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Modern aspects on *Shigella* pathogenecity and vaccine development - A Review

Partha Pal

Department of Zoology, Scottish Church College,
1 & 3 Urquhart Square, Kolkata - 700006, India
Phone: 91-33-2350-3862

E-mail address: parthapal_iicb@yahoo.co.in

ABSTRACT

Shigella species are intracytosolic Gram-negative invasive enteropathogenic bacteria, causing the rupture, invasion and inflammatory destruction of the human colonic epithelium. They utilize the host cytoskeletal components to form propulsive actin tails. The so-called invasive phenotype of *Shigella* is linked to expression of a type III secretory system (TTSS) injecting effector proteins into the epithelial cell membrane and cytoplasm, thereby inducing local but massive changes in the cell cytoskeleton that lead to bacterial internalization into non-phagocytic intestinal epithelial cells. The molecular and cellular bases of this invasive phenotype essentially encompass crossing of the epithelial lining, apoptotic killing of macrophages, entry into epithelial cells, and escape into the cytoplasm, followed by cell-to-cell spread. Intracellular colonization is likely to protect the micro-organisms from killing by humoral and cellular effectors of the innate immune response. Concurrently, the capacity of *Shigella* to reprogram invaded epithelial cells to produce proinflammatory mediators plays a major role in the strong inflammatory profile of the disease. This profile is likely to impact on the nature and quality of the adaptive response, which is dominated by humoral protection at the mucosal level. In recent years, a large amount of information has been generated regarding the host, pathogen and environmental factors that impact the pathogenesis of shigellosis at the cellular and molecular level. This review summarizes what is currently known about *Shigella*, detailing those factors that contribute to pathogenesis and examining the current progress in the development of a vaccine.

Keywords**:** *Shigella*; Enteropathogen; TTS-System; Pathogenesis; Vaccine

1. INTRODUCTION

Species of the genus *Shigella* are among the bacterial pathogens most frequently isolated from patients with diarrhea. Five to fifteen percent of all diarrheal episodes worldwide can be attributed to an infection with *Shigella*, including 1.1 million fatal cases (Kotloff et al., 1999). Two-thirds of all episodes and deaths occur in children under 5 years. The emergence of multidrug-resistant *Shigella* strains and a continuous high disease incidence imply that shigellosis is an unsolved global health problem (Sansonetti, 2006). Shigellosis is an acute intestinal infection, the symptoms of which can range from mild watery diarrhea to severe inflammatory bacillary dysentery characterized by strong abdominal cramps, fever, and stools containing blood and mucus. A combination of oral

rehydration and antibiotics leads to the rapid resolution of infection. Currently, there is no protective *Shigella* vaccine available, but several vaccines using bacterial components or killed or live-attenuated bacteria for immunization are under development and are being tested in different clinical phases (WHO, 2006). Here, we cover recent progress in our understanding of the evolution of *Shigella* virulence, current concepts on the mode of pathogenesis on cellular and molecular level, and the recent status on the development of vaccine against the disease.

2. EVOLUTION OF *SHIGELLA* VIRULENCE

Shigella spp. are gram-negative, nonsporulating, rod-shaped bacteria that belong to the family *Enterobacteriaceae*. The first report on the isolation and characterization of bacteria causing bacillary dysentery, later named *Shigella*, was published by Kiyoshi Shiga at the end of the 19th century (Shiga, 1897). Descriptions of numerous differing strains followed over the next decades, with all of them being closely related to the nonpathogenic bacterium *Escherichia coli*. To distinguish the pathogenic strains of high clinical relevance from less-pathogenic or nonpathogenic strains, the genus *Shigella* was defined based on biochemical, serological, and clinical phenotypes (Ewing, 1949). The genus *Shigella* includes the four species *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C), and *S. sonnei* (serogroup D). According to variations in their O antigens, the species were further divided into several serotypes. *S. flexneri* and, to a lesser extent, *S. sonnei* are endemic and cause the majority of all infections (Kotloff, 1999). *S. dysenteriae* accounts for epidemic disease outbreaks and the most severe form of dysentery, which causes the majority of fatal shigellosis cases.

