

Clinical trials of *Shigella* vaccines: two steps forward and one step back on a long, hard road

Myron M. Levine, Karen L. Kotloff, Eileen M. Barry, Marcela F. Pasetti and Marcelo B. Sztein

Abstract | More than 50 years of research has yielded numerous *Shigella* vaccine candidates that have exemplified both the promise of vaccine-induced prevention of shigellosis and the impediments to developing a safe and effective vaccine for widespread use, a goal that has yet to be attained. This Review discusses the most advanced strategies for *Shigella* vaccine development, the immune responses that are elicited following disease or vaccination, the factors that have accelerated or impeded *Shigella* vaccine development and our ideas for the way forward.

At the end of the 19th century, as epidemics of bacillary dysentery accompanied by high mortality spread across Japan, the young microbiologist Kiyoshi Shiga examined dysenteric stools and isolated a bacterium that was agglutinated by serum from convalescent patients but not from patients with acute disease^{1–3} (FIG. 1). That bacterium — known today as *Shigella dysenteriae* 1 — was the first identified member of the genus *Shigella*. Four *Shigella* species (or groups) are now recognized: *S. dysenteriae* (group A), which has 15 serotypes; *Shigella flexneri* (group B), which has 14 classical serotypes and subserotypes; *Shigella boydii* (group C), which has 20 serotypes; and *Shigella sonnei* (group D), which has a single serotype⁴ (TABLE 1).

There has been resurgent interest in *Shigella* as a human pathogen, driven by the availability of more precise data on the disease burden^{5–8}, emerging antibiotic resistance^{9,10} and the fact that mucosally invasive *Shigella*, which often cause dysentery (gross blood in diarrhoeal stools), are less amenable to the salutary effects of oral rehydration than non-invasive pathogens that cause watery diarrhoea, such as *Vibrio cholerae* and enterotoxigenic *Escherichia coli*. The target populations for the use of *Shigella* vaccines include infants and young children in developing countries (in whom the peak incidence occurs at 12–47 months of age and the *S. flexneri* serotypes predominate)^{5–7}.

S. dysenteriae 1, which produces Shiga toxin and typically carries R factors that encode resistance to multiple antibiotics, waxes and wanes as a cause of epidemic severe disease in the world's least developed countries^{11–14}.

Pandemics of Shiga dysentery, as occurred in Central America from ~1968–1972 (REF. 11), South Asia in the 1970s¹², Central Africa in the 1980s¹³ and East Africa in the 1990s^{14,15} (FIG. 1), profoundly influence the global mortality burden that can be attributed to *Shigella*^{5,15}. The lack of *S. dysenteriae* 1 endemicity results in low background immunity in populations, so epidemics of Shiga dysentery affect adults and children alike and the target ages for the use of a Shiga vaccine would be similarly broad^{11,11–13}.

S. sonnei persists in developed (and transitional) countries, causing sporadic diarrhoea and occasional outbreaks in epidemiological niches (such as day-care centres) where personal hygiene can be suboptimal^{16,17}. Travellers from developed to developing regions, who mainly acquire *S. sonnei* and *S. flexneri* infections¹⁸, represent another target population for *Shigella* vaccines. Shigellosis due to *S. boydii* or *S. dysenteriae* serotypes other than type 1 is uncommon^{5–7} (although increases in *S. boydii* have been reported in some foci⁷). Consequently, a *Shigella* vaccine that can provide a high level of protection against *S. dysenteriae* 1, all *S. flexneri* serotypes and *S. sonnei* would constitute an epidemiologically valid 'global' vaccine^{5,19} (TABLE 1; BOX 1; FIG. 2).

