An update on vaccines against *Shigella*

Ther Adv Vaccines
(2013) 1(3) 113–123
DOI: 10.1177/2051013613500428

Shai Ashkenazi and Dani Cohen

Abstract: Despite intensive research efforts for more than 60 years, utilizing diverse vaccine strategies, a safe and efficacious vaccine against shigellosis is not available yet. We are currently witnessing innovative approaches based on elucidation of the virulence mechanisms of *Shigella*, understanding the immune response to the pathogen and progress in molecular technology for developing *Shigella* vaccines. It is hoped that these will lead to a licensed effective *Shigella* vaccine to protect humans against the significant worldwide morbidity and mortality caused by this microorganism.

Keyword: Shigellosis, diarrhea, gastroenteritis, prevention, vaccine

Correspondence to:
Shai Ashkenazi, MD, MSc
Department of Pediatrics A, Schneider Children's Medical Center, 14 Kaplan Street, Petach Tikva 49202, Israel
ashai@post.tau.ac.il, sashkenazi@clalit.org.il

Dani Cohen, PhD, MPH
School of Public Health, Sackler Faculty of Medicine, Tel Aviv University, Israel

Background

Epidemiology

Shigella spp. cause acute gastrointestinal infections by invasion of the mucosa, toxin production and induction of local inflammation [Page *et al.* 1999; Sansonetti *et al.* 2001; Ingersoll *et al.* 2002], sometimes with extraintestinal manifestations [Ashkenazi *et al.* 1990; Baiulescu *et al.* 2002; Anatoliotaki *et al.* 2003]. These infections are of major public health relevance, especially in developing countries, where they cause significant pediatric morbidity and mortality [Bennish *et al.* 1990; Goren *et al.* 1992]. It is estimated that approximately 1.1 million deaths result from the 164.7 million annual cases worldwide, with about 70% of episodes and 60% of deaths involving children younger than 5 years [Kotloff *et al.* 1999]. In developed countries the infection is associated with considerable morbidity; the incidence of *Shigella* infections in the United States is 4–8 per 100,000, with 10,000–15,000 cases reported annually [Gupta *et al.* 2004; Centers for Disease Control and Prevention, 2011; Shiferaw *et al.* 2012]. The age group with the highest risk is children between 1 and 4 years old (particularly those in the second and third years of life), followed by those between 5 and 9 years [Ashkenazi, 2004]. *Shigella* spp. are also important etiologic agents of diarrhea in travelers and among soldiers deployed to endemic regions [DuPont 2009; Putman *et al.* 2006; Cohen *et al.* 2001]. In the United States, seasonality of shigellosis has changed from a peak in summer to a higher incidence in the late summer and autumn. In developing countries with tropical climates, *Shigella* infection is common throughout the year, but higher isolation rates are found during the summer and rainy seasons.

About 50 serotypes of *Shigella* have been identified, belonging to four serogroups (or species): group A (*S. dysenteriae*), group B (*S. flexneri*), group C (*S. boydii*), and group D (*S. sonnei*). The relative prevalence of the *Shigella* serotypes varies over time and by geography. Currently, *S. sonnei* consists of about 80% of *Shigella* isolates in developed countries, with an increasing relative prevalence in the last decades. By contrast, in developing countries, *S. flexneri* is the most common cause of bacillary dysentery [von Seidlein *et al.* 2006], while epidemics caused by *S. dysenteriae* serotype 1 that have occasionally occurred in the past have not been reported recently.

Clinical presentation

The typical incubation period is 12–48 h, but it may last for up to a week. In mild infections, especially in adults, the only complaints may be watery or loose stools for few days with minimal constitutional symptoms of fever. In contrast, children in particular have acute onset of high fever, malaise, abdominal cramps, and watery diarrhea that is followed by appearance of nausea, vomiting, and passage of frequent, mucous, bloody stools associated with severe abdominal pain and tenesmus. Other children have bloody mucous diarrhea from

the onset of illness [Ashkenazi, 2004]. Physical findings include increased body temperature, usually 38.5°C or higher, general toxicity, mild dehydration (<30 ml/kg/day of diarrheal fluid is usually lost in the dysenteric phase), lower abdominal tenderness, and increased bowel sounds. Rectal examination elicits severe pain. In most cases, symptoms resolve without antibiotic therapy in about a week, but therapy shortens significantly the clinical illness. Neonates and children with underlying immune deficiency (including human immunodeficiency virus infection) or malnutrition are at increased risk of bacteremia and other complications of shigellosis [Struelens *et al.* 1985; Martin *et al.* 1983; Viner *et al.* 2001; Greenberg *et al.* 2003].

Immunity to shigellosis

Although mucosal secretory immunoglobulin (Ig) A antibodies and serum IgG antibodies develop against the virulence invasion plasmid antigens (*ipa*) *ABCD* after natural infection [Hayani *et al.* 1991; Levine *et al.* 2007], it has been shown that acquired natural immunity is serotype [lipopolysaccharide (LPS)] specific [Ferreccio *et al.* 1991; Cohen *et al.* 1991]. While there is no established correlate of immunity, case–control and prospective studies documented a strong association between pre-existing *Shigella* LPS IgG antibodies and protection against serotype-specific infection [Cohen *et al.* 1988, 1991]. The level of serum *Shigella* IgG antibodies induced by *S. sonnei* conjugate vaccine correlated with the extent of protection conferred by the vaccine among young adults and children [Cohen *et al.* 1997; Passwell *et al.* 2010]. Recent analysis of data generated by challenge studies showed an inverse correlation between the magnitude of prechallenge IgG antibodies to LPS and IpaB, as well as IgA IpaB B memory cells (B_M) and postchallenge IgA LPS B_M with disease severity, suggesting a role for antigen-specific B_M in protection [Wahid *et al.* 2013]. It is unclear how occasional naïve children can be infected with *Shigella* and yet remain well [Guerrero *et al.* 1994]. Cellular immunity and cytokine production, which also develop during acute illness, may be an explanation. Tumor necrosis factors α and β, interleukin (IL)-1α, IL-1β, IL-1ra, IL-4, IL-8, IL-6, and transforming growth factor β are induced while interferon γ is suppressed [Raqib *et al.* 1997].

