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Progress and pitfalls in *Shigella* vaccine research

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Abstract

Renewed awareness of the significant morbidity and mortality that *Shigella* causes among young children in developing countries combined with technological innovations in vaccinology has led to the development of novel vaccine strategies in the past five years. Along with advancement of classical vaccines in clinical trials and new sophisticated measurements of immunological responses, much new data has been produced lending promise to the potential for production of safe and effective *Shigella* vaccines. Herein we review the recent progress in *Shigella* vaccine development within the framework of persistent obstacles.

Introduction

Shigella diarrheal illness remains an important cause of morbidity and mortality globally, particularly among children < 5 years of age in developing countries. In 1999, it was estimated that *Shigella* was causing annually ~ 113 million episodes and 0.6 million deaths[1]. As a pathogen that invades and destroys intestinal mucosa, *Shigella* is less amenable to the salutory effects of oral rehydration compared to enterotoxigenic pathogens that cause dehydrating diarrhea. Antibiotics are the standard of care for shigellosis but therapeutic options are limited by the widespread prevalence of resistant strains, as in Asia where resistance to ciprofloxacin has become common[2, 3]. Resistance has increased to the three second line choices, reaching moderate levels (30–50%) for pivmecillinam and azithromycin, and has appeared to third generation cephalosporins mediated by extended spectrum β-lactamases[2, 4–6]. As therapeutic options narrow, the need for a safe and effective *Shigella* vaccine becomes more pressing. Herein we review advances in *Shigella* vaccine development during the past 5 years, highlighting new vaccine technologies.

Current taxonomy and epidemiology of *Shigella*

Shigella is an antigenically diverse pathogen whose taxonomy undergoes periodic modifications. The current official taxonomy encompasses four species (or Groups) and 49 serotypes and subtypes that include *S. dysenteriae* (Group A types), *S. flexneri* (Group B, 13 types and subtypes), *S. boydii* (Group C, 20 types) and *S. sonnei* (Group D, 1 type). There are also more than a dozen putative new type or subtype strains that are being considered for possible official classification. Among these, arguably, the most important are *S. flexneri* provisional serotype variants Y394 and 88–893 that are likely to be designated *S. flexneri* 7a and 7b, respectively[7].

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Competing interests

Myron M. Levine is the co-inventor of the patent for the attenuated *Shigella flexneri 2a* vector vaccine expressing two putative protective antigens of Enterotoxigenic *Escherichia coli*. However, heretofore no company has licensed this technology, thus there is no commercial “product” in development. The patent exists on paper only.

Since the ingestion of minute inocula (10 organisms) can lead to shigellosis, *Shigella* disseminates easily in settings where there is overcrowding, limited access to water, compromise of personal hygiene and inadequate sanitation. *S. flexneri* serotypes are the major agents of endemic shigellosis among children in developing countries, while *S. sonnei* is the predominant serotype associated with *Shigella* diarrheal illness in sub-populations in industrialized country settings where *Shigella* infections persist and in transitional countries; *S. sonnei* is also an important agent of travelers' diarrhea[8]. *S. boydii* serotypes are uncommonly associated with diarrheal illness. The Global Enteric Multicenter Study (GEMS) of moderate and severe diarrhea (MSD) among children < 60 months of age in 7 developing countries in sub-Saharan Africa and South Asia determined the serotypes of *Shigella* isolates[9, 10]. Among > 1,100 isolates of *Shigella* associated with cases of MSD, 66% were *S. flexneri* and 24% were *S. sonnei; S. boydii* and *S. dysenteriae* serotypes collectively accounted for only 10% of the isolates. Four serotypes, *S. flexneri* 2a, *S. flexneri* 3a, *S. flexneri* 6 and *S. sonnei*, comprised 65% of all the isolates.

S. dysenteriae type 1 (the "Shiga bacillus"), the only serotype that elaborates Shiga toxin, can cause severe clinical disease with complications, including hemolytic uremic syndrome and historically has led to devastating epidemics and pandemics with high case fatality in all age groups. Shiga dysentery typically appears in developing countries experiencing upheaval of civil society or natural disaster[11]. Although Shiga disease has virtually disappeared within the past decade, it can reappear at any time.

For vaccine developers preparing a broad-based *Shigella* vaccine based on serotypes, the GEMS and other large survey data suggest that a quadravalent vaccine containing strains or antigens from *S. sonnei* and *S. flexneri* 2a, 3a, and 6, would directly cover ~65% of current circulating strains; with cross protection based on shared *S. flexneri* group antigens, such a quadravalent vaccine could cover >85% of currently circulating *Shigella* strains[12, 13]. Many argue for including *S. dysenteriae* 1 coverage in a serotype-based vaccine, in the expectation that pandemic Shiga dysentery will return and a vaccine could constitute an important public health tool.

Genomics

Genomic and proteomic technologies have elucidated complete *Shigella* genomes and protein profiles[14–18], providing information that impacts vaccine development. The revelations made have encouraged vaccine development strategies based on the identification of proteins conserved among *Shigella* and related *E. coli* enteropathogens (*vide infra*). Moreover, the genome analyses that document the phylogenetic relatedness of *Shigella* and *E. coli* are prompting a possible reclassification of *Shigella* as a member of the *E. coli* species. Such a taxonomic revision will have to include input from clinicians and epidemiologists to minimize confusion in the clinical and disease control arenas.

Pathogenesis and clinical features of *Shigella* infection

All serotypes follow a similar pathogenesis which involves translocation through ileal and colonic M cells, macrophage uptake basolateral invasion of epithelial cells, and dissemination within the mucosa (reviewed in[19, 20]). After an incubation period of 1 to 4 days, shigellosis usually begins with systemic symptoms, including fever, headache, malaise, anorexia, and occasional vomiting. Watery diarrhea typically precedes dysentery[21] and may be the only clinical manifestation of mild infection[22]. Watery diarrhea arises from the action of enterotoxins in the jejunum, whereas bloody diarrhea results from invasion of the colonic epithelium. Frank dysentery manifests as frequent scanty stools of containing blood and mucus, accompanied by lower abdominal cramps and rectal tenesmus. Patients with severe infection may pass more than 20 dysenteric stools daily. Shigellosis in otherwise

healthy individuals is generally self-limited and resolves within 5 to 7 days, without sequelae. However, extraintestinal complications may occur[23] including generalized convulsions and encephalopathy. Hemolytic uremic syndrome (HUS) can accompany *S. dysenteriae* 1 infection[24]. Acute, life-threatening complications are sometimes seen in malnourished children in developing countries. In the United States, *Shigella* bacteremia has rarely been reported among HIV-infected and other immunocompromised patients[25].

Whereas the molecular mechanisms that determine *Shigella* invasiveness, rupture of the phagocytic vacuole, movement through the host-cell cytoplasm, and modulation of the innate immune response have been intensively studied, less is known about how the organism evokes diarrhea. Nevertheless, secretogenic proteins elaborated by *Shigella* strains have been identified (Table 1) and serve as targets for attenuating mutations or as new vaccine antigens.

***Shigella* enterotoxins—**In 1995 we identified *Shigella* enterotoxins 1 and 2 (ShET1 and ShET2,) by demonstrating their ability to cause fluid accumulation in rabbit ileal loops (ShET1), and greater potential difference and short circuit current (Isc) in Ussing chambers (both measures of electrolytes and water secretion)[26, 27]. ShET2 secretion requires the T3SS in *S. flexneri* 2a. Functional studies of a ShET2 mutant demonstrating a reduction in interleukin-8 (IL-8) secretion following invasion suggests that this toxin might also participate in *Shigella*-induced inflammation in epithelial cells[28]. A role for these two toxins in disease was determined in clinical trials where a reduction in reactogenicity in live attenuated *S. flexneri* 2a vaccine strains containing mutations in these two toxins was demonstrated[29]. ShET1 and ShET2 continue to serve as targets for attenuating mutations in multiple vaccine candidates[30–32].