Few bacterial pathogens have had their pathogenesis or interactions with mammalian tissues elucidated so precisely at the cellular and subcellular levels as *Shigella* spp.^{20,21} Nevertheless, progress in attaining safe and effective *Shigella* vaccines has faltered. Herein, we review recent and old clinical trials that have evaluated the safety, immunogenicity and efficacy of candidate *Shigella* vaccines. We relate the basis for the most popular

Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland 21201, USA. Correspondence to M.L. e-mail: mlevine@medicine.umaryland.edu
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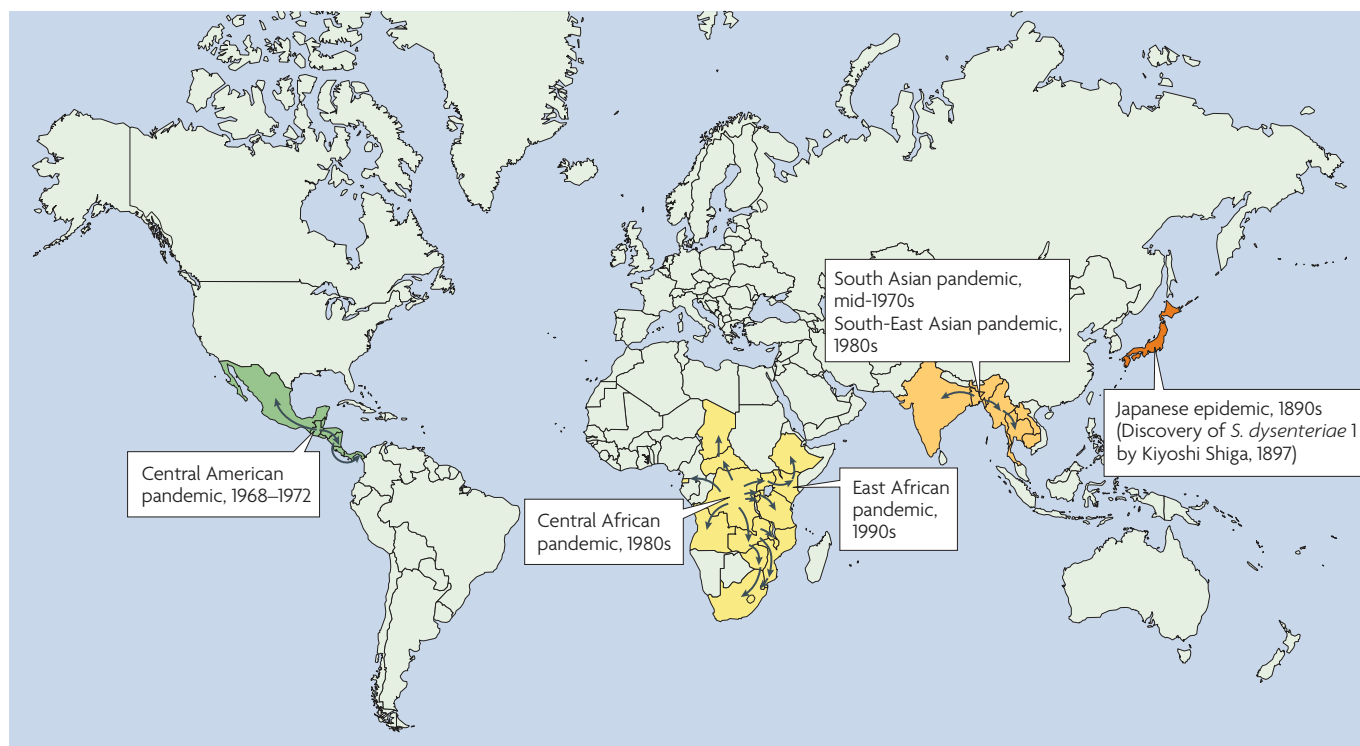


Figure 1 | Pandemics of Shiga dysentery. The first member of the genus *Shigella* to be identified, *Shigella dysenteriae* 1, was isolated by Kiyoshi Shiga during epidemics that occurred in Japan in the 1890s. Beginning in Central America in the late 1960s, pandemics of *S. dysenteriae* 1 caused severe disease with many complications and high case-fatality rates in all age groups. Subsequent pandemics occurred in Asia and Africa over the following 3 decades.

strategies (BOX 2), the relevance of the different immune responses measured, the factors that have favoured or impeded vaccine development and, most importantly, the lessons that must be learned for the future.