Treatment

Rapid correction of fluid and electrolyte deficits, early reinstitution of feeding (<12 h after initiation of treatment), and continued replacement of ongoing losses are key elements to therapy. Oral rehydration is the treatment of choice, although patients in shock or coma, as well as those with severe vomiting or ileus, should be treated with intravenous replacement of fluids and electrolytes.

Appropriate antibiotic therapy of shigellosis shortens the duration of fever, diarrhea and fecal excretion of the pathogen (thereby reducing infectivity) and apparently also reduces the risk of complications [Varsano *et al.* 1991; Eidlitz-Marcus *et al.* 1993; Ashkenazi *et al.* 1993; Martin *et al.*, 2000; Basualdo and Arbo, 2003]. Empiric antibiotic treatment is generally recommended for people with colitis or dysentery until results of culture and clinical response are known [Ashkenazi, 2004; Christopher *et al.* 2010]. The major problem, however, is the worldwide increasing antibiotic resistance of *Shigella* spp. [Chuang *et al.* 2006; Centers for Disease Control and Prevention, 2013]. Antimotility agents should be avoided in shigellosis as well as in other infectious causes of colitis [Ashkenazi, 2004].

Need for a vaccine

Clean running water and appropriate sanitation are crucial for preventing infectious diarrhea, including shigellosis, in developing countries. For toddlers and older children, good hygiene practices are helpful but are difficult to implement [Khan, 1982]. It has been shown that prolonged breastfeeding is an important strategy to reduce shigellosis in young children [Clemens *et al.* 1986; Mata *et al.* 1969]. Specific protective antibodies present in the milk of women living in endemic areas decrease the severity of infection in infants [Hayani *et al.* 1991].

Because of the very low infectious dose, control of shigellosis only by hygiene measures is limited. As shown for other infectious disease, prevention by active vaccination is optimal, and can present an important measure in controlling the worldwide morbidity and mortality of shigellosis. Since *Shigella* spp. naturally infect mainly humans, an efficacious vaccine can potentially lead to a significant global reduction, or even to eradication, of shigellosis.

Approaches to development of *Shigella* vaccines

Diverse vaccine strategies (Table 1) have been utilized over several decades in an attempt to develop a safe and efficacious *Shigella* vaccine [Levine *et al.* 2007; Camacho *et al.* 2013; Barry *et al.* 2013]. Although a licensed vaccine is not available yet, these attempts have helped in better understanding the immune response to *Shigella*, and together with recent innovative strategies led to promising vaccines [Barry *et al.* 2013].

Observational and challenge studies have shown that natural *Shigella* infection confers protection for a limited duration against the homologous serotype [Ferreccio *et al.* 1991; Cohen *et al.* 1991]. Therefore, the main attempts to develop *Shigella* vaccines were targeted towards inducing a good immune response against *Shigella* O polysaccharide, which determines the *Shigella* serogroup and serotype. 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. These include young children in developing countries, children living in developed countries but under conditions of particular crowding, travelers from industrialized countries to highly endemic countries, military personnel, men who have sex with men, and so on. 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]. This is based on the assumption (from the analysis of *Shigella* O antigens and cross protection studies) that inclusion of *S. flexneri* 2a, 3a, and 6 in the vaccine will provide cross protection against the other 11 *S. flexneri* serotypes because of shared group antigens [Noriega *et al.* 1999; Levine *et al.* 2007].

There are two basic approaches to attain such a wide range in protection against shigellosis: either by combining efficacious serotype-targeted vaccines in a multivalent vaccine or by using a cross-reactive antigen which will confer extended cross protection against *Shigella* strains. To reach this ultimate goal, it is first necessary that monovalent *Shigella* candidate vaccines will demonstrate protection in clinical trials. Regrettably, only two prototype *Shigella* vaccines, one which uses attenuated strains as live oral vaccines [Mel *et al.* 1974; Meitert *et al.* 1984] and another which uses parenteral conjugates of *Shigella* O polysaccharide covalently linked to a carrier protein [Cohen *et al.* 1997; Passwell *et al.* 2010], have conferred significant protection in controlled field trials.

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).

Live-attenuated Shigella strains

Attenuated mutants obtained by serial passages in vitro. In the early 1960s, in Yugoslavia, David Mel serially passed different *Shigella* serotypes on streptomycin-containing media until they became streptomycin resistant and streptomycin dependent (SmD), losing in parallel the capability of mucosal invasiveness [Sereny, 1957]. These vaccine strains were well tolerated; in controlled field trials, 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 [Mel *et al.* 1971, 1974]. 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. Large doses of 5×10^{10}–2×10^{11} colony-forming units (CFUs) were shown to be well tolerated and significantly protective in large field studies in Romania and China [Meitert *et al.* 1984]. The field trials also suggested that T32 conferred significant (albeit lower level) 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 [Venkatesen *et al.* 1991].

These studies provided proof of concept for future modern multivalent vaccines that aim to confer broad protection. Unfortunately, they had drawbacks that prevented licensure and further large-scale use, such as the multiple doses needed for primary vaccination, need for annual boosters, lack of clear knowledge on the exact segment of

Table 1. Shigella vaccine development strategies (past and present).

