakan oleh Formal *et al.* Laporan tersebut menunjukkan bahawa perlindungan kepada jangkitan telah berlaku ke atas moyet yang diberi dengan organisma yang dilemahkan. Sejak laporan tersebut dikemukakan, strain *Shigella* yang hidup dan dilemahkan merupakan pendekatan yang utama dalam pembangunan vaksin *Shigella*.

S. flexneri mempunyai satu gen struktur yang dikenali sebagai *ompA*. Gen *ompA* mengekodkan kepada protein membran luaran A. Fungsi protein luaran A adalah untuk memberikan kestabilan kepada sampul sel dengan menghubungkan membran luaran kepada peptidoglikan. Jadi, dalam kajian ini, *ompA* adalah gen yang akan dimutasikan. Mutan yang terhasil dipercayai akan merangsangkan imuniti terhadap shigellosis. Dalam kajian ini,

ompA dimutasikan dengan memasukkan gen rintangan kanamycin (*aphA*) ke dalam gen tersebut. Penggunaan gen rintangan antibiotik ini adalah untuk memudahkan proses manipulasi gen. Selain itu, gen rintangan kanamycin juga berfungsi sebagai "selective marker" yang akan memudahkan proses pemilihan konstruk *ompA::aphA*. Pembentukan konstruk *ompA::aphA* merupakan satu pendekatan kepada pembangunan vaksin *Shigella* yang berpotensi.

Chapter 1 Introduction

1.1 Introduction to *Shigella*

Shigella was discovered over 100 years ago by a Japanese microbiologist named K. Shiga. *Shigella* is Gram-negative, non-motile, facultatively anaerobic, non-spore-forming rods in the family *Enterobacteriaceae*. It is differentiated from the closely related *Escherichia coli* on the basis of pathogenicity, physiology (failure to ferment lactose or decarboxylate lysine) and serology. The genus *Shigella* consist of four species: *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C) and *S. sonnei* (subgroup D) (http://vm.cfsan.fda.gov/~ebam/bam-6.html).

Shigella is the causative agent of human shigellosis, however *Shigella* also causes disease in other primates, but not in other mammals. Shigellosis is spread by means of fecal-oral transmission. Other modes of transmission include ingestion of contaminated food or water, contact with a contaminated inanimate object and sexual contact. Vectors like the housefly can spread the disease by physically transporting the infected feces. The infectivity dose (ID) is extremely low. As few as 10 *S. dysenteriae* bacilli can cause clinical disease, whereas 100-200 bacilli are needed for *S. sonnei* or *S. flexneri* to cause infection. The incubation period varies from 12 hours to 7 days, but typically it is 2-4 days and it is inversely proportional to the load of ingested bacteria. Bacterial shedding usually ceases within 4 weeks of the onset of illness. A chronic carrier state beyond 1 year is rare (http://www.emedicine.com/ped/topic2085.htm).

Shigellosis or bacterial dysentery is a syndrome characterized by frequent, but small volume, loose stools, consisting largely of blood and mucus. Fever, pain and tenesmus (unproductive straining) are frequently present. Unlike secretory diarrhoeas, this syndrome

is a result of invasion of the distal small bowel and/or colon by bacteria. Shigellosis usually resolves in 5 to 7 days, but in some persons, especially young children and the elderly, the diarrhoea can be so severe that the patient needs to be hospitalized. A severe infection with high fever may also be associated with seizures in children less than 2 years old. Some persons who are infected may have no symptoms at all, but may still transmit the *Shigella* bacteria to others (http://www.textbookofbacteriology.net/*Shigella*.html).