Shigellosis models and vaccine development

The ability of *Shigella* to cause diarrhoeal illness is restricted to human and higher non-human primate (NHP) hosts. Experimental models of shigellosis in adult human volunteers and NHPs have been invaluable for studying *Shigella* pathogenesis, infection and immunity as well as for evaluating candidate *Shigella* vaccines. Epidemiologically, shigellosis is a low-inoculum infection that is readily transmitted by direct faecal-oral contact. This has been corroborated by volunteer studies in which the ingestion of as few as ten *S. dysenteriae* 1 organisms induced a clinical infection rate of 10%²² and 200 organisms of different serotypes caused infection rates that were >25%²²⁻²⁶. In a volunteer model of experimental shigellosis that was developed in the late 1960s, the *Shigella* inoculum was administered in skimmed milk without buffer^{22,23,26,27}. However, this was accompanied by considerable variability in the infection rate, particularly at lower inocula levels. A helpful modification was to administer the inoculum with NaHCO₃ buffer²⁵, which achieved consistency in the rate of shigellosis^{25,28} (TABLE 2). Volunteers develop classical bacillary dysentery, which commences with fever, malaise, abdominal cramps and (often watery) diarrhoea (for ~18-24 hours). These symptoms are followed by scanty stools that contain blood and mucus and by tenesmus²⁵.

With early antibiotic therapy, volunteers rapidly respond clinically and microbiologically (their cultures are often negative within 24 hours)²⁵.

In contrast to the disease in humans, a large inoculum (~ 10¹⁰ colony-forming units (CFU)) administered with NaHCO₃ is required to reliably induce shigellosis in NHPs²⁹⁻³¹. So, although the NHP model is helpful for studying pathogenesis, infection-derived immunity and perhaps the efficacy of vaccines, it is not useful for predicting the clinical acceptability of putatively attenuated live vaccine candidates for humans.

The gross pathology of shigellosis in the human colon and the terminal ileum reflects the mucosal invasiveness of this pathogen. Understandably, most work on the molecular pathogenesis of *Shigella* infections has focused on the genes and gene products that are involved in invading epithelial cells and the host response to invasion^{20,21}. The early watery diarrhoeal phase of shigellosis was largely ignored until the NHP and volunteer model studies began to shed light on this stage of pathogenesis. Clinicians recognize that some patients with shigellosis manifest only watery diarrhoea, and even dysentery patients passing scanty bloody stools usually recount a day of watery diarrhoea without blood before the onset of their dysentery. When Rout *et al.*³² fed virulent *S. flexneri* 2a to NHPs, some NHPs developed only watery diarrhoea, whereas others developed only dysentery and some manifested both. *In vivo* perfusion studies revealed that colonic net transport of water occurred in all clinically ill NHPs, whereas only NHPs with watery

diarrhoea exhibited net secretion of water, sodium and chloride ions in the jejunum, indicating the effect of an enterotoxin³². Histological examination demonstrated no jejunal abnormalities, corroborating the hypothesis that an enterotoxin (or toxins) was probably responsible for the watery diarrhoea. Kinsey *et al.*³³ proved that the initiation of watery diarrhoea required that *S. flexneri* 2a be present in the jejunum. When they bypassed the jejunum and inoculated NHPs directly in the cecum, the animals developed dysentery but not watery diarrhoea, and no jejunal secretion was observed³³. The early watery diarrhoea phase, which can clearly be discerned in the volunteer model of shigellosis, prompted Fasano *et al.* to look for enterotoxins that could elicit small intestinal secretion in the absence of invasion^{34–37}. Their efforts led to the discovery and characterization of *Shigella* enterotoxin 1 (ShET1; encoded by the chromosomal gene *set*) and ShET2 (encoded by the plasmid-borne gene *sen*)^{34–37}. ShET1, a classical enterotoxin formed by a single A subunit that combines with five B subunits, is encoded on a chromosomal pathogenicity island³⁸ and is restricted to isolates of *S. flexneri* 2a^{37–41}. By contrast, ShET2, which is encoded by a gene on an invasiveness plasmid, is expressed by all *Shigella* serotypes^{36,39–41}. The identification of these enterotoxins has had enormous repercussions for the development of well-tolerated live oral *Shigella* vaccine strains (TABLE 3).