Serine Protease Autotransporters of Enterobacteriaceae (SPATES)—The most common secretion mechanism across the Gram negative envelope is the autotransporter system, in which the full length species is passed across the inner membrane by virtue of the Sec apparatus, and then employs its own C-terminus to effect translocation across the outer membrane. A large and growing family of SPATEs has been identified, produced almost exclusively by pathogenic *E. coli*, *Shigella* and *Salmonella* strains[33]. Three SPATES have been identified as potential contributors to the enterotoxic activity of *Shigella* (Table 1).

SigA is a chromosomally-encoded class I SPATE (cytotoxic to epithelial cells) in *S. flexneri* 2a that exerts cytopathic effect in HEp-2 cells[34], suggesting that it may be a cell-altering toxin with a role in pathogenesis. SigA was demonstrated to be partly responsible for the ability of *S. flexneri* to stimulate fluid accumulation in ligated rabbit ileal loops[34]. A fragment of SigA is the basis for one conserved protein vaccine strategy [35].

SepA is a class II non-cytotoxic SPATE with a potential role demonstrated in the rabbit ligated ileal loop assay, in which a mutant in SepA exhibited significantly less inflammation and tissue damage than the wild type parent strain[36]. We have demonstrated in Ussing chamber studies that SepA exerts enterotoxic activity (unpublished).

Pic is a SPATE encoded on the chromosomes of EAEC 042, urinary pathogenic *E. coli* and *Shigella flexneri* 2a[37, 38]. Functional analyses of the Pic protein implicate this factor in mucinase activity, serum resistance, hemagglutination, enterotoxicity and immune modulation in targeting a broad range of human leukocyte adhesion proteins[39]. The gene encoding Pic is located as overlapping DNA on the opposite strand as *set1AB*, encoding ShET1[38]. Thus, live attenuated strains containing mutations in ShET1 also contain mutations in Pic.

Whereas most commensal and diarrheagenic *E. coli* encode few SPATEs, most *Shigella* strains harbor one or more SPATE-encoding gene(s)[40], suggesting that class I SPATEs are important in *Shigella* pathogenesis. Moreover, the most common *Shigella* serotypes (e.g., *S. flexneri* 2a, 2b) generally carry the greatest number of SPATE-encoding genes. The class II Pic protease is largely found in *S. flexneri* 2a, which is globally the most important *Shigella* serotype. As more is learned about the precise roles of the SPATEs, their inclusion in vaccine strategies may be expanded.

Animal Models

No small animal model recapitulates all aspects of *Shigella* pathogenesis, as seen in humans. Non-human primates (NHP) constitute one useful model, since they exhibit diarrhea and dysentery following oral infection with virulent *Shigella* strains and have aided efficacy evaluation of vaccine candidates[41–43]. However, the cost and availability of NHPs, combined with the fact that enormous inocula ($>10^9$ CFU) are required to consistently induce shigellosis, are recognized drawbacks. The guinea pig keratoconjunctivitis model described by Sereny, which correlates with the ability of the organism to invade cells, spread through a single cell layer, and induce inflammation[44], is helpful for evaluating the safety of live vaccines, as well as for testing the efficacy of many types of vaccines. The mouse lung model is employed for the same purposes, i.e., to reveal the inflammatory potential of live vaccines and its mutants and to demonstrate protection conferred by *Shigella* vaccines[44, 45]. A model involving intrarectal inoculation of guinea pigs[46], which leads to severe acute rectocolitis and a robust inflammatory response, is also useful in vaccine evaluation.

We have shown that *Shigella* strains and their extracellular enterotoxins induce ion flux (correlating with a secretory state) in rabbit tissue mounted in Ussing chambers[26, 47]. Ussing chambers provide a far more quantitative readout than rabbit ileal loops of pathophysiologic effects on mucosal epithelium. We have also used mouse small intestine to study *Shigella* enterotoxic activity in *ex vivo* models.

Vaccine Candidates

The lack of an ideal small animal model of *Shigella* infection represents one hurdle in vaccine development. Despite this obstacle, multiple vaccine strategies have been advanced in the past 5 years, buoyed by increased knowledge of *Shigella* epidemiology and pathogenesis and of human immune responses to the pathogen, as well as by new vaccinology technologies. These efforts can be categorized into two broad approaches including: 1) serotype based vaccines, or 2) conserved antigen vaccines (Table 2). Serotype-specific strategies extend the demonstration that an initial clinical infection stimulates acquired immunity and serotype-specific protection against shigellosis. This has been well documented in challenge studies in non-human primates[48], in adult volunteers experimentally infected with virulent strains[49, 50] and in epidemiological studies in endemic regions[51].

Serotype-targeted vaccines—Serotype-specific candidates include conjugate vaccines composed of purified *Shigella* O polysaccharides conjugated to a protein carrier, genetically engineered O polysaccharide protein fusions and live attenuated strains. The most advanced conjugate vaccines, developed by investigators at the National Institute of Child Health and Human Development, include *S. flexneri* 2a LPS conjugated to recombinant *Pseudomonas* exoprotein A (rEPA) and *S. sonnei* LPS conjugated to rEPA. These conjugates were shown to be safe and immunogenic in adults and young children[52]. Furthermore, the *S. sonnei* conjugate was efficacious against disease when tested in Israeli soldiers in field trials [53–55]. Recently the *S. sonnei* and *S. flexneri* 2a conjugate vaccines were tested for efficacy in Israeli children aged 1–4 yrs. There was not enough disease due to *S. flexneri* to calculate

efficacy. The *S. sonnei* conjugate did not meet the primary aim of the study in providing significant efficacy overall in children <4 years of age. However, when sub-group analyses by age were undertaken it was revealed that 71% efficacy was observed against *S. sonnei* infection in the 3–4 yr old age group, 35.5% efficacy in 2–3 yr olds but no efficacy was found in children aged 1–2 yrs at the time of vaccination[56]. Efficacy paralleled the age-related immune responses induced by the vaccine. One interpretation of these data is that the *Shigella* conjugate vaccine boosted children old enough to have had likely previous exposure but was unable to prime and protect immunologically naïve young children under 2 years of age.

A novel bioconjugate vaccine technology has been advanced by investigators at GlycoVaxyn that utilizes recombinant DNA technology to catalyze the *in vivo* synthesis of conjugate vaccines. The glycosylation machinery from *Campylobacter* was cloned in an *E. coli* production strain to generate a protein carrier glycosylated with the O-antigen specific *Shigella* LPS, which was then purified as a conjugate vaccine [57, 58]. A *S. dysenteriae* 1 O-antigen-EPA conjugate vaccine was produced in this system and tested in a Phase 1 trial where it was found to be safe and immunogenic following two doses [59].

Investigators at the Institut Pasteur have formulated carbohydrate vaccines encompassing synthetic oligosaccharides mimicking the protective determinants carried by the *Shigella* O antigen. The synthetic oligosaccharides fused to tetanus toxoid resulted in a functional O-antigen mimic recognized by human serum [60, 61]. The use of synthetic technology may allow great flexibility in the production of vaccine antigens.

Live attenuated vaccines represent another strategy based on the serotype specificity of the human protective response and include the advantage of presenting much more of the antigenic repertoire of the bacteria to the host immune system. Live attenuated vaccines have induced protective responses against virulent challenge in volunteer studies and have protected adult and pediatric populations against disease in controlled field trials[62–64]. While sophisticated genetic techniques allow the introduction of specifically targeted modifications into vaccine strains, achieving the correct balance of safety and immunogenicity has been a formidable challenge[65, 66]. Two attenuating strategies continue to progress through clinical trials. We have engineered a series of *Shigella* strains containing mutations in *guaBA,* encoding critical enzymes for bacterial metabolism, and in the *sen* and *set* loci, encoding ShET1 and ShET2. *S. flexneri* 2a strain CVD 1208S appeared safe and immunogenic in Phase 1 studies[13, 30] and has advanced through process development, cGMP manufacture and to Phase 2 clinical studies. The CVD is advancing an overall strategy utilizing five attenuated strains including *S. dysenteriae* 1, *S. sonnei*, *S. flexneri* 2a, *S. flexneri* 3a and *S. flexneri* 6 to encompass the most important strains and the type- and group-specific antigens found on all *Shigella* isolates[12, 13].