1.2 Epidemiology of shigellosis

Shigella is the primary causative agent of bacillary dysentery throughout the developing world. According to the World Health Organization, the annual number of *Shigella* episodes throughout the world was estimated to be 164.7 million, of which 163.2 million were in developing countries (with 1.1 million deaths) and 1.5 million in industrialized countries (WHO 2002). The burden of disease in Asia was estimated by reviewing studies initiated in Asian countries after 1990 and of which the results were published. Total morbidity and mortality attributable to *Shigella* were estimated through extrapolation. Overall, shigellosis remains a common disease in the Asia continent. The annual numbers of episodes and deaths due to *Shigella* in Asia were estimated to be 91 million and 414,000 respectively. *S. flexneri* is the commonest serotype, followed by *S. sonnei* (Legros, 2004). A total of 69% of all episodes and 61% of all deaths attributable to shigellosis involved children under 5 years of age (Kotloff *et al.*, 1999), particularly if there is malnutrition. In epidemic situations a mortality rate as high as 3.9% in children under age 1 and 19.3% for infants under 4 months of age has been reported. The case fatality rate declines with increasing age (Bennish *et al.*, 1990; Bennish, 1991). Normally, in normal healthy individual, shigellosis is a condition characterized by bloody diarrhoea, fever and

severe abdominal cramps. Whereas in the immunocompromised humans results in more severe manifestations of *Shigella* infection, including persistent and recurrent intestinal diseases and bacteremia (Angulo and Swerdlow, 1995).

There are four species of *Shigella: boydii, dysenteriae, flexneri and sonnei.* Two of the four species of *Shigella* are common in developing countries. *Shigella flexneri* is endemic (present at all times) in most communities. Whereas, *S. dysenteriae* type 1 often occurs in an epidemic pattern; the organism can be absent for a number of years, only to reappear and infect a large proportion of the population. These two species of *Shigella* generally produce the most severe illness. In developed countries, *Shigella sonnei* is the most common and is the least virulent *Shigella* bacterium. *Shigella boydii* causes disease of intermediate severity and is least common of the four, except in the Indian sub-continent (http://www.rehydrate.org/dd/su44.htm).

1.3 Pathogenesis of *Shigella*

Humans are the only known natural host of *Shigella.* However, *in vitro* and *in vivo* studies have used various cell types (epithelial cells, macrophages, monocytes, fibroblasts and red blood cells) (Oaks *et al.*, 1985) and animal models of infection, such as guinea pigs, rabbit ligated ileal loops and mouse lungs, to study various aspects of the molecular basis for pathogenesis of *Shigella. Shigella* has a very low infectious dose, requiring as few as 10-200 bacteria to establish infection in a healthy adult (DuPont *et al.*, 1989). This is mainly due to the ability of *Shigella* to survive the low pH of the human stomach (Gorden and Small, 1993). After passing through the acidic conditions in the stomach, *Shigella* ultimately reaches the colon. Experiments using polarized cell lines have demonstrated that *S. flexneri* invades colonic epithelial cells from the basolateral side (Mounier *et al.*, 1992).

Invasion of epithelial cells requires the invasion plasmid antigens (Ipa) secreted by the virulence plasmid encoded type three secretion system in *S. flexneri*. In addition to the virulence factors discussed above, endotoxins contribute to the pathogenicity of *Shigella* spp. and may be responsible for many of the systemic symptoms of shigellosis, such as fever, malaise and body aches. *S. dysenteriae* type 1 produces a potent protein cytotoxin known as Shiga toxin.

There are three known mechanisms by which *Shigella* gains access to the submucosal layer of epithelial cells. First, *S. flexneri* can disrupt the tight junction proteins on epithelial cells, allowing paracellular movement of bacteria into the sub-mucosa (Sakaguchi *et al.*, 2002). Second, polymorphonuclear leukocytes recruited by interleukins produced in response to *S. flexneri* invasion create gaps between epithelial cells, through which the bacteria can transmigrate into the sub-mucosa (Beatty and Sansonetti, 1997). Third, in the colonic mucosa, *Shigella* crosses the epithelial layer barrier by invading the M cells overlying the lymphoid follicles (Wassef *et al.*, 1989). From the M cells, the bacteria are released into an intraepithelial pocket filled with lymphocytes and macrophages. Macrophages phagocytose the bacteria but *S. flexneri* escapes the phagosome and induces apoptosis in the macrophage. After release from the macrophage, bacteria enter the submucosal layer and invade the epithelial cells from the basolateral side.