Immunity following wild-type infection

Convincing evidence that an initial clinical *Shigella* infection elicits serotype-homologous protection comes from three sources: NHP challenge studies³¹, volunteer model re-challenge studies^{24,25,42} and prospective epidemiological surveillance of a cohort of children in an endemic area⁶. Formal *et al.* reported that an initial *S. flexneri* 2a diarrhoeal infection in NHPs conferred 100% protection against re-challenge with *S. flexneri* 2a, but offered no protection against challenge with *S. sonnei*³¹. For 30 months, Ferreccio *et al.* prospectively followed a cohort of children <4 years of age in a poor community in Santiago, Chile, where shigellosis was endemic. They found that 3 serotypes, *S. sonnei*, *S. flexneri* 2a and *S. flexneri* 6, caused 79% of the cases of shigellosis⁶. As these serotypes do not share O-antigen determinants (BOX 1), this provided a unique opportunity to investigate the immunity that follows natural clinical

exposure to each of these serotypes. By comparing the incidence of subsequent episodes of shigellosis caused by a homologous serotype against the overall incidence of subsequent episodes, it was calculated that an initial bout of shigellosis afforded 72% protection against a second clinical illness due to the homologous serotype ($p=0.05$)⁶. Protection against a second clinical illness due to a heterologous serotype was low (<30%; $p>0.25$). Collectively, these data suggest that antibodies to *Shigella* O-antigen have a key role in protection (FIG. 3).

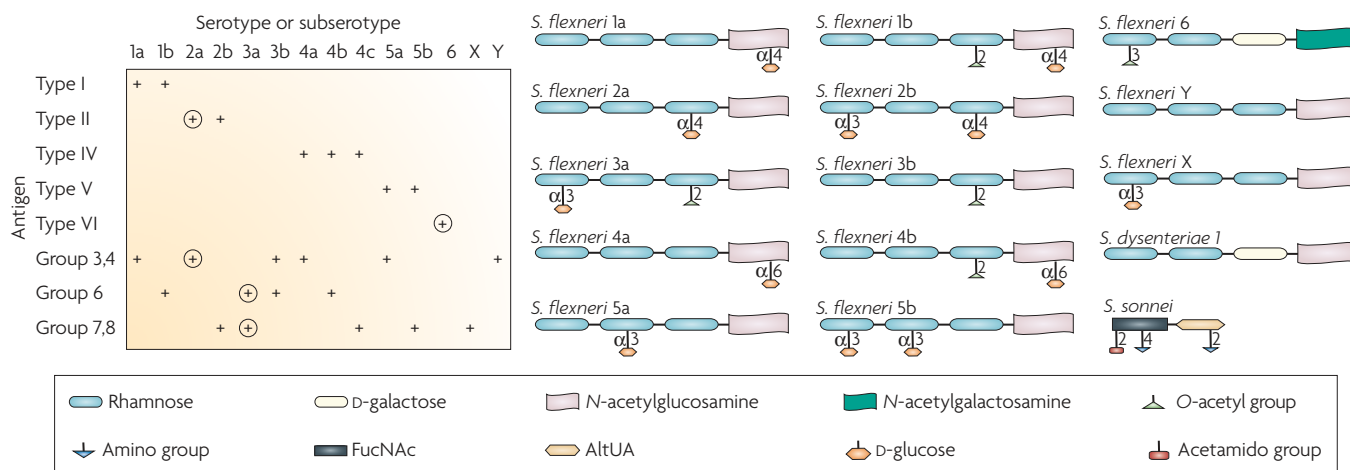
The debate over whether protection is predominantly mediated by mucosal secretory IgA (sIgA) anti-O-antigen antibodies, by serum IgG antibodies or by both (perhaps with contributions from other immune effector mechanisms) is more contentious. Strong mucosal (faecal and urinary) sIgA anti-O-antigen antibody responses are observed following wild-type infection^{25,28,43–47} and experimental challenge^{25,28}. Moreover, priming of the mucosal immune system following an initial wild-type *Shigella* infection has also been documented by enumerating gut-derived IgA anti-O-antigen antibody-secreting cells (ASCs)^{25,28,46,48} (FIG. 3). On the one hand, although IgA anti-O-antigen ASCs and serum anti-O-antigen IgG have generally been considered key elements for protection, some studies have failed to show an association between these responses and resistance to illness following challenge^{49,50}. In volunteer re-challenge studies, the substantial clinical protection exhibited by veterans^{24,25,42} is accompanied by an ~1 log reduction in the level of excretion of *Shigella*²⁵. In one endemic situation, initial clinical infection by *S. flexneri* 2a was followed by repetitive asymptomatic infections by that serotype and ultimately by sterile intestinal immunity such that even asymptomatic carriage was no longer detected⁵¹. On the other hand, the importance of mucosal antibodies in preventing shigellosis was demonstrated by Tacket *et al.* who reported that passive oral administration of cow's milk immunoglobulin that contained high titres of anti-*S. flexneri* 2a antibodies protected volunteers from experimental challenge with wild-type *S. flexneri* 2a strain 2457T⁵². Notably, cow's milk immunoglobulin that contained low titres of anti-*S. flexneri* 2a antibodies did not provide protection⁵². Additional research is needed to define the roles of intestinal sIgA and serum IgG antibodies in mediating protection against *Shigella*.