Live attenuated vaccines			
Vaccine strain	**Attenuation/mutation**	**Phase of development**	**Reference**
Attenuated mutants obtained by serial passages *in vitro*			
SmD in Yugoslavia	Serial passages *in vitro*	Phase III	Mel *et al.* [1971, 1974]
Istrati T32 in Romania	Serial passages *in vitro*	Phase III and IV	Meitert *et al.* [1984]
Hybrid live vector *Shigella* vaccine			
Escherichia coli K12–*Shigella flexneri* 2a	*Escherichia coli* K12 vector of *Shigella* invasiveness plasmid; aroD	Phase IIb	Cohen *et al.* [1994];
Attenuated mutants obtained by recombinant DNA technology			
S. flexneri 2a CVD 1208S	guaBA, sen, set	Phase II	Kotloff *et al.* [2007]
Shigella sonnei WRSs1	virG	Phase II	Orr *et al.* [2005]
S. sonnei WRSs2, WRSs3	virG, senA, senB, msbB2	Preclinical	Kotloff *et al.* [2002]; Barnoy *et al.* [2011]; Bedford *et al.* [2011]
S. flexneri 2a SC602	virG, iuc	Phase IIb	Sansonetti and Arondel [1991]; Coster *et al.* [1999]; Rahman *et al.* [2011]
S. flexneri 2a WRSf2G11, 12, 15	virG, senA, senB, msbB2	Preclinical	Ranallo *et al.* [2012]
Shigella dysenteriae 1 WRSd1	virG, stxAB	Phase I	McKenzie *et al.* [2006]
Inactivated vaccines	**Vaccine candidate**	**Phase of development**	**Reference**
Whole cell killed vaccines	*S. flexneri* 2a, 3a, *S. sonnei*	Preclinical	McKenzie *et al.* [2006]
Subunit vaccines			
Chemical glycoconjugates	*S. flexneri* 2a, *S. sonnei*, PS-rec. exoprotein A	Preclinical and phases I, II and III	Cohen *et al.* [1997]; Ashkenazi *et al.* [1999]; Passwell *et al.* [2010]
Synthetic glycoconjugates	*S. flexneri* 2a synt O ag-tetanus toxoid	Preclinical	Pozsgay *et al.* [2007]; Phalipon *et al.* [2009]
Bioglycoconjugates	*S. dysenteriae* 1 LPS exoprotein A	Phase I	Fernandez *et al.* [2009]
Proteosomes LPS	*S. flexneri* 2a, *S. sonnei*	Phase II	Fries *et al.* [2001]
Ribosomes LPS	*S. sonnei, S. flexneri*	Preclinical	Levenson *et al.* [1995]; Shim *et al.* [2007]
Purified Ipa proteins	*Shigella* spp.	Preclinical	Martinez-Becerra *et al.* [2013]
GMMA protein vesicles	*S. flexneri* 2a, *S. sonnei*	Preclinical	Scorza *et al.* [2012]
Combined LPS and common proteins	*S. flexneri* 2a	Phase I	Oaks and Turbyfill [2006]; Riddle *et al.* [2011]

Ipa, invasion plasmid antigen; LPS, lipopolysaccharide; SmD, streptomycin dependent; GMMA, generalized modules of membrane antigens.

the bacterial genome which had been modified by the serial passages *in vitro* and the occasional back mutations, reverting the streptomycin-dependent strain to streptomycin-independent strains in the case of the SmD vaccine strain [Levine, 1975; Levine *et al.* 2007].

Attenuated mutants obtained by recombinant DNA technology. 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 multiplication (icsA or virG), altering key metabolic pathways, such as *aro* and guaAB that introduce severe auxotrophy, impairing synthesis of nucleic acids,

impairing the capacity to compete for ferric iron, via the production of siderophores (i.e. aerobactin). The knowledge on the exact changes in the bacterial genome associated with different levels of attenuation together with the various advantages of manufacturing and delivery of oral live-attenuated vaccines strongly supported investment of research efforts and funding in the development of this vaccination strategy. This has been the leading approach in *Shigella* vaccine development at the Walter Reed Army Institute of Research (WRAIR), University of Maryland Center for Vaccine Development (CVD), Institut Pasteur and Karolinska Institute.

Two major obstacles emerged and significantly slowed down the process of development of these promising candidates. The first was the narrow window between immunogenicity and safety of these candidates. The development process of the hybrid *Escherichia coli* K12–*S. flexneri* 2a (EcSf2a1 and 2), *S. flexneri* 2a SC602 vaccine strain, *S. dysenteriae* 1 (SC599 and WRSd1), the series of *S. sonnei* WRSs1, WRSs2, and WRSs3, and the series of CVD 1204, CVD 1207, CVD 1208, and CVD 1208S exemplified this delicate balance along the way.

The invasiveness plasmid of *S. flexneri* 5 and the genes that allow expression of *S. flexneri* 2a type and group-specific O antigens were introduced into *E. coli* K12, producing a hybrid strain EcSf2a-1 which was further attenuated by an aroD mutation, becoming the vaccine candidate EcSf2a-2. At high dosage levels, this strain, with the ability to penetrate epithelial cells, was immunogenic but also caused some adverse reactions [Cohen *et al.* 1994]. The vaccine conferred only 36% protection against illness (fever, diarrhea, or dysentery) upon experimental challenge.

Deletions in the aerobactin-encoding system (iuc iut) and icsA in *S. flexneri* 2a led to the development of a vaccine candidate SC602 [Sansonetti and Arondel, 1991] that has undergone phase I and II clinical trials, with encouraging results in Western volunteers [Coster *et al.* 1999]. SC602 was reactogenic at 2×10^6CFUs, but relatively safe at 10^4 with seroconversion (IgG or IgA) and significant antibody-secreting cell (ASC) response in 60–70% of the volunteers, and protection when vaccinees were challenged with a wild type pathogenic *S. flexneri* strain of a similar serotype. Subsequent studies demonstrated the absence of accidental transmission of the live vaccine strain. A delta icsA *S. sonnei* and a delta icsA and delta sxtA and sxtB (encoding Shiga toxin) *S. dysenteriae* type 1vaccine candidate constructed by scientists at WRAIR showed similar results with regard to tolerance and immunogenicity balance [Orr *et al.* 2006].