Upon contact with epithelial cells, *Shigella* induces the formation of cell extensions that reach several tens of microns in length, rise above the apical cell surface at the site of bacterial interaction and engulf the bacterium in a large vacuole in a process reminiscent of macropinocytosis (Sansonetti and Egile, 1998). The uptake of bacteria by the epithelial cells involves membrane ruffling due to rearrangement of the eukaryotic cell cytoskeleton. Once internalized, *Shigella* lyses the phagosomal membrane and multiplies freely in the

cell cytosol. During this multiplication phase, *Shigella* moves intracellularly by polymerizing actin at one pole of the bacterial body (Sansonetti and Egile, 1998). Using this actin-based motility, the bacterium induces protrusions that invade neighboring cells. After lysis of both protrusion and recipient cell membranes, *Shigella* reinitiates its intercellular cycle and can spread within the cell monolayer without an extracellular step. Both cell entry and intracellular motility are examples of bacterial manipulation of processes controlling the host cytoskeletal dynamics (Sansonetti, 1998, Parsot and Sansonetti, 1996, Sansonetti and Egile, 1998, Nhieu and Sansonetti, 1999).

The ability of *Shigella* to colonize the epithelial layer protects the bacteria from exposure to the extracellular environment. Intracellular growth and multiplication ultimately result in the death of epithelial cells, with resultant ulceration and mucosal inflammation (Keusch and Bennish, 1998; Keusch, 1998).

1.4 *Shigella* transmission

Shigellosis is spread by means of fecal-oral transmission. Other modes of transmission include ingestion of contaminated food or water, contact with a contaminated inanimate object and sexual contact especially man who have sex with man. Vectors like the housefly can spread the disease by physically transporting infected feces. Several investigations demonstrated that the transmission of the disease increases with poor hand-hygiene, contaminated drinking-water, inadequate sanitation and poor toileting behaviours (Legros, 2004). Indeed, poor toileting practices may lead to contamination of hands and subsequently of stored drinking-water and food.

1.5 Diagnosis of shigellosis

The physical signs and symptoms of shigellosis include abdominal cramps, fever and chills, malaise, diarrhoea and/or dysentery and abdominal tenderness. Examination of the rectal mucosa shows it to be inflamed and friable, with ulcers present in severe cases. Blood and mucus in the stool are frequent manifestations of shigellosis and abundant faecal leukocytes are generally noted on examination due to the inflammatory and invasive characteristics of the organism.

Until now, there is no commercially available serologic assay to aid in the diagnosis of shigellosis. Definitive diagnosis of *Shigella* infection is made by culturing the organism from a stool specimen. Areas of faecal mucus are optimal for sampling. If whole stool is not available, comparable culture results can be obtained by gently swabbing the rectal mucosa with a cotton-tip applicator inserted into the anus (Adkins and Santiago, 1987).

Shigella are extremely fastidious and survive poorly in stool samples that are left in ambient temperature; therefore, the sample should be fresh. If it cannot be plated quickly onto solid media, the specimen should be inoculated into transport media and refrigerated. Buffered glycerol saline may be preferable to Cary Blair as a transport media (Wells and Morris, 1981). A variety of mildly selective as well as highly selective media are appropriate for culturing *Shigella*. Media that inhibit the growth of Gram-positive bacteria, such as MacConkey, *Shigella-Salmonella* (SS) agar or xylose-lysine-deoxycholate (XLD), are necessary for isolation of *Shigella* from clinical specimens. After overnight incubation at 37°C *Shigella* appear as pale, non-lactose-fermenting colonies on MacConkey agar and as pink colonies on XLD medium (Shears, 1996). Yield of culture can be enhanced by the addition of enrichment media such as Gram negative (GN) broth (Clemens, Kotloff and Kay, 1999). The identity of suspected colonies is confirmed using standard methods,

including agglutination with species specific antiserum. However, the traditional identification method by culture lacks of sensitivity due to the low number of causative microorganisms, competition with commensal organisms and deleterious changes in ambient temperature and pH during specimen transportation (Taylor and Schelhart, 1975; Shears, 1996; Thiem *et al.*, 2004). The detection is also frequently impaired by the use of antibiotics prior to specimen collection.