Table 1 | *Shigella* species and serotypes

<i>Shigella</i> species (group)	Number of serotypes and subserotypes	Most important epidemiological niche	Serotypes that a global vaccine must protect against
<i>S. dysenteriae</i> (group A)	15	Epidemics and pandemics of severe Shiga (<i>S. dysenteriae</i> 1) dysentery	<i>S. dysenteriae</i> 1
<i>S. flexneri</i> (group B)	14*	Endemic paediatric shigellosis in developing countries	All 14 types
<i>S. boydii</i> (group C)	20	Scattered endemic foci in developing countries	None
<i>S. sonnei</i> (group D)	1	Travellers' diarrhoea; endemic shigellosis and outbreaks in developed and transitional countries	<i>S. sonnei</i>

*There are 14 classical *Shigella flexneri* serotypes and subserotypes⁴.

Box 1 | **Shigella O-antigens**



The lipopolysaccharide (LPS) of *Shigella*, a virulence factor^{119,120}, consists of a toxic lipid A moiety embedded in the bacterial outer membrane, a core sugar region and an exposed terminal O polysaccharide. Whereas there is little diversity in lipid A and core regions among *Shigella*, the terminal O polysaccharide structures vary greatly, thereby giving rise to the immunological specificity that results in distinct serotypes (and subserotypes)⁴. Serotyping of *Shigella flexneri* is complicated. Current taxonomy recognizes 14 classical *S. flexneri* serotypes or subserotypes⁴, defined by type (I, II, IV–VI) and group (3, 4; 6; 7, 8) antigens (see left panel of the figure). Notably, the group antigens are shared among different serotypes. Type III antigen is not shown in the figure, as no structure can be assigned to it¹²¹. Moreover, absorbed rabbit ‘type III’ antisera actually bind group 6 antigen, indicating that type III antigen is identical to group 6 antigen, which is present in other serotypes¹²¹. Nevertheless, re-classification is unnecessary as strains heretofore characterized as *S. flexneri* 3a and 3b can be readily identified with absorbed or monoclonal typing sera^{4,121,122}; *S. flexneri* 3a agglutinate with antisera to group 6 and 7,8 antigens and *S. flexneri* 3b agglutinate with antisera to group 6 and 3,4 antigens (left panel)¹²¹.

Thirteen of the 14 classical *S. flexneri* serotypes or subserotypes (all except *S. flexneri* 6) share a common backbone of tetrasaccharide repeats that contain three rhamnose residues and one N-acetylglucosamine (right panel). Genes that encode proteins involved in the synthesis of the linear backbone are located in the *rfb* locus of the chromosome, whereas lysogenic phages encode the enzymes that link O-acetyl groups or D-glucose at various

sites on the tetrasaccharide backbone, thereby creating or ablating antigenic epitopes. The resultant O-antigens, the structures of which are displayed schematically in the right panel of the figure, provide the basis for the distinct *S. flexneri* serotypes or subserotypes. *S. flexneri* 6 has D-galactose as the third sugar of the tetrasaccharide and N-acetylgalactosamine as the terminal residue (right panel). The structure of *S. flexneri* 4c is not shown as it has not been reported.

As *Shigella dysenteriae* 1 and *Shigella sonnei* are two serotypes that should be included in a global *Shigella* vaccine, the basic structures of their O polysaccharide repeats are also shown^{103,123}. In contrast to the other O-antigens that have tetrasaccharide repeats, the O-antigen repeat of *S. sonnei* is a disaccharide (FucNAc, 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose; AltUA, 2-amino-2-deoxy-L-altruronic acid)¹⁰³. The *S. dysenteriae* 1 O polysaccharide repeat most closely resembles the structures of *S. flexneri* 6 and *S. flexneri* Y of the *S. flexneri* subtypes.