A second generation of more attenuated *S. sonnei* mutants, WRSs2 and WRSs3, were constructed at WRAIR [Barnoy *et al.* 2011; Bedford *et al.* 2011]. Besides the loss of VirG (IcsA), WRSs2, and WRSs3, the mutants also lacked plasmid-encoded enterotoxins ShET2-1 and ShET2-2. WRSs3 further lacks MsbB2 that reduces the endotoxicity of the lipid A portion of the bacterial LPS. Studies in cell cultures and in gnotobiotic piglets demonstrate that WRSs2 and WRSs3 have the potential to cause less diarrhea due to loss of ShET2-1 and ShET2-2, as well as alleviate febrile symptoms by loss of MsbB2 [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].

A series of strains were constructed at the CVD. CVD 1203 strain, incorporating aroA and virG deletions, was too reactogenic at doses of 10^8 and 10^9 CFUs when it showed good immunogenicity and strain 1207, which harbored deletion mutations in guaBA, virG, set, and sen, was well tolerated at 10^8 and 10^9 CFUs but poorly immunogenic [Kotloff *et al.* 2000]. Recently, *S. flexneri* 2a strain CVD 1208 with a guaAB mutation that has been combined with a sen and a set mutation leaving the virG gene unknocked showed excellent tolerance, thereby allowing administration of vaccine doses up to 10^9 CFUs without side effects. CVD 1208 induced a geometric mean IgA ASC of 62 per 100 peripheral blood mononuclear cells, with 71% of subjects exhibiting fourfold rises in serum IgA or IgG, and 86% exhibiting fourfold rises in fecal IgA at 10^9 CFUs [Kotloff *et al.* 2004]. Similar good safety and immunogenicity results were generated when the same vaccine strain (CVD 1208) was reconstructed using animal-free media to conform to regulatory guidelines, and designated CVD1208S [Kotloff *et al.* 2007].

The studies on the new generations of live-attenuated vaccine strains delivered orally to volunteers from industrialized countries are encouraging in their achievement of a broader and safer interval between immunogenicity and safety following

immunization with oral live-attenuated *Shigella* vaccine strains.

A second and emerging concern related to orally delivered live-attenuated *Shigella* vaccine strains is associated with their performance in terms of immunogenicity and efficacy in children in developing countries. A series of clinical trials with the live-attenuated *S. flexneri* 2a SC602 strain, which was well tolerated and performed excellently in North American volunteers at a dose of 10^4 CFUs in terms of replication, immunogenicity, and protection, demonstrated very poor excretion and very low immunogenicity in Bangladeshi adults and children [Rahman *et al.* 2011]. We must be cautious with generalization of these results to other live-attenuated *Shigella* strains administered orally, especially those with different attenuating gene deletions (e.g. *S. flexneri* 2a strain CVD 1208). Nevertheless, we have to keep in mind that *S. flexneri* 2a SC602 was the strain which showed very good local and systemic immunogenicity and some residual reactogenicity in North American volunteers while the other current *Shigella* live candidate vaccine strains are more attenuated albeit sometimes with other attenuating gene deletions. The immunogenicity data related to *S. flexneri* 2a SC602 vaccine strain in Bangladesh corroborate findings on weaker immunogenicity and efficacy in children in developing countries compared with developed countries documented with other oral enteric vaccines, including the two recently licensed live-attenuated rotavirus vaccines [Levine, 2010]. Different hypotheses have been raised to explain the 'intestinal barrier' of volunteers in developing countries against this *S. flexneri* 2a SC602 live vaccine strain [Rahman *et al.* 2011] and other oral enteric vaccines [Levine, 2010], and solutions proposed to overcome this obstacle. There is no doubt that a significant research effort will be invested to resolve this complex issue.

Inactivated Shigella vaccine candidates

Whole-cell vaccines. In the early attempts to develop a vaccine, inactivated, whole-cell preparations of *Shigella* were developed and administered parenterally [Levine *et al.* 2007]. Although serum antibodies were elicited, unfortunately no significant protection was observed either in challenge studies among volunteers or in field trials. McKenzie *et al.* [2006] examined an orally delivered, formalin-inactivated, whole-cell *Shigella* vaccine candidate; there were no significant adverse events, but the immune response to several *Shigella* antigens was only moderate. Because of these limitations, the whole-cell inactivated approach to develop an efficacious *Shigella* vaccine was not continued.

Lipopolysaccharide-based vaccines. 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]. Since the lipid A moiety of the LPS is associated with significant adverse events, the O-specific polysaccharides (O-PS) were used as vaccine candidates; however, as the immune response to sugars is limited, particularly in infants and young children, methods to increase the immunogenicity were needed.

Shigella conjugate vaccines. 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. Conjugates against *S. sonnei, S. flexneri* 2a, and *S. dysenteriae* 1 were examined first in mice, and then in adults, children aged 4–7 years, and infants and children aged 1–4 years [Cohen *et al.* 1997; Ashkenazi *et al.* 1999; Passwell *et al.* 2003]. They were found as very safe, with minimal systemic adverse events (mainly fever in 2–5%, which was usually of low level and self limited) and local adverse events (mainly mild local pain and swelling) [Ashkenazi *et al.* 1999; Passwell *et al.* 2003]. They were highly immunogenic in terms of inducing homologous serum LPS antibodies, although the immune response in younger children was lower than that obtained in older children and in adults [Ashkenazi *et al.* 1999; Passwell *et al.* 2003].

The efficacy of these conjugates was examined in two field studies. A randomized, double-blind, double-dummy study in Israeli soldiers demonstrated that a single dose of *S. sonnei* conjugate conferred 74% protection against *S. sonnei* gastroenteritis [Cohen *et al.* 1997]. A randomized, double-blind efficacy study was done in 1–4-year-old children at 15 sites throughout Israel [Passwell *et al.* 2010]. The 2799 children enrolled received two doses of either *S. sonnei* or *S. flexneri* 2a conjugate vaccines, each serving as a control for the

other, and followed for 2 years. 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]. The reduced efficacy of the *Shigella* conjugate vaccines in young children is probably related to the lower antibody levels against *S. sonnei* LPS that have been elicited in this age group [Passwell *et al.* 2003]. It is well known from other conjugate vaccines, such as *Haemophilus Influenzae* type b and *Streptococcus pneumoniae* vaccines, that a higher number of doses is needed to protect infants and young children. The number of *S. flexneri* 2a cases cultured during the study was too low to enable conclusions regarding its efficacy [Passwell *et al.* 2010].