Comparative genomics have challenged the traditional serological classification recently. Several studies using different technical approaches clearly prove that *Shigella* spp. belong to the species *E. coli*, rather than forming a separate genus (Fukushima et al., 2002; Ochman et al., 1983; Pupo et al., 1997; Rolland et al., 1998). Moreover, diarrheagenic enteroinvasive *E. coli* (EIEC) strains share biochemical characteristics, essential virulence factors, and clinical symptoms with *Shigella* spp. While EIEC does not completely fulfill the definition of the genus *Shigella*, genome analysis revealed a closer relationship to *Shigella* spp. than to commensal *E. coli* strains (Lan et al., 2004; Yang et al., 2007).

Comparative genomics clearly indicates that *Shigella* spp. and EIEC evolved from multiple *E. coli* strains by convergent evolution (Pupo et al., 2000; Yang et al., 2007). Phylogenetic studies of *Shigella* deduced either from numerical and phenotypic taxonomy or from comparative genomics showed three main *Shigella* clusters, each containing strains from the traditionally defined species, are identified. *S. sonnei* and some *S. dysenteriae* strains are more distantly related to these main clusters but still group with *E. coli*. The three main *Shigella* clusters started to diverge from *E. coli* 35,000 to 270,000 years ago (Lan and Reeves, 2002). *S. sonnei* is of more recent origin and separated from the other strains about 10,000 years ago (Shepherd et al., 2000). Since EIEC retained more characteristics of commensal *E. coli* than *Shigella* spp., these strains apparently acquired the virulence machinery more recently and might reflect an earlier stage of the evolutionary process undergone by *Shigella* spp. (Lan et al., 2004; Yang et al., 2005).

In addition to a reassignment of the phylogenetic relationships between *Shigella* strains, comparative genomics provides insight into the genetic basis of *Shigella* virulence. The genetic information constituting the phenotypes of *Shigella* spp. is encoded on the bacterial

chromosome and on a large virulence plasmid. The virulence plasmid is an essential virulence determinant of all *Shigella* spp. and encodes the molecular machinery necessary for tissue invasion and the intracellular lifestyle (Sansonetti et al., 1983; Sansonetti et al., 1982; Sasakawa et al., 1986). The central element of this machinery is a TTS-system. The TTS-system enables the bacteria to translocate a set of approximately 25 proteins from the bacterial cytoplasm directly into the eukaryotic host cell, where these "effector" proteins interfere with various host cell processes (Ogawa and Sasakawa, 2006; Parsot, 2005; Tran Van Nhieu et al., 2005).

The complete sequences of the virulence plasmids and chromosomes of several *Shigella* strains including all four species are currently available (Buchrieser et al., 2000; Jiang et al., 2005; Jin et al., 2002; Nie et al., 2006; Venkatesan et al., 2001; Wei et al., 2003; Yang et al., 2005). Furthermore, the genomes of a strain collection representing all serotypes of *Shigella* were characterized by comparative genomic hybridization (Peng et al., 2006). This vast amount of genetic information allows the identification of the successive genetic events that led to the evolution of pathogenic *Shigella* from nonpathogenic *E. coli* and provides insight into how variations in the virulence traits of different *Shigella* strains developed.

3. PATHOGENESIS OF *SHIGELLA* INFECTION

Shigella spp. are transmitted by the fecal-oral route and enter the human body via the ingestion of contaminated food or water. They are highly infectious, since as few as 10 to 100 microorganisms are sufficient to cause disease (Dupont et al., 1989). Furthermore, it was shown that *Shigella* spp. are able to down regulate the expression of antimicrobial peptides, which are important antibacterial effectors constantly released from the mucosal surfaces of the intestinal tract (Islam et al., 2001). After passage through the stomach and small intestine, the bacteria reach the large intestine, where they establish an infection. *Shigella* entry into cells is affected by a type three secretion system, which is a needle-like apparatus that injects effector proteins directly into the host cell cytoplasm to modulate host cell functions. Delivery of early effector proteins induce rearrangements of actin cytoskeleton resulting in membrane remodelling and bacteria uptake, which is followed shortly by bacterial escape from the vacuole (Clerc and Sansonetti, 1987; Menard et al., 1994). Intracellular *Shigella* triggers the polymerization of polymerized host actin and leads to dissemination of *Shigella* in the intestinal epithelium (Goldberg, 2001). For efficient actin tail formation, the *Shigella* outer membrane protein IcsA, the host actin nucleation-promoting factor N-WASP, and Toca-1 are required (Lommel et al., 2001; Snapper et al., 2001; Egile et al., 1999; Suzuki et al., 1998; Leung et al., 2008). N-WASP is maintained in an auto-inhibited state in the resting cells (Ho et al., 2004; Bompard and Caron, 2004). Toca-1 activates N-WASP by relieving N-WASP auto-inhibition during *Shigella* actin tail formation. The recruitment of Toca-1 to bacteria is dependant on type three secretion system (Bompard and Caron, 2004) but the protein responsible for the recruitment process is not yet known.