The left panel displays the 14 *S. flexneri* serotypes and subserotypes along the horizontal axis and the type and group antigens in the vertical axis. Below each serotype or subserotype, the specific type and group antigens expressed are indicated by a plus sign. As the O-antigens (circled) of *S. flexneri* 2a and *S. flexneri* 3a crossreact with 10 other *S. flexneri* serotypes or subserotypes, *S. flexneri* 2a, *S. flexneri* 3a and *S. flexneri* 6 have been proposed as three components of a pentavalent vaccine (along with *S. dysenteriae* 1 and *S. sonnei*) that could confer broad protection. Cross-protection among *S. flexneri* subserotypes has been demonstrated in a guinea pig model¹¹⁸. A high priority is to test this strategy in humans.

Other immune effector mechanisms have also been described in shigellosis (FIG. 3). Increases in serum anti-Ipa (invasion plasmid antigen) antibodies have been demonstrated after natural disease and experimental challenge^{25,43,53}, and in endemic areas the seroprevalence of anti-Ipa antibodies increases with age^{43,54}. A few studies have also measured cell-mediated immunity (CMI) elicited by wild-type *Shigella* infection. Rectal biopsies from Bangladeshis during acute and convalescent *S. flexneri* and *S. dysenteriae* 1 infection revealed an increased frequency of cytokine-producing cells and mononuclear cell infiltration in the rectal mucosa⁵⁵. Increased levels of interferon- γ (IFN- γ) in plasma and stool, upregulation of IFN- γ receptor expression in patients with shigellosis and increased IFN- γ production during convalescence suggest that this cytokine might play a role in both

pathogenesis and protection^{55–59}. Upon stimulation with *Shigella* antigens (including Ipa), the production of IFN- γ and interleukin-10 (IL-10), but not IL-4, IL-5, IL-12 or IL-15, was demonstrated in peripheral blood mononuclear cells (PBMC) from volunteers challenged with modified virulent *S. dysenteriae* 1 strain SC595, indicating a predominant T helper 1 (T_H1)-type response⁶⁰. Also, increased proportions of memory T cells (CD45RO⁺), the expression of T-cell activation molecules (for example, CD25, CD38, HLA-DR and CD54) and the expansion of defined T-cell receptor V β families have been reported in patients with shigellosis^{61,62}. Despite these findings, the contribution to protection from *Shigella* that can be attributed to CMI or to antibodies directed at *Shigella* proteins (such as Ipa and VirG) rather than O polysaccharide antigens remains controversial (FIG. 3).

T helper 1
(T_H1 cell). A type of activated T_H cell that promotes responses associated with the production of a particular set of cytokines, including interleukin-2 and interferon- γ , the main function of which is to stimulate phagocytosis-mediated defences against intracellular pathogens.