Attempts are currently being made to prepare a more immunogenic conjugate *Shigella* vaccine. One approach is based on synthetic oligosaccharides and another one involves core fragments of *S. sonnei* O-PS, both further bound to carrier proteins. Such conjugates have been developed by investigators at the National Institutes of Health in the USA [Pozsgay *et al.* 2007] and at the Pasteur Institute in France [Phalipon *et al.* 2009]. The use of the synthetic technology allows flexibility and control in the production of vaccine antigens which mimic the protective antigenic configuration carried by *Shigella* O antigen, and showed promising preliminary results in animal studies [Pozsgay *et al.* 2007; Phalipon *et al.* 2009]. A synthetic pentadecasaccharide, representing three biological repeating units of the O-PS of *S. flexneri* 2a recognized by sera of naturally infected subjects, elicited a protective serum anti-LPS 2a response in mice [Phalipon *et al.* 2009]. Further studies with these new conjugates, especially testing in human field trials, are needed to prove their efficacy.

Other subunit Shigella vaccines. *S. flexneri* 2a and *S. sonnei* LPS were hydrophobically complexed with proteosomes, meningococcal outer membrane proteins, for another approach to increase the immunogenicity of LPS-based vaccines [Fries *et al.* 2001; Kweon 2008]. These vaccines were first administered orally, and then intranasally, with a good immune response. Relatively purified *Shigella* ribosomal subunit vaccines, composed of *Shigella* O-PS and ribosomes, were initially developed by Levenson and his group in the former USSR and later on in the USA (WRAIR) [Levenson *et al.* 1995]. New preparations of a *Shigella* ribosomal vaccine candidate were prepared in South Korea. They were safe, immunogenic, and demonstrated protection against *Shigella* pneumonia in a murine model following intranasal administration [Shim *et al.* 2007].

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*. Oaks and and Turbyfill prepared a macromolecular complex composed of *S. flexneri* 2a LPS, ipaB, ipaC, and ipaD [Oaks and Turbyfill 2006]. When given intranasally, this experimental vaccine induced serum and mucosal antibodies and also antibody-secreting cells [Riddle *et al.* 2011]. In a non-LPS-based approach, outer membrane vesicles released from shigellae and encapsulated into nanoparticles were immunogenic and protected mice against experimental *Shigella* infection [Camacho *et al.* 2013]. Genetically derived outer membrane particles composed of predicted *Shigella* outer membrane and periplasmic proteins without LPS, obtained by the novel protein vesicle technology, represent another promising subunit vaccine candidate with expected wide coverage [Scorza *et al.* 2012].

Conclusion

We currently witness diverse innovative approaches based on progress in molecular technology, elucidation of the virulence mechanisms of *Shigella*, and lessons learnt from previous vaccine studies for developing *Shigella* vaccines. It is hoped that these will lead to a licensed safe and efficacious *Shigella* vaccine to protect humans against this pathogen and the related morbidity and mortality.

Funding

The study was supported in part by the European Commission (grant agreement number 261472 STOPENTERICS).

Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

References

Anatoliotaki, M., Galanakis, E., Tsekoura, T., Schinaki, A., Stefanaki, S. and Tsilimigaki, A. (2003) Urinary tract infection caused by *Shigella* sonnei. *Scand J Infect Dis* 35: 431–233.

Ashkenazi, S. (2004) *Shigella* infections in children: new insights. *Semin Pediatr Infect Dis* 5: 246–252.

Ashkenazi, S., Amir, J., Waisman, Y., Rachmel, A., Garty, B.Z. and Samra, Z. (1993) A randomized, double-blind study comparing cefixime and TMP-SMX in the treatment of childhood shigellosis. *J Pediatr* 123: 817–821.

Ashkenazi, S., Cleary, K., Pickering, L., Murray, B.E. and Cleary, T.G. (1990) The association of Shiga toxin and other cytotoxins with the neurologic manifestations of shigellosis. *J Infect Dis* 161: 961–965.

Ashkenazi, S., Dinari, G., Zevulunov, A. and Nitzan, M. (1987) Convulsions in childhood shigellosis. *Am J Dis Child* 141: 208–210.

Ashkenazi, S., Passwell, J., Harlev, E., Miron, D., Ramon, R. and Farzam, N.. (1999) Safety and immunogenicity of *Shigella sonnei* and *Shigella flexneri* 2a O-specific polysaccharide conjugates in children. *J Infect Dis* 179: 1565–1568.

Baiulescu, M., Hannon, P., Marcinak, J. (2002) Chronic vulvovaginitis caused by antibiotic-resistant *Shigella* flexneri in a prepubertal child. *Pediatr Infect Dis J* 21: 170–172.

Barnoy, S., Baqar, S., Kaminski, R. (2011) *Shigella* sonnei vaccine candidates WRSs2 and WRSs3 are as immunogenic as WRSS1, a clinical tested vaccine candidate in a primate model of infection. *Vaccine* 29: 6371–6378.

Barnoy, S., Jeong, K., Helm, R., Suvarnapunya, A., Ranallo, R., Tzipori, S. *et al.* (2010) Characterization of WRSs2 and WRSs3 new second-generation virG (icsA)-based Shigella sonnei vaccine candidate with potential for reduced reactogenicity. *Vaccine* 28: 1642–1654.

Barry, E., Pasetti, M., Sztein, M., Fasano, A., Kotloff, K. and Levine, M. (2013) Progress and pitfalls in Shigella vaccine research. *Nat Rev Gastroenterol Hepatol* 10: 245–255.

Basualdo, W. and Arbo, A. (2003) Randomized comparison of azithromycin versus cefixime for treatment of shigellosis in children. *Pediatr Infect Dis J* 22: 374–376.