Investigators at the Walter Reed Army Institute of Research (WRAIR) have developed a series of live attenuated vaccine candidates containing a fundamental mutation in the *virG* (*icsA*) gene which is required for cell to cell spread of the bacteria. Additional mutations in some strains include genes encoding ShET1 and ShET2 as well as the *msbB* gene which is thought to detoxify lipid A of LPS and render the strain less reactogenic[31, 32, 67, 68]. *S. flexneri* 2a vaccine SC602, containing mutations in *virG* and *iuc* (encoding aerobactin), was previously demonstrated to be immunogenic and protective against challenge in North American volunteers although reactogenic at moderate and high doses [63, 69]. This vaccine was subsequently tested in healthy adults and school age children (8-10 yrs) in a *Shigella*-endemic region of Bangladesh, where single oral doses of 10^4, 10^5, or 10^6 CFU resulted in minimal vaccine shedding, minimal reactogenicity, no transmission and low immune stimulation[70].

This study underscores a critical point surrounding the use of orally administered live attenuated strains in *Shigella*-endemic regions where the nutritional and immune status as well as the microbiota of individuals may well affect vaccine performance[71]. Other oral live attenuated vaccines including polio, cholera and rotavirus have engendered lower immune responses among vaccinees in developing countries compared to those in industrialized countries[72–75]. Nonetheless, some of these vaccines have provided protection against severe illness in these populations[75, 76] and it is expected that the optimal formulation and vaccination regimen of a live attenuated *Shigella* vaccine will be equally successful[72, 77, 78].

Conserved antigen vaccines—The concept of using an antigen conserved among *Shigella* strains as an immunogen to provide broad protection is the basis for several new vaccine candidates. The most advanced vaccine that contains components of conserved proteins plus serotype specific O-antigen has been established by investigators at WRAIR. *Shigella* Invaplex was initially formulated from a bacterial extract composed of invasion plasmid antigen proteins (Ipa) which are highly conserved among all *Shigella* serotypes, and LPS. In animals, only serotype-specific protection has been demonstrated, mediated by the LPS component[79]. Invaplex has been shown to be safe and immunogenic following intranasal delivery in volunteers[80, 81] Current studies are underway to optimize formulation and delivery.

In related efforts, a vaccine composed of purified IpaB plus IpaD has been demonstrated to confer homologous as well as heterologous protection in a mouse model of *Shigella* infection when delivered with adjuvant[82]. Antibodies against invasion plasmid antigens (Ipas) are produced after natural and experimental human infection and are believed to contribute to protection[83, 84]. A vaccine that could induce protective anti-Ipa responses could provide protection against all *Shigella* strains expressing these highly conserved antigens.

The use of outer membrane protein preparations as vaccine formulations has also been explored[85, 86]. A novel protein vesicle technology named Generalized Modules of Membrane Antigens (GMMA) is an industrial, high yielding production process for genetically derived outer membrane particles composed of predicted *Shigella* outer membrane and periplasmic proteins without LPS[87]. In preclinical mouse studies, immunization with GMMA provided 65–100% protection against lethal challenge.

Taking advantage of genomic and proteomic data, investigators at the International Vaccine Institute (IVI) have identified two conserved protein candidates including IcsP2, an outer membrane protease which cleaves IcsA from the surface and which is present on all *Shigella* species and EIEC, and SigA2, a SPATE present on all *S. flexneri* 2a, *S. boydii* and *S. sonnei*. These antigens have demonstrated protection in animal models [35]

Immune Responses

The evaluation of vaccine candidates relies on an understanding of which responses are critical for protection. Studies from humans and non-human primates following natural infection and vaccination provide the most relevant data and suggest that a complex series of responses engaging multiple arms of the immune system are involved in immunity to disease caused by *Shigella*.

Immunity induced by natural infection or vaccination—Humans develop an array of immune responses following *Shigella* infection, including humoral and cell-mediated immune (CMI) responses. Of particular importance are the high levels of serum IgG and IgA antibodies against *Shigella* O-antigen, which appear 1–2 weeks following primary exposure. Results from multiple epidemiological[51] and seroepidemiological studies [88–90]

suggest that O-specific antibodies play a critical role in protection. Antibodies against Ipas are also produced after natural and experimental infection and believed to contribute to protection[83, 84, 91].

In addition to systemic immunity, strong mucosal immune responses are induced [63, 92]. Gut-derived O-specific IgA antibody secreting cells (ASC) are believed to play a critical role in protection against *Shigella*. These cells are detected in peripheral blood 7 to 10 days after exposure to the organism or vaccine and are believed to represent a pool of transiently migrating antigen-specific B cells with the capacity to home to mucosal effector sites where they will participate in host defenses by producing local antibodies. IgA O-antigen ASCs represent a measure of oral priming that has been associated with efficacy of live attenuated vaccines[63, 93]. The number of O-specific IgA ASCs and the levels of O-specific serum IgG are commonly used as primary readouts of immunogenicity in clinical trials of attenuated live and non-living whole cell oral vaccines and serum IgG O antibody for parenteral O polysaccharide-protein conjugate vaccines; moreover, these are generally considered to be predictors of the efficacy of these vaccines. Secretory IgA (sIgA) also appears to have a major role limiting the duration of illness[94, 95]. However, measurements of sIgA in mucosal secretions (e.g., stool and saliva) can be variable and there is no consensus in the literature showing a clear association with resistance to infection[93].

The efficacy of conjugate vaccines has been correlated with high levels of IgG O-antibody[56, 96]. It has been speculated that the organism may be inactivated by parenterally induced IgG leaked into the intestinal lumen, possibly through complement-mediated lysis in the epithelial cell surface[97]. It is reasonable to assume that the presence of a critical level of protective IgG antibodies implies the presence of strong underlying T helper immunity, yet there are no reports of T cell measurements in *Shigella*-conjugate vaccine studies.

Shigella infection has also been shown to induce CMI including upregulation of interferon (IFN)-γ receptor expression and production of pro-inflammatory cytokines, including IFN-γ[98, 99]. Moreover, an expansion of T cells, particularly $CD8^+$ and T-cell receptor (TCR) $\gamma\delta^+$ T-cell subsets in the gut mucosa[100], has been described in the rectal mucosa of shigellosis patients. Of note, increased levels of activated and memory $CD4^+$ and $CD8^+$ T cells and expansion of defined TCR Vβ families have been reported in peripheral blood of patients with shigellosis[101, 102]. However, a direct association between CMI responses and protection has not been demonstrated and the extent to which these responses contribute to clearing infection and to the pathogenesis of shigellosis remains unknown.

Mucosal immunological priming and effector responses induced by wild type or attenuated *Shigella* vaccine strains—Our current understanding of the processes involved in *Shigella* pathogenesis offers some insights into how the organism may interact with the immune cells in the gastrointestinal mucosa and trigger immune responses following infection (Figure 1A). Upon re-exposure to the organism, the host displays a plethora of immunological effector mechanisms to resist the infection (Figure 1B). Of particular importance are antibodies and immune cells in the gut (e.g., O-specific ASC and plasma cells, sIgA, memory B cells (B_M), Th1 and Th2 T cells) which will provide the first line of defense to prevent the organism from invading the epithelial barrier.