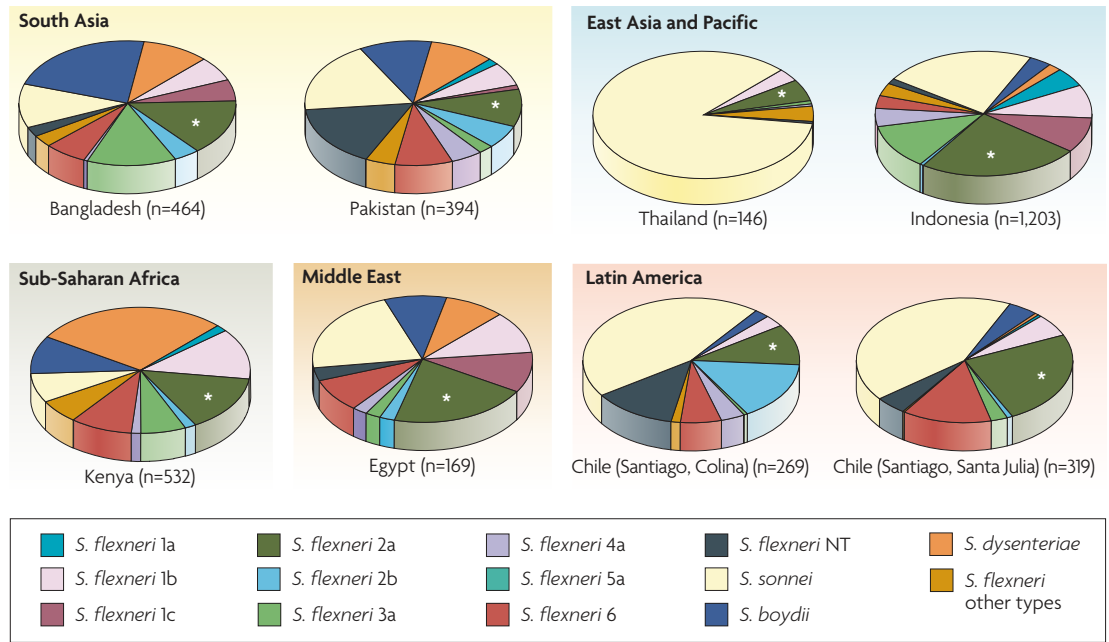


Figure 2 | The distribution of *Shigella* species and *Shigella flexneri* serotypes from selected studies in Latin America, Asia and Africa. Several population-based surveillance studies lasting at least 2 years used extensive serotyping methods (including either commercial absorbed animal sera or monoclonal antibodies for *Shigella flexneri* typing)^{6–9,122,129,130}. In all countries, *S. flexneri* 2a (marked with an asterisk) is the single most important *S. flexneri* serotype. The most developed of the countries, Chile and Thailand, show a notable proportion of cases due to *Shigella sonnei*. In any individual country, 3–4 serotypes constitute ~75% of all cases. However, the other important *S. flexneri* serotypes, in addition to *S. flexneri* 2a, differ from country to country. Serotype 1c described in some studies is a provisional serotype. If the Center for Vaccine Development (CVD) pentavalent vaccine strategy that incorporates *S. flexneri* 2a, *S. flexneri* 3a and *S. flexneri* 6 (along with *Shigella dysenteriae* 1 and *S. sonnei*) succeeds in conferring cross-protection against the other 11 classical *S. flexneri* serotypes in future field trials, it should be possible to achieve a broad-spectrum global vaccine against shigellosis. *S. dysenteriae* type 1 were identified only in Kenya, where they comprised 67% of *S. dysenteriae* isolates. NT, non-typable.

Protective prototype vaccines

Two prototype *Shigella* vaccines, one which uses attenuated strains as live oral vaccines and the other which uses parenteral conjugates of *Shigella* O polysaccharide covalently linked to a carrier protein, have conferred significant protection in controlled Phase III trials. As these vaccines are furthest along in development, and as the results of the multiple clinical trials that have been carried out collectively provide insights, they will be discussed in depth. Other publications address promising alternative strategies (BOX 2) to develop *Shigella* vaccines⁶³.

In the 1960s, Mel of the Military Medical Institute, Belgrade, Yugoslavia (Serbia), serially passaged different *Shigella* serotypes on streptomycin-containing media until they became streptomycin-resistant and streptomycin-dependent (SmD; that is, they could not grow in the absence of exogenous streptomycin)^{64,65}. These SmD strains lost their ability to cause purulent keratoconjunctivitis in guinea pigs, a reliable test for *Shigella* mucosal invasiveness, intercellular spread and propensity to cause inflammation⁶⁶. At the time, the genetic changes responsible for the attenuation were unknown. In addition to testing the vaccines in adults and children in Yugoslavia^{67–70}, SmD *Shigella* vaccines were tested in adults and children in institutions in the United States where the risk of acquiring shigellosis was high^{42,51,71–73}.