Bedford, L., Fonseka, S., Boren, T., Ranallo, R., Suvarnapunya, A., Lee, J. *et al.* (2011) Further characterization of Shigella sonnei live vaccine candidates WRSs2 and WRSs3 – plasmid composition, invasion assays and Sereny reactions. *Gut Microbes* 2: 244–51.

Bennish, M., Harris, J., Wojtyniak, B. and Struelens, M. (1990) Death in shigellosis: incidence and risk factors in hospitalized patients. *J Infect Dis* 161: 500–506.

Camacho, A., Irache, J. and Gamazo, C. (2013) Recent progress towards development of *Shigella* vaccine. *Expert Rev Vaccines* 12: 43–55.

Camacho, A., Souza-Reboucas, J., Irache, J. and Gamazo, C. (2013) Towards a non-living vaccine against *Shigella* flexneri: from the inactivation procedure to protection studies. *Methods* 60: 264–268.

Centers for Disease Control and Prevention (2011) Final 2010 report of notifiable diseases. *MMWR Morb Mortal Wkly Rep* 60: 1088–1092.

Centers for Disease Control and Prevention (2013) Notes from the field: outbreak of infections caused by *Shigella* sonnei with decreased susceptibility to azithromycin – Los Angeles, California, 2012. *MMWR Morb Mortal Wkly Rep* 62: 171–173.

Christopher, P., David, K., John, S. and Sankarapandian, V. (2010) Antibiotic therapy for *Shigella* dysentery. *Cochrane Database Syst Rev* (4): CD006784.

Chuang, Y., Huang, Y. and Lin, S. (2006) Outbreak of *Shigella* sonnei gastroenteritis in northern Taiwan. *Pediatr Infect Dis J* 25: 92–94.

Clemens, J., Stanton, B., Stohl, B., Shahid, N.S., Banu, H. and Chowdhury, A.K. (1986) Breast-feeding as a determinant of severity in shigellosis. *Am J Epidemiol* 123: 710–720.

Cohen, D., Ashkenazi, S., Green, M., Gdalevich, M., Robin, G. and Slepon, R. (1997) Double-blind, vaccine-controlled randomised efficacy trial of an investigational *Shigella sonnei* conjugate vaccine in young adults. *Lancet* 349: 155–159.

Cohen, D., Ashkenazi, S., Green, M., Yavzori, M., Orr, N., Slepon, R. *et al.* (1994) Safety and immunogenicity of the oral E. coli k12-S. flexneri 2a vaccine (EcSf2a-2) among Israeli soldiers. *Vaccine* 12: 1436–1442.

Cohen, D., Green, M., Block, C., Rouach, T. and Ofek, I. (1988) Serum antibodies to lipopolysaccharide and natural immunity to shigellosis in an Israeli military population. *J Infec Dis* 157: 1068–1071.

Cohen, D., Green, M., Block, C., Slepon, R. and Ofek, I. (1991) Prospective study of the association between serum antibodies to Lipopolysaccharide O antigen and the attack rate of shigellosis. *J Clin Microbiol* 29: 386–389.

Cohen, D., Sela, T., Slepon, R., Yavzori, M., Ambar, R., Orr, N. *et al.* (2001) Prospective cohort studies of shigellosis during military field training. *Eur J Clin Microbiol Infect Dis* 20: 123–126.

Coster, T., Hoge, C., VanDeVerg, L. *et al.* (1999) Vaccination against shigellosis with attenuated *Shigella* flexneri 2a strain SC602. *Infect Immun* 67: 3437–3443.

DuPont, H. (2009) Systematic review: the epidemiology and clinical features of travellers' diarrhoea. *Aliment Pharmacol Ther* 30: 187–196.

Eidlitz-Marcus, T., Cohen, Y., Nussinovitch, M., Elian, I. and Varsano, I. (1993) Comparative efficacy of two and five day courses of ceftriaxone for treatment of severe shigellosis in children. *J Pediatr* 123: 822–824.

Fernandez F, Wetter M, Kowarik M, *et al.* A novel approach to produce multivalent glycoconjugated vaccines against shigellosis using recombinant bacterial cells that directly produce immunogenic bioconjugates. *Vaccines for Enteric Diseases Conference*, Malaga , Spain, 9–11 September 2009, Session 6-Shigellosis.

Ferreccio, C., Prado, V., Ojeda, A. (1991) Epidemiologic patterns of acute diarrhea and endemic *Shigella* infections in children in a poor periurban setting in Santiago, Chile. *Am J Epidemiol* 134: 614–627.

Fries, L., Montemarano, A., Mallett, C., Taylor, D.N., Hale, T.L. and Lowell, G.H. (2001) Safety and immunogenicity of a proteosome-Shigella flexneri 2a lipopolysaccharide vaccine administered intranasally to healthy adults. *Infect Immun* 69: 4545–4553.

Goren, A., Freier, S. and Passwell, J. (1992) Lethal toxic encephalopathy due to childhood shigellosis in a developed country. *Pediatrics* 89: 1189–1193.

Greenberg, D., Marcu, S., Melamed, R. and Lifshitz, M. (2003) *Shigella* septicemia: prevalence, presentation, risk factors and outcome. *Clin Pediatr* 42: 411–415.

Guerrero, L., Calva, J., Morrow, A. (1994) Asymptomatic *Shigella* infections in a cohort of Mexican children younger than two years of age. *Pediatr Infect Dis J* 13: 597–602.

Gupta, A., Polyak, C., Bishop, R., Sobel, J. and Mintz, E. (2004) Laboratory-confirmed shigellosis in the United States, 1989–2002: epidemiologic trends and patterns. *Clin Infect Dis* 15: 1372–1377.

Hayani, K., Guerrero, M., Ruiz-Palacios, G. (1991) Evidence for long-term memory of the mucosal immune system: milk secretory immunoglobulin A against *Shigella* lipopolysaccharides. *J Clin Microbiol* 29: 2599–2603.