A key step in *Shigella* pathogenesis is the ability of this intracellular pathogen to mediate its own uptake of into normally non-phagocytic epithelial cells. Several type 3 secreted effectors including IpgB1 are involved in this process. IpgB1 is GEF (GTP exchange factor) (Huang et al., 2009) that localizes to host cell membranes at bacterial entry sites where it activates the small GTPase Rac and Cdc42 to induce membrane ruffling which promote the uptake of *Shigella* into host cells (Hachani et al., 2008; Alto et al., 2006; Handa

et al., 2007; Ohya et al., 2005). *Shigella* strains that no longer encode IpgB1 are impaired in invasion are attenuated in virulence (Ohya et al., 2005). The translocation of IpgB1 into host cells is dependent on Spa15, a class IB type 3 secretion chaperone (Page et al., 2002). In addition to IpgB1, Spa 15 binds to and mediates the secretion of eight additional *Shigella* effectors (Page et al., 2002; Niebuhr et al., 2000; Ogawa et al., 2003; Schmitz et al., 2009). Interestingly, these nine effectors share a conserved amino acid sequence, the conserved chaperone binding domain (CCBD) sequence, within their first 50 residues that mediates interactions with Spa 15 (Costa et al., 2012).

The most frequently linked with endemic outbreaks of shigellosis are caused by *S. flexneri* strains. They invade the colonic and rectal epithelium of their host and cause severe tissue damage. Recent findings elucidated the contribution of the periplasmic enzyme, L-asparaginase (AnsB) to the pathogenesis of *S. flexneri*. The bacterial OmpA, is a prominent outer membrane protein whose activity has been found to be required for bacterial pathogenesis, and is reported to be up-regulated in *ansB* mutant cells. Overexpression of OmpA in wild type *S. flexneri* serotype 3b resulted in decreasing the adherence of this virulent strain, suggesting that the up-regulation of OmpA in *ansB* mutants contributes to the reduced adherence of this mutant strain (George et al., 2014).

4. CURRENT APPROACHES FOR DEVELOPMENT OF *SHIGELLA* VACCINE

The diversity of the worldwide *Shigella* serotype isolates and their variable relative importance in developing *versus* developed countries led to the conclusion that a multivalent *Shigella* vaccine will have to be developed to address the needs of the different potential target populations for an efficacious *Shigella* vaccine. It is expected that a vaccine which will include *S. dysenteriae* type 1, *S. sonnei, S. flexneri* 2a, *S. flexneri* 3, and *S. flexneri* 6 will cover more than 75 % of the global *Shigella*-associated episodes of diarrhea (Levine et al., 2007). The *Shigella* vaccine development strategies of the last 50 years and the current ones include the two main distinct categories of live-attenuated vaccine strains and inactivated *Shigella* vaccine candidates (subunit and whole cell).

4. 1. Live-attenuated *Shigella* strains

Mel and colleagues demonstrated the efficacy of the SmD vaccines, showed that multiple strains could be mixed together in combination vaccines and reported that protection was serotype specific. Protection persisted for a year following primary immunization of children, but administration of a single booster extended the protection for an additional year (Mel et al., 1971; 1974).