Anamnestic humoral immune responses, largely dependent on the presence of B memory (B_M) cells, are generally faster and higher in magnitude than primary responses and are crucial for protection from subsequent infections[103]. We have recently provided direct evidence for the presence of B_M cells specific for *Shigella* antigens in volunteers immunized with a single oral dose of *S. flexneri 2a* CVD 1204 or CVD 1208 attenuated vaccine candidates[104, 105]. Using a modified B_M ELISPOT assay we found that volunteers

developed IgA and IgG B_M to *S. flexneri* 2a LPS and IpaB antigens which were correlated with serum antibody responses. Moreover, we found elevated frequencies of IgA^+, but not IgG^+, $CD19^+$ $CD27^+$ $CD20^+$ and $CD19^+$ $CD27^+$ $CD20^+$ $CD20^{-/dim}$ B cell subsets expressing the gut homing receptor integrin α4/β7 28 days after oral vaccination, suggesting an increase in the circulating pool of *Shigella*-specific IgA B_M and plasmablasts with gut homing potential in individuals that developed humoral responses to *Shigella*. These responses are finely regulated, with mucosal DC playing a central role in imprinting mucosal homing receptors on T and B cells and inducing IgA differentiation and regulatory T cells (Treg). A competent immune system in a healthy individual can clear the pathogen within 7–10 days of infection.

These findings are important for the development of effective vaccines since the presence of B_M are likely to contribute to the persistence of systemic and mucosal antibodies and the ability to mount an anamnestic response when circulating antibody levels have already declined[106]. We therefore suggest including measurements of specific B_M to relevant antigens in future vaccine studies since they might represent an important immunological correlate of protection and indicate long-term immunity.

Conclusions

Technological advancements including genomic technologies, synthetic carbohydrate chemistry, *in vivo* conjugation techniques and sophisticated assays to measure immune responses have facilitated the development and evaluation of a new generation of *Shigella* vaccines. In parallel, the progression of clinical trials assessing classical conjugate and live attenuated vaccines is revealing new information about relevant immune responses associated with protection. Despite these advances, there is still no consensus on what constitute critical immunological correlates of protection; this remains an obstacle impeding the development of safe and efficacious vaccines. This issue is complicated by the lack of an optimal small animal model for studying disease and the effects of vaccines leading to reliance, ultimately, on clinical trials for relevant data. Notwithstanding these difficulties, this is a time for optimism as in recent years there has been a renewed recognition of the excessive morbidity and mortality that *Shigella* causes among young children in developing countries and a commitment to diminish this burden. This has translated to new funding partners joining the efforts of other traditional supporters in providing resources for *Shigella* vaccine development. As a consequence, consortia of scientists have been contributing their expertise toward the common goal of developing safe and efficacious *Shigella* vaccines that might in the future become widely utilized public health tools to diminish the burden of shigellosis in populations most in need.

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Reference List

1. Kotloff KL, et al. Global burden of Shigella infections: implications for vaccine development and implementation of control strategies. Bull World Health Organ. 1999; 77:651–666. [PubMed: 10516787]
2. Zhang W, et al. Wide dissemination of multidrug-resistant Shigella isolates in China. J Antimicrob Chemother. 2011; 66:2527–2535. [PubMed: 21859815]

3. Ghosh S, et al. Genetic characteristics and changing antimicrobial resistance among Shigella spp. isolated from hospitalized diarrhoeal patients in Kolkata, India. J Med Microbiol. 2011; 60:1460–1466. [PubMed: 21659504]
4. Vinh H, et al. A changing picture of shigellosis in southern Vietnam: shifting species dominance, antimicrobial susceptibility and clinical presentation. BMC Infect Dis. 2009; 9:204. [PubMed: 20003464]
5. Khatun F, et al. Changing species distribution and antimicrobial susceptibility pattern of Shigella over a 29-year period (1980–2008). Epidemiol Infect. 2011; 139:446–452. [PubMed: 20478088]
6. Taneja N, Mewara A, Kumar A, Verma G, Sharma M. Cephalosporin-resistant Shigella flexneri over 9 years (2001–09) in India. J Antimicrob Chemother. 2012; 67:1347–1353. [PubMed: 22410619]
7. Foster RA, et al. Structural elucidation of the O-antigen of the Shigella flexneri provisional serotype 88–893: structural and serological similarities with S. flexneri provisional serotype Y394 (1c). Carbohydr Res. 2011; 346:872–876. [PubMed: 21392735]
8. Hyams KC, et al. Diarrheal disease during Operation Desert Shield. N Engl J Med. 1991; 325:1423–1428. [PubMed: 1656260]
9. Kotloff K, Blackwelder WC, Nasrin D, et al. The Global Enterics Multicenter Study (GEMS) of Diarrheal Disease in Young CHildren in Dveeloping Countries: Epidemiologic and Clinical Methods of the Case-Control Study. Clinical Infectious Diseases. 2012 In press.
10. Panchalingam S, Antonio M, Hossain A, et al. Diagnostic Microbiology Methods in the GEMS-1 Case/Control Study. Clinical Infectious Diseases. 2012 In press.
11. Centers for Disease Control Prevention. Health Status of displaced persons following Cival War - Burundi Dcemebr 1993 – January 1994. 1994; 43:701–703.
12. Noriega FR, et al. Strategy for cross-protection among Shigella flexneri serotypes. Infect Immun. 1999; 67:782–788. [PubMed: 9916090]
13. Levine MM, Kotloff KL, Barry EM, Pasetti MF, Sztein MB. Clinical trials of Shigella vaccines: two steps forward and one step back on a long, hard road. Nat Rev Microbiol. 2007; 5:540–553. [PubMed: 17558427]
14. Jin Q, et al. Genome sequence of Shigella flexneri 2a: insights into pathogenicity through comparison with genomes of Escherichia coli K12 and O157. Nucleic Acids Res. 2002; 30:4432–4441. [PubMed: 12384590]
15. Yang F, et al. Genome dynamics and diversity of Shigella species, the etiologic agents of bacillary dysentery. Nucleic Acids Res. 2005; 33:6445–6458. [PubMed: 16275786]
16. Yang J, Chen L, Yu J, Sun L, Jin Q. ShiBASE: an integrated database for comparative genomics of Shigella. Nucleic Acids Res. 2006; 34:D398–D401. [PubMed: 16381896]
17. Wei J, et al. Complete genome sequence and comparative genomics of Shigella flexneri serotype 2a strain 2457T. Infect Immun. 2003; 71:2775–2786. [PubMed: 12704152]
18. Ying TY, et al. Immunoproteomics of membrane proteins of Shigella flexneri 2a 2457T. World J Gastroenterol. 2005; 11:6880–6883. [PubMed: 16425402]
19. Phalipon A, Sansonetti PJ. Shigella's ways of manipulating the host intestinal innate and adaptive immune system: a tool box for survival? Immunol Cell Biol. 2007; 85:119–129. [PubMed: 17213832]
20. Schroeder GN, Hilbi H. Molecular pathogenesis of Shigella spp.: controlling host cell signaling, invasion, and death by type III secretion. Clin Microbiol Rev. 2008; 21:134–156. [PubMed: 18202440]
21. DuPont HL, Hornick RB, Dawkins AT, Snyder MJ, Formal SB. The response of man to virulent Shigella flexneri 2a. J Infect Dis. 1969; 119:296–299. [PubMed: 5780532]
22. Taylor DN, et al. The role of Shigella spp., enteroinvasive Escherichia coli, and other enteropathogens as causes of childhood dysentery in Thailand. J Infect Dis. 1986; 153:1132–1138. [PubMed: 3517189]
23. Ashkenazi S, Dinari G, Zevulunov A, Nitzan M. Convulsions in childhood shigellosis. Clinical and laboratory features in 153 children. Am J Dis Child. 1987; 141:208–210. [PubMed: 3544808]
24. Koster F, et al. Hemolytic-uremic syndrome after shigellosis. Relation to endotoxemia and circulating immune complexes. N Engl J Med. 1978; 298:927–933. [PubMed: 642973]