Since there are a lot of disadvantages of using traditional method, more rapid and sensitive techniques for detecting *Shigella* have been developed. The new techniques for detecting *Shigella* should be robust, quick, reliable (sensitive and specific), efficient on fecal samples and easy to use at patient's bedside. Two approaches have emerged: polymerase chain reaction (PCR) detection (Frankel *et al.*, 1990; Sethabutr *et al.*, 1994; Houng *et al.*, 1997; Thiem *et al.*, 2004; von Seidlein *et al.*, 2006) and immunochromatographic techniques, for example, dipsticks based on the recognition of pathogen-specific antigens by monoclonal antibodies (mAbs).

For PCR detection, the designed primers are directed towards virulence genes such as the invasion plasmid locus (*ipl*) or that encoding the IpaH antigen virulence factor. Although more sensitive than the conventional diagnostic methods, this technique requires a sophisticated laboratory and is not widely used in clinical laboratories (Keusch and Bennish, 1998; Keusch, 1998). While, for immunochromatographic techniques, it utilizes the principle of antigen-antibody reaction. The dipstick is based on the detection of lipopolysaccharide (LPS), the major bacterial surface antigen (Nato *et al.*, 2007). Indeed, *Shigella* serotypes are defined by the structure of the oligosaccharide repeating unit (RU) that forms the O-antigen (O-Ag), the polysaccharide moiety of LPS (Linberg *et al.*, 2005). For *S. flexneri* serotype 2a, the biological RU is a branched pentasaccharide. It is composed

of a linear tetrasaccharide backbone made of three L-rhamnose residues, A, B and C and a N-acetyl-D-glucosamine residue D, that is common to all *S. flexneri*, except serotype 6 and of an *a*-D glucose residue E, branched at position 4 of rhamnose C that specifies serotype 2a (Linberg *et al.*, 2005; Phalipon *et al.*, 2006). The monoclonal antibody (mAb) coated at the test region will recognize serotype 2a-specific determinants carried by the LPS O-Ag in stool samples. Production of band in test region indicates the presence of LPS O-Ag in the stool sample.

1.6 Treatment for shigellosis

Although severe dehydration is uncommon in shigellosis, the first consideration in treating any diarrhoeal disease is correction of abnormalities that result from isotonic dehydration, metabolic acidosis and significant potassium loss. The oral rehydration treatment developed by the World Health Organization has proven effective and safe in the treatment of acute diarrhoea, provided that the patient is not vomiting or in shock from severe dehydration. In the latter case, intravenous fluid replacement is required until initial fluid and electrolyte losses are corrected. With proper hydration, shigellosis is generally a self-limiting disease and the decision to prescribe antibiotics is predicated on the severity of disease, the age of the patient and the likelihood of further transmission of the infection.

The use of antimicrobial therapy requires the knowledge of the antimicrobial resistance pattern of *Shigella* strains circulating locally. The choice of antimicrobials in treating shigellosis is now limited. Originally, both sulfonamides and tetracycline were effective, but *Shigella* strains rapidly developed resistance to these agents. Ampicillin and Trimethoprim-Sulfamethozazole (TMP-SMZ) were then used and continue to be effective in many industrialized countries. Unfortunately, in many parts of the world strains of all

species of *Shigella* have become resistant to these low-cost agents and neither can now be confidently used as empiric therapy for shigellosis (Tauxe *et al.*, 1990; Haltalin *et al.*, 1967; Nelson *et al.*, 1976; Chang *et al.*, 1977; DuPont and Steele, 1987; DuPont *et al.*, 1987; Bennish and Salam, 1992; Bennish *et al.*, 1992). To date, tetracycline, ampicillin, cotrimoxazole and nalidixic acid are no longer effective in many countries, especially in patients infected with *S. dysenteriae* type 1 (Legros, 2004). The antimicrobials that remain effective are mecillinam, ciprofloxacin, other fluoroquinolones, ceftriaxone and azithromycin. However, in 2003 and 2004, a few cases of ciprofloxacin and other fluoroquinolones-resistant *S. dysenteriae* type 1-associated infection have been reported from India, Bangladesh and Nepal (Legros, 2004).