A similar live-attenuated *Shigella* vaccine (*S. flexneri* 2a strain T32) was developed in Romania by repeated subculturing (Meitert et al., 1984). The field trials also suggested that T32 conferred significant protection against shigellosis due to *S. sonnei, S. flexneri* 1b and *S. boydii* 1–6. Later, it was shown that T32 harbored a large deletion in the invasiveness plasmid, resulting in the loss of three loci, ipaADCB, invA, and virG, which diminished the ability of this strain to invade epithelial cells (Venkatesan et al., 1991).

Advances in recombinant DNA technology and more recently whole genome sequencing of shigellae enabled the development of live-attenuated oral *Shigella* candidates with defined deletion mutations, knocking out virulence genes on the invasiveness plasmid that encode for intracellular spread and altering key metabolic pathways, impairing synthesis of nucleic acids, impairing the capacity to compete for ferric iron, via the production of

siderophores (i.e. aerobactin). A second generation of more attenuated *S. sonnei* mutants, WRSs2 and WRSs3, were constructed at WRAIR (Barnoy et al., 2011; Bedford et al., 2011). A combination of these gene deletions with the addition of the knockout of the chromosomal *set* locus encoding for the ShET1 were applied for *S. flexneri* 2a candidate vaccine strains WRSf2G11, WRSf2G12, and WRSf2G15 (Ranallo et al., 2012).

4. 2. Inactivated *Shigella* vaccine candidate

In the early attempts to develop a vaccine, inactivated, whole-cell preparations of *Shigella* were developed and administered parenterally (Levine et al., 2007).

LPS-based vaccines were developed in concert with case control and prospective studies demonstrating an association between serum LPS IgG antibodies and serotype-specific protection against shigellosis (Cohen et al., 1991; Passwell et al., 1995).

Investigators at the National Institutes of Health developed parenterally administered conjugate *Shigella* vaccines by covalently binding the serotype-specific polysaccharides of *Shigella* to a carrier protein, thus obtaining T-cell-dependent antigens, immune memory, and a better immune response. They were found as very safe, with minimal systemic adverse and local adverse events (Ashkenazi et al., 1999; Passwell et al., 2003).

The efficacy of these conjugates was examined where a randomized, double-blind efficacy study was done in 1-4-year-old children at 15 sites throughout Israel (Passwell et al., 2010). The study demonstrated that the *S. sonnei* conjugate had a protective efficacy of 71 % in 3-4 year-old children, but not in younger ones (Passwell et al., 2010).

With the idea of developing a multivalent subunit *Shigella* vaccine, candidate vaccines based on the type three secretion system, which is utilized by all shigellae, were developed. IpaB- and IpaD-based *Shigella* vaccines, with adjuvants, given to mice intranasally (Martinez-Becerra et al., 2012), parenterally (Martinez-Becerra et al., 2013), or orally (Heine et al., 2013), were promising, shown as immunogenic and protective against lethal pulmonary infection with *Shigella*.

5. DISCUSSION AND CONCLUSION

More than 100 years after their discovery, *Shigella* spp. still poses a worldwide major health problem due to the devastating diarrhea that the bacteria may cause. Research performed over the last 25 years has tremendously contributed to our understanding of shigellosis on molecular, cellular, and organismic levels. Thus, we gained profound insight into the evolution of *Shigella* spp. originating from harmless enterobacterial relatives, the structure and function of the major virulence factors, including a TTS-System and their cognate effectors which are multi-domain proteins optimized not only to be recognized as bacterial secreted substrates, but also to act within eukaryotic host cells (Costa and Lesser, 2014). An in-depth understanding of the molecular mechanisms underlying the mode of pathogenesis is a prerequisite to design a protective, live-attenuated *Shigella* vaccine strain that is safe and efficient. In concert with ongoing efforts to improve hygiene standards, such a vaccine would offer substantial relief from what is still one of the most dreadful bacterial pathogens.

In the present review, it was concluded that current researches are focussing on the diverse innovative approaches based on progress in molecular technology, elucidation of the virulence mechanisms in understanding the molecular basis of pathogenesis of *Shigella* and development of a licensed safe and efficacious *Shigella* vaccine to protect humans against

this pathogen and the related morbidity and mortality. This present review will also help for further research in understanding the evolutionary mechanisms of new *Shigella* isolates having epidemic potential, the mode of pathogenesis of virulent strains and the development of a suitable vaccine strain for this bacterial pathogen.

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