25. Nelson MR, Shanson DC, Hawkins DA, Gazzard BG. Salmonella, Campylobacter and Shigella in HIV-seropositive patients. AIDS. 1992; 6:1495–1498. [PubMed: 1362879]
26. Fasano A, Noriega FR, Liao FM, Wang W, Levine MM. Effect of shigella enterotoxin 1 (ShET1) on rabbit intestine in vitro and in vivo. Gut. 1997; 40:505–511. [PubMed: 9176079]
27. Fasano A, et al. Shigella enterotoxin 1: an enterotoxin of Shigella flexneri 2a active in rabbit small intestine in vivo and in vitro. J Clin Invest. 1995; 95:2853–2861. [PubMed: 7769126]
28. Farfan MJ, Toro CS, Barry EM, Nataro JP. Shigella enterotoxin-2 is a type III effector that participates in Shigella-induced interleukin 8 secretion by epithelial cells. FEMS Immunol Med Microbiol. 2011; 61:332–339. [PubMed: 21219446]
29. Kotloff KL, et al. 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. 2004; 190:1745–1754. [PubMed: 15499528]
30. Kotloff KL, et al. Safety and Immunogenicity of CVD 1208S, a Live, Oral DeltaguaBA Deltasen Deltaset Shigella flexneri 2a Vaccine Grown on Animal-Free Media. Hum Vaccin. 2007; 3
31. Ranallo RT, et al. Two live attenuated Shigella flexneri 2a strains WRSf2G12 and WRSf2G15: A new combination of gene deletions for 2nd generation live attenuated vaccine candidates. Vaccine. 2012; 30:5159–5171. [PubMed: 22658966]
32. Bedford L, et al. Further characterization of Shigella sonnei live vaccine candidates WRSs2 and WRSs3-plasmid composition, invasion assays and Sereny reactions. Gut Microbes. 2011; 2:244–251. [PubMed: 21983066]
33. Dutta PR, Cappello R, Navarro-Garcia F, Nataro JP. Functional comparison of serine protease autotransporters of enterobacteriaceae. Infect Immun. 2002; 70:7105–7113. [PubMed: 12438392]
34. Al-Hasani K, et al. The sigA gene which is borne on the she pathogenicity island of Shigella flexneri 2a encodes an exported cytopathic protease involved in intestinal fluid accumulation. Infect Immun. 2000; 68:2457–2463. [PubMed: 10768931]
35. Czerkinsky, c; Kim, DW. Novel Shigella Protein Antigens and Methods US2010/0136045. 2010. p. 187
36. Benjelloun-Touimi Z, Si TM, Montecucco C, Sansonetti PJ, Parsot C. SepA, the 110 kDa protein secreted by Shigella flexneri: two-domain structure and proteolytic activity. Microbiology. 1998; 144 (Pt 7):1815–1822. [PubMed: 9695914]
37. Henderson IR, Czeczulin J, Eslava C, Noriega F, Nataro JP. Characterization of pic, a secreted protease of Shigella flexneri and enteroaggregative Escherichia coli. Infect Immun. 1999; 67:5587–5596. [PubMed: 10531204]
38. Behrens M, Sheikh J, Nataro JP. Regulation of the overlapping pic/set locus in Shigella flexneri and enteroaggregative Escherichia coli. Infect Immun. 2002; 70:2915–2925. [PubMed: 12010980]
39. Ruiz-Perez F, et al. Serine protease autotransporters from Shigella flexneri and pathogenic Escherichia coli target a broad range of leukocyte glycoproteins. Proc Natl Acad Sci U S A. 2011; 108:12881–12886. [PubMed: 21768350]
40. Boisen N, Ruiz-Perez F, Scheutz F, Krogfelt KA, Nataro JP. Short report: high prevalence of serine protease autotransporter cytotoxins among strains of enteroaggregative Escherichia coli. Am J Trop Med Hyg. 2009; 80:294–301. [PubMed: 19190229]
41. Formal SB, et al. Protection of monkeys against experimental shigellosis with a living attenuated oral polyvalent dysentery vaccine. J Bacteriol. 1966; 92:17–22. [PubMed: 4957431]
42. Formal SB, LaBrec EH, Palmer A, Falkow S. Protection of Monkeys Against Experimental Shigellosis with Attenuated Vaccines. J Bacteriol. 1965; 90:63–68. [PubMed: 16562044]
43. Shipley ST, et al. A challenge model for Shigella dysenteriae 1 in cynomolgus monkeys (Macaca fascicularis). Comp Med. 2010; 60:54–61. [PubMed: 20158950]
44. Sereny B. Experimental shigella keratoconjunctivitis; a preliminary report. Acta Microbiol Acad Sci Hung. 1955; 2:293–296. [PubMed: 14387586]
45. Mallett CP, VanDeVerg L, Collins HH, Hale TL. Evaluation of Shigella vaccine safety and efficacy in an intranasally challenged mouse model. Vaccine. 1993; 11:190–196. [PubMed: 8438617]
46. Shim DH, et al. New animal model of shigellosis in the Guinea pig: its usefulness for protective efficacy studies. J Immunol. 2007; 178:2476–2482. [PubMed: 17277155]

47. Nataro JP, et al. Identification and cloning of a novel plasmid-encoded enterotoxin of enteroinvasive Escherichia coli and Shigella strains. Infect Immun. 1995; 63:4721–4728. [PubMed: 7591128]
48. Formal SB, et al. Effect of prior infection with virulent Shigella flexneri 2a on the resistance of monkeys to subsequent infection with Shigella sonnei. J Infect Dis. 1991; 164:533–537. [PubMed: 1869840]
49. Herrington DA, et al. Studies in volunteers to evaluate candidate Shigella vaccines: further experience with a bivalent Salmonella typhi-Shigella sonnei vaccine and protection conferred by previous Shigella sonnei disease. Vaccine. 1990; 8:353–357. [PubMed: 2204243]
50. Kotloff KL, et al. A modified Shigella volunteer challenge model in which the inoculum is administered with bicarbonate buffer: clinical experience and implications for Shigella infectivity. Vaccine. 1995; 13:1488–1494. [PubMed: 8578831]
51. Ferreccio C, et al. Epidemiologic patterns of acute diarrhea and endemic Shigella infections in children in a poor periurban setting in Santiago, Chile. Am J Epidemiol. 1991; 134:614–627. [PubMed: 1951266]
52. Passwell JH, et al. 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. 2003; 22:701–706. [PubMed: 12913770]
53. Robbins JB, Chu C, Schneerson R. Hypothesis for vaccine development: protective immunity to enteric diseases caused by nontyphoidal salmonellae and shigellae may be conferred by serum IgG antibodies to the O-specific polysaccharide of their lipopolysaccharides. Clin Infect Dis. 1992; 15:346–361. [PubMed: 1381621]
54. Taylor DN, et al. Synthesis, characterization, and clinical evaluation of conjugate vaccines composed of the O-specific polysaccharides of Shigella dysenteriae type 1, Shigella flexneri type 2a, and Shigella sonnei (Plesiomonas shigelloides) bound to bacterial toxoids. Infect Immun. 1993; 61:3678–3687. [PubMed: 8359890]
55. Cohen D, et al. Safety and immunogenicity of investigational Shigella conjugate vaccines in Israeli volunteers. Infect Immun. 1996; 64:4074–4077. [PubMed: 8926071]
56. Passwell JH, et al. Age-related efficacy of Shigella O-specific polysaccharide conjugates in 1-4-year-old Israeli children. Vaccine. 2010; 28:2231–2235. [PubMed: 20056180]
57. Ihssen J, et al. Production of glycoprotein vaccines in Escherichia coli. Microb Cell Fact. 2010; 9:61. [PubMed: 20701771]
58. Feldman MF, et al. Engineering N-linked protein glycosylation with diverse O antigen lipopolysaccharide structures in Escherichia coli. Proc Natl Acad Sci U S A. 2005; 102:3016–3021. [PubMed: 15703289]
59. Dro, P.; Sinclair, M. GlycoVaxyn Phase I Clinical Study Shows Positive Data with Shigella dysenteriae Vaccine Candidate. 2010. http://www.glycovaxyn.com/content/news/releases/10%2010%2008.pdf[http://www.glycovaxyn.com/content/news/releases/10%2010%2008.pdf]
60. Phalipon A, et al. A synthetic carbohydrate-protein conjugate vaccine candidate against Shigella flexneri 2a infection. J Immunol. 2009; 182:2241–2247. [PubMed: 19201878]
61. Kubler-Kielb J, Vinogradov E, Chu C, Schneerson R. O-Acetylation in the O-specific polysaccharide isolated from Shigella flexneri serotype 2a. Carbohydr Res. 2007; 342:643–647. [PubMed: 17087926]
62. Porter CK, Thura N, Ranallo RT, Riddle MS. The Shigella human challenge model. Epidemiol Infect. 2012:1–10. [PubMed: 22906296]
63. Coster TS, et al. Vaccination against shigellosis with attenuated Shigella flexneri 2a strain SC602. Infect Immun. 1999; 67:3437–3443. [PubMed: 10377124]
64. Mel DM, Terzin AL, Vuksic L. Studies on vaccination against bacillary dysentery. 3. Effective oral immunization against Shigella flexneri 2a in a field trial. Bull World Health Organ. 1965; 32:647–655. [PubMed: 5294180]
65. Ranallo RT, Barnoy S, Thakkar S, Urick T, Venkatesan MM. Developing live Shigella vaccines using lambda Red recombineering. FEMS Immunol Med Microbiol. 2006; 47:462–469. [PubMed: 16872384]