Heine, S., Diaz-McNair, J., Martinez-Becerra, F., Choudhari, S., Clemnts, J., Picking, W. *et al.* (2013) Evaluation of immunogenicity and protective efficacy of orally delivered Shigella type 3 secretion system proteins IpaB and IpaD. *Vaccine* 31: 2919–2929.

Ingersoll, M., Groisman, E. and Zychlinsky, A. (2002) Pathogenicity islands of *Shigella*. *Curr Top Microbiol Immunol* 264: 49–65.

Khan, M. (1982) Interruption of shigellosis by hand washing. *Trans R Soc Trop Med Hyg* 76: 164–168.

Kotloff, K., Pasetti, M., Barry, E., Nataro, J.D., Wasserman, S.S. and Sztein, M.B. (2004) Deletion in the *Shigella* enterotoxin genes further attenuates *Shigella* flexneri 2a bearing guanine auxotrophy in a phase 1 trial of CVD 1204 and CVD 1208. *J Infect Dis* 190: 1745–1754.

Kotloff, K., Simon, J., Pasetti, M., Sztein, M., Wooden, S., Livio, S. *et al.* (2007) Safety and immunogenicity of CVD 1208S, a live, oral DeltaguaBA Deltasen Deltaset Shigella flexneri 2a vaccine grown on animal-free media. *Hum Vaccine* 3: 268–275.

Kotloff, K., Taylor, D., Sztein, M., Wasserman, S.S., Losonsky, G.A. and Nataro, J.D. (2002) Phase 1 evaluation of a virG deleted *Shigella sonnei* live, attenuated vaccine (strain WRSS1) in healthy adult volunteers. *Infect Immun* 70: 2016–2021.

Kotloff, K., Winickoff, J., Ivanoff, B., Clemens, J.D., Swerdlow, D.L. and Sansonetti, P.J. (1999) Global burden of *Shigella* infections: implication for vaccine development and implementation of control strategies. *Bull WHO* 77: 651–666.

Kweon, M. (2008) Shigellosis: the current status of vaccine development. *Curr Opin Infect Dis* 21: 313–318.

Levenson, V., Mallett, C. and Hale, T. (1995) Protection against local Shigella sonnei infection in mice by parenteral immunization with nucleoprotein subcapsular vaccine. *Infect Immun* 63: 2762–2765.

Levine, M. (1975) Shigellosis in custodial institutions. IV. In vivo stability and transmissibility of oral attenuated streptomycin-dependent *Shigella* vaccines. *J Infect Dis* 131: 704–707.

Levine, M. (2010) Immunogenicity and efficacy of oral vaccines in developing countries. *BMC Biol* 8: 129–139.

Levine, M., Kotloff, K., Barry, W., Pasetti, M. and Sztein, M. (2007) Clinical trials of *Shigella* vaccines: two steps forward and one step back on a long, hard road. *Nat Rev Microbiol* 5: 540–553.

Martin, J., Pitetti, R., Maffei, F., Tritt, J., Smail, K. and Wald, E.R. (2000) Treatment of shigellosis with cefixime: two days vs. five days. *Pediatr Infect Dis J* 19: 522–526.

Martin, T., Habbick, B. and Nyssen, J. (1983) Shigellosis with bacteremia: a report of two cases and a review of the literature. *Pediatr Infect Dis J* 2: 21–26.

Martinez-Becerra, F., Kissmann, J., McNair, J., Choudhari, S., Quick, A., Mellado-Sanchez, G. *et al.* (2012) Broadly protective Shigella vaccine based on type 3 secretion apparatus proteins. *Infect Immun* 80: 1222–1231.

Martinez-Becerra, F., Scobey, M., Harrison, K., Choudhari, S., Quick, A., Loshi, S. *et al.* (2013) Parenteral immunization with IpaB/IpaD protects mice against lethal pulmonary infection by Shigella. *Vaccine* 31: 2667–2672.

Mata, L., Urrutia, J., Garcia, B., Fernandez, R. and Behar, M. (1969) *Shigella* infections in breast fed Guatemalan Indian neonates. *Am J Dis Child* 117: 142–146.

McKenzie, R., Walker, R., Nabors, G., Van De Berg, C.C., Carpenter, C. and Gomez, G. (2006) Safety and immunogenicity of an oral, inactivated, whole-cell vaccine for *Shigella* sonnei: preclinical studies and phase 1 trial. *Vaccine* 24: 3735–3745.

Meitert, T., Pencu, E., Ciudin, L. and Tonciu, M. (1984) Vaccine strain Sh. flexneri T32-Istrati. Studies in animals and in volunteers. Antidysentery immunoprophylaxis and immunotherapy by live vaccine Vadizen (Sh. flexneri T32-Istrati). *Arch Roum Pathol Exp Microbiol* 43: 251–278.

Mel, D., Arsic, B., Radovanovic, M. and Litvinjenko, S. (1974) Live oral *Shigella* vaccine: vaccination schedule and the effect of booster dose. *Acta Microbiol Acad Sci Hung* 21: 109–114.

Mel, D., Gangarosa, E., Radovanovic, M., Arsic, B. and Litvinjenko, S. (1971) Studies on vaccination against bacillary dysentery. 6. Protection of children by oral immunization with streptomycin-dependent *Shigella* strains. *Bull World Health Organ* 45: 457–464.

Noriega, F., Liao, F., Maneval, D., Ren, S., Formal, S. and Levine, M. (1999) Strategy for cross-protection among Shigella flexneri serotypes. *Infect Immun* 67: 782–788.

Oaks, E. and Turbyfill, K. (2006) Development and evaluation of Shigella flexneri 2a and S. sonnei bivalent invasin complex (Invaplex) vaccine. *Vaccine* 24: 2290–2301.

Orr, N., Katz, D., Atsmon, J., Radu, P., Yavzori, M. and Halperin, T. (2005) Community-based safety, immunogenicity, and transmissibility study of the *Shigella sonnei* WRSS1 vaccine in Israeli volunteers. *Infect Immun* 73: 8027–8032.