In the wake of an alarming increase in antibiotic resistance in *Shigella* spp., alternative medicines are an attractive approach. Antibacterial peptides, such as LL-37, are the first line of host defense that prevents microbial intruders (Legros, 2004). Sodium butyrate, a short-chain fatty acid normally produced by the gut flora, was shown to induce up-regulation of this peptide in the colonic cell lines. In the rabbit model of shigellosis, butyrate treatment was shown to enhance expression of CAP-18, the precursor of LL-37, in the surface epithelial cells and reduction in inflammation, reduction in bacterial count in stool and improved clinical recovery (Legros, 2004). In addition, supplementing protein-rich diet during convalescence significantly enhances catch-up growth, while prolonged supplementation of zinc has both therapeutic and preventive impacts on diarrhoeal illnesses (Legros, 2004).

1.7 Control measures and prevention for *Shigella* infection

As is the case with other intestinal infections, the most effective methods for controlling shigellosis are provision of safe and abundant water and effective feces disposal. These public health measures are, at best, long range strategies for control of enteric infections in developing countries.

Since there is no vaccine, shigellosis can be prevented by washing hands thoroughly with soap and warm water after using bathroom and before eating and preparing food.

If a child in diapers has shigellosis, individual who changes the child's diapers should dispose the diapers properly in a closed-lid garbage can and should wash their hands carefully with soap and warm water immediately after changing the diapers. After use, the diaper changing area should be wiped down with disinfectant, such as household bleach or bactericidal wipes.

At swimming pools, maintaining a chlorine level of at least 0.5-PPM will kill *Shigella*. Children with diarrhoea should not be permitted to public swimming areas.

In addition, basic food safety precautions will also help to prevent shigellosis. *Shigella* organisms are killed if the food in properly cooked. Shigellosis also can be prevented by consuming washed and peeled fruit. Individual who has shigellosis or any diarrhoea should not prepare food for others until they have been shown to no longer be carrying the bacterium. Besides that, shigellosis can also be prevented by drinking chlorinated water and consume only pasteurized dairy products.

When travelling to the developing world, it is suggested to drink imported, carbonated or boiled beverages. By drinking imported, carbonated or boiled beverages, the chances of getting *Shigella* infection could be reduced.

1.8 Vaccine candidates under development

Shigellosis or bacillary dysentery is an important cause of childhood morbidity and mortality and it remains a serious disease throughout the world (Dan *et. al.*, 1988; DuPont *et. al.*, 1976; Echeveriia *et. al.*, 1992; Ghosh *et. al.*, 1996; Green at. al., 1991; Hyams *et. al.*, 1991; Parry *et. al.*, 2001; Thomson A.W., 1998; Garcia-Fulgueiras *et. al.*, 2001). Emergence of *Shigella* strains resistant to multiple antibiotics (Ashkenazi *et. al.*, 2003; Sarkar *et. al.*, 2003; Taylor *et. al.*, 1986) and the increasing number of infected persons in some areas of the world emphasize the need for an effective vaccine. Out of all *Shigella* strains, *S. flexneri* 2a, *S. dysenteriae* 1 and *S. sonnei* are the most important *Shigella* strains to be targeted for vaccine development. To date, candidate vaccine for shigellosis include both subunit and live attenuated vaccines. The subunit vaccine approach includes parenteral conjugate vaccine, parenteral nuclear protein/ribosomal vaccine and proteosome vaccine.

1.8.1 Subunit vaccine

1.8.1.1 Parenteral conjugate vaccine

Previous report indicated that a critical level of serum IgG anti-lipopolysaccharide (anti-LPS) confered immunity to shigellosis. Hence, a study of *Shigella* conjugate vaccine composed of the O-specific polysaccharide of *S. sonnei* bound to *Pseudomonas aeruginosa* recombinant exoprotein A (rEPA) has been conducted in Israel by National Institutes of Health (NIH).