66. Venkatesan MM, Ranallo RT. Live-attenuated Shigella vaccines. Expert Rev Vaccines. 2006; 5:669–686. [PubMed: 17181440]

67. Barnoy S, et al. Shigella sonnei vaccine candidates WRSs2 and WRSs3 are as immunogenic as WRSS1, a clinically tested vaccine candidate, in a primate model of infection. Vaccine. 2011; 29:6371–6378. [PubMed: 21596086]

68. Ranallo RT, et al. Virulence, inflammatory potential, and adaptive immunity induced by Shigella flexneri msbB mutants. Infect Immun. 2010; 78:400–412. [PubMed: 19884336]

69. Katz DE, et al. Two studies evaluating the safety and immunogenicity of a live, attenuated Shigella flexneri 2a vaccine (SC602) and excretion of vaccine organisms in North American volunteers. Infect Immun. 2004; 72:923–930. [PubMed: 14742537]

70. Rahman KM, et al. 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. 2011; 29:1347–1354. [PubMed: 21040694]

71. Gordon JI, Dewey KG, Mills DA, Medzhitov RM. The human gut microbiota and undernutrition. Sci Transl Med. 2012; 4:137ps12.

72. Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. BMC Biol. 2010; 8:129. [PubMed: 20920375]

73. Hanlon P, et al. Trial of an attenuated bovine rotavirus vaccine (RIT 4237) in Gambian infants. Lancet. 1987; 1:1342–1345. [PubMed: 2884455]

74. John TJ, Jayabal P. Oral polio vaccination of children in the tropics. I. The poor seroconversion rates and the absence of viral interference. Am J Epidemiol. 1972; 96:263–269. [PubMed: 4342327]

75. Armah GE, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. Lancet. 2010; 376:606–614. [PubMed: 20692030]

76. Balraj V, John TJ, Thomas M, Mukundan S. Efficacy of oral poliovirus vaccine in rural communities of North Arcot District, India. Int J Epidemiol. 1990; 19:711–714. [PubMed: 2262269]

77. Madhi SA, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. N Engl J Med. 2010; 362:289–298. [PubMed: 20107214]

78. Su-Arehawaratana P, et al. Safety and immunogenicity of different immunization regimens of CVD 103-HgR live oral cholera vaccine in soldiers and civilians in Thailand. J Infect Dis. 1992; 165:1042–1048. [PubMed: 1583321]

79. Turbyfill KR, Kaminski RW, Oaks EV. Immunogenicity and efficacy of highly purified invasin complex vaccine from Shigella flexneri 2a. Vaccine. 2008; 26:1353–1364. [PubMed: 18276045]

80. Riddle MS, et al. Safety and immunogenicity of an intranasal Shigella flexneri 2a Invaplex 50 vaccine. Vaccine. 2011; 29:7009–7019. [PubMed: 21787825]

81. Tribble D, et al. Safety and immunogenicity of a Shigella flexneri 2a Invaplex 50 intranasal vaccine in adult volunteers. Vaccine. 2010; 28:6076–6085. [PubMed: 20619378]

82. Martinez-Becerra FJ, et al. Broadly protective Shigella vaccine based on type III secretion apparatus proteins. Infect Immun. 2012; 80:1222–1231. [PubMed: 22202122]

83. Li A, Zhao CR, Ekwall E, Lindberg AA. Serum IgG antibody responses to Shigella invasion plasmid-coded antigens detected by immunoblot. Scand J Infect Dis. 1994; 26:435–445. [PubMed: 7984976]

84. Oaks EV, Hale TL, Formal SB. Serum immune response to Shigella protein antigens in rhesus monkeys and humans infected with Shigella spp. Infect Immun. 1986; 53:57–63. [PubMed: 3721580]

85. Adamus G, Mulczyk M, Witkowska D, Romanowska E. Protection against keratoconjunctivitis shigellosa induced by immunization with outer membrane proteins of Shigella spp. Infect Immun. 1980; 30:321–324. [PubMed: 7002794]

86. Pore D, Mahata N, Pal A, Chakrabarti MK. Outer membrane protein A (OmpA) of Shigella flexneri 2a, induces protective immune response in a mouse model. PLoS One. 2011; 6:e22663. [PubMed: 21818362]