Page, A., Ohayon, H., Sansonetti, P. and Parsot, C. (1999) The secreted IpaB and IpaC invasins and their cytoplasmic chaperone IpgC invasins are required for intercellular dissemination of *Shigella flexneri. Cell Microbiol* 1: 183–193.

Passwell, J., Ashkenazi, S., Banet-Levi, Y., Ramon, R., Earzan, N. and Lerner-Geva, L. (2010) Age-related efficacy of Shigella sonnei conjugate vaccine in children. *Vaccine* 28: 231–235.

Passwell, J., Ashkenazi, S., Harlev, E., Miron, D., Ramon, R. and Farzan, N. (2003) Safety and immunogenicity of *Shigella sonnei*-CRM9 and *Shigella flexneri* type 2a-rEPAsucc conjugate vaccines in one-to-four year-old children. *Pediatr Infect Dis J* 22: 701–706.

Passwell, J., Freier, S., Shor, R., Farzan, N., Block, C. and Lison, M. (1995) *Shigella* lipopolysaccharide antibodies in pediatric populations. *Pediatr Infect Dis J* 14: 859–865.

Phalipon, A., Tanguy, M., Grandjean, C., Guerreiro, C., Belot, F., Cohen, D. *et al.* (2009) A synthetic carbohydrate-protein conjugate vaccine candidate against *Shigella flexneri* 2a infection. *J Immunol* 182: 2241–2247.

Pozsgay, V., Kubler-Kielb, J., Schneerson, R. and Robbins, J. (2007) Effect of the nonreducing end of *Shigella dysenteriae* type 1 O-specific oligosaccharides on their immunogenicity as conjugates in mice. *Proc Natl Acad Sci U S A* 104: 14478–14482.

Putman, S., Sanders, J., Frenck, R., Monteville, M., Riddle, M., Rockabrand, D. *et al.* (2006) Self-reported description of diarrhea among military populations in operations Iraqi Freedom and Enduring Freedom. *J Travel Med* 13: 92–99.

Rahman, K., Arifeen, S., Zaman, K., Rahman, M., Raqib, R., Yunus, M. *et al.* (2011) Safety, dose, immunogenicity and transmissibility of an oral live-attenuated *Shigella flexneri* 2a vaccine candidate (SC602) among healthy adults and school children in Matlab, Bangladesh. *Vaccine* 29: 1347–1354.

Ranallo, R., Fonseka, S., Boren, T., Bedford, L., Kaminski, R., Thakkar, S. *et al.* (2012) Two live attenuated Shigella flexneri 2a strains WRSf2G12 and WRSf2G15: a new combination of gene deletions for 2nd generation live attenuated vaccine candidates. *Vaccine* 30: 5159–5171.

Raqib, R., Gustafsson, A., Andersson, J. and Bakhiet, M. (1997) A systemic down regulation of gamma interferon production is associated with acute shigellosis. *Infect Immun* 65: 5338–5341.

Riddle, M., Kaminski, R., Williams, C., Porter, C., Bagar, S. and Kordis, A. (2011) Safety and immunogenicity of an intranasal *Shigella flexneri* 2a Invaplex 50 vaccine. *Vaccine* 29: 7009–7019.

Sansonetti, P. (2001) Rupture, invasion, and inflammatory destruction of intestinal barrier by *Shigella*, making sense of prokaryote-eukaryote cross-talks. *Microbiol Rev* 25: 3–14.

Sansonetti, P. and Arondel, J. (1991) Construction and evaluation of a double mutant of *Shigella flexneri* as a candidate for oral vaccination against shigellosis. *Vaccine* 7: e443–e450.

Scorza, F., Colucci, A., Maggiore, L., Sanzone, S., Rossi, O., Ferlenghi, I. *et al.* (2012) High yield production process for *Shigella* outer membrane particles. *PLoS ONE* 7: e35616.

Sereny, B. (1957) Experimental keratoconjunctivitis shigellosa. *Acta Microbiol Acad Sci Hung* 4: 367–376.

Shiferaw, B., Solghan, S., Palmer, A., Joyce, R., Barzilay, E.J. and Kruger, A. (2012) Antimicrobial susceptibility patterns of *Shigella* isolates in Foodborne Diseases Active Surveillance Network (FoodNet) sites, 2000–2010. *Clin Infect Dis* 54(Suppl. 5): S458–S462.

Shim, D., Chang, S., Park, S., Jang, H., Carbis, R., Czerkinsky C. (2007) Immunogenicity and protective efficacy offered by a ribosomal-based vaccine from *Shigella flexneri* 2a. *Vaccine* 25: 4828–4836.

Struelens, M., Patte, D., Kabir, I., Salam, A., Nath, S.K. and Butler, T. (1985) *Shigella* septicemia: prevalence, presentation, risk factors, and outcome. *J Infect Dis* 152: 784–790.

Varsano, I., Eidlitz-Marcus, T., Nussinovitch, M. and Elian, I. (1991) Comparative efficacy of ceftriaxone and ampicillin for treatment of severe shigellosis in children. *J Pediatr* 118: 627–632.

Venkatesan, M., Fernandez Prada, C., Buysse, J., Formal, S. and Hale, T. (1991) Virulence phenotype and genetic characteristics of the T32- ISTRATI *Shigella flexneri* 2a vaccine strain. *Vaccine* 9: 358–363.

Viner, Y., Miron, D., Gottfried, E., Segal, D. and Luder, A. (2001) Neonatal shigellosis. *Isr Med Assoc J* 3: 964–966.

von Seidlein, L., Kim, D., Ali, M., Lee, H., Wang, X. and Thiem, V.D. (2006) A multicentre study of *Shigella* diarrhoea in six Asian countries: disease burden, clinical manifestations, and microbiology. *PLoS Med* 3: e353–e358.

Wahid, R., Simon, J., Picking, W., Kotloff, K., Levine, M. and Sztein, M. (2013) Shigella antigen-specific B memory cells are associated with decreased disease severity in subjects challenged with wild-type Shigella flexneri 2a. *Clin Immunol* 148: 35–43.