The randomized, double-blind, vaccine-controlled study has shown that the *S. sonnei*-rEPA elicited 74% protection against shigellosis occurring about 3 months after vaccination. Besides, the vaccine also conferred 43% protection in adult volunteers during

an *S. sonnei* outbreak up to 14 days following vaccination. It is reported that the efficacy of *S. sonnei*-rEPA was correlated with the level of vaccine-induced IgG antibodies.

Furthermore, the safety and immunogenicity of these *Shigella* conjugates are also demonstrated in 4 to 6 years old children in Israel. The study also found that serum antibody responses to polysaccharide-based vaccine is age-dependent and infants as well as young children responded poorly to both disease and vaccination. Although the fold rise in anti-LPS was similar among the children, the level of anti-LPS elicited by the conjugates was lower than if compared to adults. Thus, NIH has improved the immunogenicity of *Shigella* conjugates and the study regarding to the safety, immunogenicity and efficacy of these improved conjugates in 1 to 4 years-old children in Israel is being evaluated.

1.8.1.2 Parenteral nuclear protein/ribosomal vaccine

Ribosomal-based vaccines developed from organisms such as bacteria, fungi, and protozoa have been shown to possess strong immunogenicity and protective efficacy (Gregory, 1986). Such vaccines have been adopted as useful tools in preventing several infectious diseases such as *Pseudomonas aeruginosa*, *Streptococcus* sp., *Klebsiella pneumoniae*, and *Heamophilus influenzae* (Lieberman *et al.*, 1980; Serrano *et al.*, 1997). Berry *et al.* suggested that the ribosomal particle serves as an efficient delivery system for weak antigen (Berry *et al.*, 2006). In 1988, first ribosomes have been isolated from avirulent *S. sonnei* and *S. flexneri* by using sonic disruption followed by differential ultracentrifugation (Levenson *et al.*, 1988). Levenson *et al.* predicted that the formation of immunogenic O-Ag and ribosome complexes would be importance because these complexes can increase the efficiency of antigen presentation (Levenson and Egorova, 1990). Study has demonstrated that parenteral vaccination with *Shigella* ribosomal vaccine

(SRV) elicited a significant level of O-Ag-specific IgG Ab in serum and of IgA Ab in the tears of guinea pigs, as well as in the saliva and bile of monkeys (Levenson *et al.*, 1987). In year of 2007, Doo-Hee *et al.* has demonstrated the immunogenicity and protective efficacy of SRV. In the study, mice were vaccinated with SRV via the intranasal (i.n.) route. The study has shown that robust levels of *Shigella*-derived LPS specific IgG and IgA Abs and antibody forming cells were elicited in systemic and mucosal compartments following two i.n. administrations of SRV. At the same time, groups of mice receiving i.n. SRV developed milder pulmonary pneumonia upon challenge with virulent *S. flexneri* 2a than did those receiving parenteral SRV.

1.8.1.3 Proteosome vaccine

Proteosome-based LPS vaccines for *Shigella* have been tolerated well by several animal species and have shown protective activity in the Se'reny test and in a murine lethal pneumonia model when delivered via mucosal routes (Orr *et al.*, 1993; Mallett *et al.*, 1995; Lowell, 1997). In addition, proteosome-based mucosal vaccines have provided protection against respiratory challenge with staphylococcal enterotoxin B and have elicited neutralizing mucosal and systemic antibody responses to human immunodeficiency virus (Lowell *et al.*, 1995; Lowell *et al.*, 1996). The term "proteosome" refers to purified preparations of meningococcal outer membrane proteins (OMPs) that form multimolecular vesicular structures with antigens noncovalently complexed to them, generally via hydrophobic interactions (Lowell, 1997).

Study regarding to the safety and immunogenicity of a *S. flexneri* 2a vaccine comprising native *S. flexneri* 2a lipopolysaccharide (LPS) complexed to meningococcal outer membrane proteins (proteosomes) has been conducted in normal, healthy adults