87. Berlanda SF, et al. High yield production process for Shigella outer membrane particles. PLoS One. 2012; 7:e35616. [PubMed: 22701551]
88. Cohen D, Green MS, Block C, Rouach T, Ofek I. Serum antibodies to lipopolysaccharide and natural immunity to shigellosis in an Israeli military population. J Infect Dis. 1988; 157:1068–1071. [PubMed: 3283258]
89. Cohen D, Green MS, Block C, Slepon R, Ofek I. Prospective study of the association between serum antibodies to lipopolysaccharide O antigen and the attack rate of shigellosis. J Clin Microbiol. 1991; 29:386–389. [PubMed: 1706731]
90. Van De Verg LL, Herrington DA, Boslego J, Lindberg AA, Levine MM. Age-specific prevalence of serum antibodies to the invasion plasmid and lipopolysaccharide antigens of Shigella species in Chilean and North American populations. J Infect Dis. 1992; 166:158–161. [PubMed: 1607690]
91. Oberhelman RA, et al. Prospective study of systemic and mucosal immune responses in dysenteric patients to specific Shigella invasion plasmid antigens and lipopolysaccharides. Infect Immun. 1991; 59:2341–2350. [PubMed: 2050402]
92. Kotloff KL, et al. Safety, immunogenicity, and transmissibility in humans of CVD 1203, a live oral Shigella flexneri 2a vaccine candidate attenuated by deletions in aroA and virG. Infect Immun. 1996; 64:4542–4548. [PubMed: 8890204]
93. Kotloff KL, et al. Evaluation of the safety, immunogenicity, and efficacy in healthy adults of four doses of live oral hybrid Escherichia coli-Shigella flexneri 2a vaccine strain EcSf2a-2. Vaccine. 1995; 13:495–502. [PubMed: 7639017]
94. Hayani KC, et al. Concentration of milk secretory immunoglobulin A against Shigella virulence plasmid-associated antigens as a predictor of symptom status in Shigella-infected breast-fed infants. J Pediatr. 1992; 121:852–856. [PubMed: 1447644]
95. Cleary TG, Winsor DK, Reich D, Ruiz-Palacios G, Calva JJ. Human milk immunoglobulin A antibodies to Shigella virulence determinants. Infect Immun. 1989; 57:1675–1679. [PubMed: 2470676]
96. Cohen D, et al. Double-blind vaccine-controlled randomised efficacy trial of an investigational Shigella sonnei conjugate vaccine in young adults. Lancet. 1997; 349:155–159. [PubMed: 9111538]
97. Robbins JB, Chu C, Schneerson R. Hypothesis for vaccine development: protective immunity to enteric diseases caused by nontyphoidal salmonellae and shigellae may be conferred by serum IgG antibodies to the O-specific polysaccharide of their lipopolysaccharides. Clin Infect Dis. 1992; 15:346–361. [PubMed: 1381621]
98. Raqib R, et al. Persistence of local cytokine production in shigellosis in acute and convalescent stages. Infect Immun. 1995; 63:289–296. [PubMed: 7806368]
99. Raqib R, et al. Delayed and reduced adaptive humoral immune responses in children with shigellosis compared with in adults. Scand J Immunol. 2002; 55:414–423. [PubMed: 11967124]
100. Islam D, Christensson B. Disease-dependent changes in T-cell populations in patients with shigellosis. APMIS. 2000; 108:251–260. [PubMed: 10843411]
101. Islam D, Bardhan PK, Lindberg AA, Christensson B. Shigella infection induces cellular activation of T and B cells and distinct species-related changes in peripheral blood lymphocyte subsets during the course of the disease. Infect Immun. 1995; 63:2941–2949. [PubMed: 7622216]
102. Islam D, Wretlind B, Lindberg AA, Christensson B. Changes in the peripheral blood T-Cell receptor V beta repertoire in vivo and in vitro during shigellosis. Infect Immun. 1996; 64:1391–1399. [PubMed: 8606106]
103. Sanz I, Wei C, Lee FE, Anolik J. Phenotypic and functional heterogeneity of human memory B cells. Semin Immunol. 2008; 20:67–82. [PubMed: 18258454]
104. Simon JK, et al. Antigen-specific B memory cell responses to lipopolysaccharide (LPS) and invasion plasmid antigen (Ipa) B elicited in volunteers vaccinated with live-attenuated Shigella flexneri 2a vaccine candidates. Vaccine. 2009; 27:565–572. [PubMed: 19022324]
105. Simon JK, et al. Antigen-specific IgA B memory cell responses to Shigella antigens elicited in volunteers immunized with live attenuated Shigella flexneri 2a oral vaccine candidates. Clin Immunol. 2011; 139:185–192. [PubMed: 21388888]

106. Traggiai E, Puzone R, Lanzavecchia A. Antigen dependent and independent mechanisms that sustain serum antibody levels. Vaccine. 2003; 21 (Suppl 2):S35–S37. [PubMed: 12763680]

107. Tyrer PC, Ruth FA, Kyd JM, Otczyk DC, Cripps AW. Receptor mediated targeting of M-cells. Vaccine. 2007; 25:3204–3209. [PubMed: 17276559]

108. Ng SC, Kamm MA, Stagg AJ, Knight SC. Intestinal dendritic cells: their role in bacterial recognition, lymphocyte homing, and intestinal inflammation. Inflamm Bowel Dis. 2010; 16:1787–1807. [PubMed: 20222140]

109. Joffre OP, Segura E, Savina A, Amigorena S. Cross-presentation by dendritic cells. Nat Rev Immunol. 2012; 12:557–569. [PubMed: 22790179]

110. Noriega FR, Liao FM, Formal SB, Fasano A, Levine MM. Prevalence of Shigella enterotoxin 1 among Shigella clinical isolates of diverse serotypes. J Infect Dis. 1995; 172:1408–1410. [PubMed: 7594690]

111. Benjelloun-Touimi Z, Sansonetti PJ, Parsot C. SepA, the major extracellular protein of Shigella flexneri: autonomous secretion and involvement in tissue invasion. Mol Microbiol. 1995; 17:123–135. [PubMed: 7476198]

112. Wu T, Grassel C, Levine MM, Barry EM. Live attenuated Shigella dysenteriae type 1 vaccine strains overexpressing shiga toxin B subunit. Infect Immun. 2011; 79:4912–4922. [PubMed: 21969003]

113. Kotloff KL, et al. Phase I evaluation of delta virG Shigella sonnei live, attenuated, oral vaccine strain WRSS1 in healthy adults. Infect Immun. 2002; 70:2016–2021. [PubMed: 11895966]

114. Barnoy S, et al. Characterization of WRSs2 and WRSs3, new second-generation virG(icsA)-based Shigella sonnei vaccine candidates with the potential for reduced reactogenicity. Vaccine. 2010; 28:1642–1654. [PubMed: 19932216]

115. Collins TA, et al. Safety and colonization of two novel VirG(IcsA)-based live Shigella sonnei vaccine strains in rhesus macaques (Macaca mulatta). Comp Med. 2008; 58:88–94. [PubMed: 19793462]

116. Ranallo RT, Thakkar S, Chen Q, Venkatesan MM. Immunogenicity and characterization of WRSF2G11: a second generation live attenuated Shigella flexneri 2a vaccine strain. Vaccine. 2007; 25:2269–2278. [PubMed: 17229494]

117. McKenzie R, et al. Safety and immunogenicity of WRSd1, a live attenuated Shigella dysenteriae type 1 vaccine candidate. Vaccine. 2008; 26:3291–3296. [PubMed: 18468742]

118. Venkatesan MM, et al. Construction, characterization, and animal testing of WRSd1, a Shigella dysenteriae 1 vaccine. Infect Immun. 2002; 70:2950–2958. [PubMed: 12010984]

119. Cohen D, et al. Clinical trials of Shigella vaccines in Israel. Adv Exp Med Biol. 1996; 397:159–167. [PubMed: 8718595]

120. Chu CY, et al. Preparation, characterization, and immunogenicity of conjugates composed of the O-specific polysaccharide of Shigella dysenteriae type 1 (Shiga's bacillus) bound to tetanus toxoid. Infect Immun. 1991; 59:4450–4458. [PubMed: 1937803]

121. Theillet FX, et al. Effects of backbone substitutions on the conformational behavior of Shigella flexneri O-antigens: implications for vaccine strategy. Glycobiology. 2011; 21:109–121. [PubMed: 21030536]

Review criteria

We searched for articles focusing on original research on *Shigella* vaccine development. A PubMed search was performed using the search terms "*Shigella*" and "vaccines". All papers identified were English-language full-text papers. We also reviewed the reference lists of identified articles for further relevant papers.

Key Points

- *Shigella* continues to be a leading cause of morbidity and mortality exerting the greatest burden in children in less industrialized countries.
- Recent epidemiological studies (GEMS) confirmed the distribution of multiple serotypes in geographical regions as important causes of infection.
- Studies on pathogenesis have revealed new virulence factors which may serve as targets for attenuation in live vaccine strains or as potential vaccine antigens.
- Vaccine strategies may be divided into serotype-targeted or conserved protein antigen approaches and there are multiple candidates in various stages of development and evaluation.
- New immunological measurements are shedding light on important protective responses.

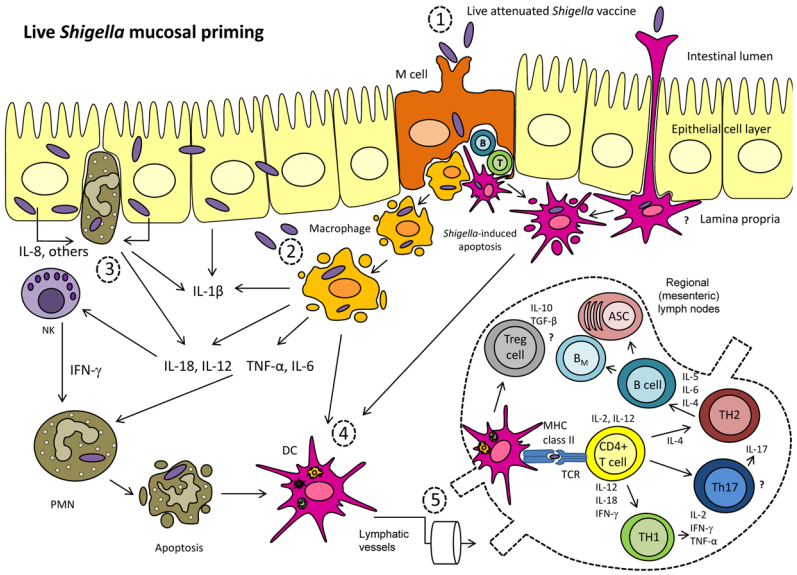

Figure 1.

Figure 1A. Live *Shigella* mucosal priming. (1) From the intestinal lumen, *Shigella* crosses the intestinal epithelial barrier through the M cells, possibly by a receptor-mediated uptake [107], and it is endocytosed by macrophages and dendritic cells in the subepithelial region of the M cell pocket. Conceivably, the organism could also be sampled from the lumen by DC residing between epithelial cells through their extended dendrites[108]. **(2)** The bacteria escape the phagocytic vacuole and induce apoptosis of the infected cells. As a result, live organisms are released and invade epithelial cells from the basolateral side, spreading from cell to cell throughout the mucosal epithelial layer. **(3)** Infected epithelial cells in turn secrete IL-8 and other chemotactic factors that will recruit polymorphonuclear (PMN) lymphocytes. Activated macrophages and PMN secrete a cascade of pro-inflammatory molecules (e.g., IL-18, IL-1β, TNF-α, IL-6, IFN-γ) further attracting phagocytic cells, which would ultimately kill and clear the organism. This initial inflammatory response sets the stage for adaptive immunological priming. **(4)** Apoptotic infected macrophages, neutrophils and other antigenic material released from infected cells may be taken up by DC, allowing for presentation and cross-presentation of bacterial antigens to T cells[109]. These antigen-loaded DC are transported to adjacent interfollicular T cell zones of mucosal lymphoid follicles or regional lymph nodes where they stimulate naïve T cells. Stimulated T cells in turn proliferate vigorously and differentiate into effector and memory T cells. This process leads to the activation of $CD4^+$ Th2 cells, which support the production of antibodies, and Th1 cells, that will facilitate and expand inflammatory responses. Being an intracellular pathogen, *Shigella* is also expected to activate cytotoxic $CD8^+$ T cells (CTL) that could eventually kill infected cells (e.g., intraepithelial -IEL- CTL) and secrete IFN-γ and other cytokines to further enhance Th1 CMI. B and T cells primed by mucosal pathogens acquire homing receptor molecules that will allow them to migrate to mucosal effector sites.

Figure 1B. Immunological effector mechanisms. A. Innate immunity. *Shigella* infection triggers an inflammatory response in the intestinal epithelium with recruitment of PMN, macrophages and NK cells, which will capture and kill the organism. These activated phagocytic cells release pro-inflammatory cytokines, which in turn contribute to the recruitment of B and T cells. **B. Adaptive immunity. (1)** IgA produced by mucosal ASC are secreted through the epithelial cells. IgG, produced by local IgG ASC or present in circulation can diffuse into the intestinal lumen or be actively transported through the FcRn

receptor. Both antibodies could block the organisms abrogating cell attachment. **(2)** Antibodies could also block the bacteria that have breached the intestinal epithelial barrier preventing further cell invasion. IgG can mediate bacterial killing through opsonophagocytosis or lysis in the presence of complement. **(3)** Th1 cells could limit bacterial dissemination through induction of IEL with cytotoxic capacity. **(4)** Th2 cells provide support for production of antibodies and contribute to B cell differentiation and induction of B_M cells. B memory cells can reactivate and mount a quick anamnestic response upon antigen exposure. Although the presence of antibodies against the O-polysaccharide and *Shigella* Ipas appear to be critical for protection, the contribution of other immunological effectors is likely necessary to clear an infection.
Abbreviations: PMN: polymorphonuclear lymphocytes; DC: dendritic cells; CTL: cytotoxic T cells (CTL); B_M: B memory cells; FcRn: Fc-γ (IgG) neonatal intestinal receptor; IEL: intraepithelial lymphocytes; NK: natural killer. Black arrows indicate soluble protein mediators (e.g cytokines); blue arrows indicate changes in cell phenotype; grey arrows depict cytokines and molecular mediators that drive cell proliferation and differentiation in the lymph nodes.

Table 1

Factors contributing to diarrhea in *Shigella*

	Gene(s)	Location	Function(s)	Reference
Enterotoxins				
ShET1	*set1A*, *set1B*	chromosome *she* PAI *S. flexneri* 2a and EAEC	Enterotoxin (Ussing chamber) Rabbit ileal loops	26, 27, 110
ShET2	*sen*, *ospD3*	virulence plasmid *Shigella*, EIEC	Enterotoxin (Ussing chamber) TTSS effector	47
SPATES				
SigA	*sigA*	Chromosome, *she* PAI *Shigella*, EIEC	Enterotoxin (rabblit ileal loop) Fodrin degradation	34, 40
Pic	*pic*	chromosome, *she* PAI EAEC042, UPEC, *S. flexneri* 2a	Mucinase, enterotoxicity, immune modulation	38–40
SepA	*sepA*	virulence plasmid *Shigella*	Rabbit ileal loop inflammation enterotoxicity	36, 111

Table 2

Shigella Vaccine Candidates

O-Antigen Directed			
Live Attenuated	**Gene Mutations**	**Development Stage**	**Reference**
S. flexneri 2a CVD 1204	*guaBA*	Phase 1	29
S. flexneri 2a CVD 1208S	*guaBA, set, sen*	Phase 2	30
S. dysenteriae 1 CVD 1256	*guaBA, sen, stxA*	Preclinical	112
S. sonnei WRSs1	*virG*	Phase 1	113
S. sonnei WRSs2, 3	*virG, senA, senB, msbB2*	Preclinical NHP	32, 67, 114, 115
S. flexneri 2a SC602	*virG, iuc*	Phase 1–2	63, 69, 70
S. flexneri 2a WRSf2G11, 12, 15	*virG, senA, senB, msbB2*	Preclinical	31, 68, 116
S. dysenteriae 1WRSd1	*virG, stxAB*	Phase 1	117, 118
Conjugate			
ChemicalConjugate	*S. flexneri 2a* LPS-rEPA	Phase 1–3	55, 56, 119
	S. sonnei LPS-rEPA	Phase 1–3	55, 56, 119
	S. dysenteriae 1 LPS-rEPA	Preclinical	120
GlycoVaxyn Bioconjugate	*S. dysenteriae* 1 LPS-exoA	Phase 1	59
Synthetic Carbohydrate			
Synthetic Oligosaccharide	O-antigen mimic-tetanus toxoid	Preclinical	60, 121
Common Protein Directed			
Purified Ipa proteins	*S. flexneri* 2a IpaB + IpaD	Preclinical	82
GMMA vesicles	OM and periplasmic proteins	Preclinical	87
Conserved Proteins IcsP, SigA	Protein fragments	Preclinical	
Combined O-Antigen Specific Plus Common Protein			
Invaplex	LPS + Ipa proteins B, C, and D	Phase 1	80, 81