These vaccine strains proved to be well tolerated clinically when administered to adults, healthy children and even debilitated institutionalized children^{42,51,71–73}, with the main adverse reaction being vomiting in a small percentage of recipients following administration of the first dose^{51,69–74}. The vaccines were typically administered in an escalating (2×10¹⁰, 3×10¹⁰, 4×10¹⁰ and 5×10¹⁰ CFU) 4-dose regimen over 11 days. In controlled field trials, Mel *et al.* demonstrated the efficacy of the SmD vaccines^{69,70}, showed that multiple strains could be mixed together in combination vaccines and reported that protection was serotype-specific^{68–70}. Protection endured for 1 year following primary immunization of children; however, administration of a single booster extended protection for an additional year⁷⁰. These pioneering studies, which involved ~36,000 adult and paediatric subjects, provided proof of concept for future modern multivalent vaccines that aim to confer broad-spectrum protection. Unfortunately, the SmD vaccine strains had drawbacks that precluded them from becoming licensed for widespread use. In addition to the fact that the basis for the attenuation was unknown, occasional lots reverted to streptomycin independence (although the organisms remained unable to invade epithelium) and difficulties were encountered in large-scale manufacture and process control^{73,75}.

Parenteral

A vaccine administered by injection into the muscle, subcutaneous tissue or dermis (as opposed to mucosal immunization via an oral or nasal route).

Phase III trial

Refers to a large-scale field trial of rigorous epidemiological design to determine the efficacy of a vaccine. When feasible, the preferred design involves a randomized, controlled, adequately powered, double-blind study in which allocation of vaccine or control regimen takes place at the level of the individual subject. Phase III trials, which are usually designated as pivotal studies for licensure, use a final formulation of the vaccine.

Box 2 | **Shigella vaccine strategies**

Strategies for which there is evidence from field trials that demonstrates efficacy:

- Attenuated strains of *Shigella* used as live oral vaccines.
- O polysaccharides of *Shigella* covalently linked to carrier proteins and used as parenteral conjugate vaccines.

Strategies that are being (or have been) pursued but for which evidence of protection in humans has not been documented:

- Proteosomes (outer membrane vesicles of group B *Neisseria meningitidis*) to which *Shigella sonnei* or *Shigella flexneri* 2a is adsorbed¹²⁴. This vaccine has been administered to humans intranasally.
- Inactivated *S. sonnei* administered orally¹²⁵.
- *Shigella* invasion complex (Invaplex); a subcellular vaccine prepared from pathogenic *S. flexneri*, containing proteins including invasion plasmid antigen B (IpaB) and IpaC and lipopolysaccharide, administered to humans intranasally¹²⁶.
- Nuclear protein–ribosomal parenteral vaccine prepared from a *Shigella* strain that has been genetically attenuated by an *msbB* mutation that partially detoxifies the lipid A portion of the *Shigella* endotoxin^{127,128}.

S. flexneri 2a strain T₃₂ is another pioneering vaccine strain developed by Istrati in Romania, where it was shown to be well tolerated and probably protective^{76,77}. Subsequently, in randomized placebo-controlled field trials in China, Bingrui *et al.* reported that T₃₂ conferred significant protection against *S. flexneri* 2a diarrhoea⁷⁸. Surprisingly, the Chinese field trials also suggested that T₃₂ conferred significant (albeit lower level) protection against shigellosis due to *S. flexneri* 1b and *S. boydii* 1–6. Investigators at the **Walter Reed Army Institute of Research** (WRAIR) revealed that T₃₂ harboured a large deletion in the invasiveness plasmid. This deletion resulted in the loss of three loci, *ipaBCDA*, *invA* and *virG* (also known as *icsA*), which crippled the ability of this strain to invade epithelial cells⁷⁹. Further investigation showed that the T₃₂ plasmid deletion encompassed *sen* (which encodes ShET2); the chromosomal *set* gene (which encodes ShET1) remained intact³⁹.

In *S. sonnei*, the *rfb* locus, which encodes O-antigen, is located on the form I invasiveness plasmid. Investigators at the Lanzhou Institute of Biological Products introduced an *S. sonnei* form I plasmid with deletions of *ipa* and *virF* into *S. flexneri* 2a T₃₂ (REF. 80). Three doses (from 2×10¹⁰ to 5×10¹⁰ CFU) of the resultant hybrid strain expressing both *S. flexneri* 2a and *S. sonnei* O-antigens provided ~65% protection against both *S. flexneri* 2a and *S. sonnei* diarrhoea⁸⁰.

Formal *et al.* at WRAIR prepared a ‘mutant-hybrid’ *S. flexneri* 2a vaccine strain by selecting a colonial mutant that had lost its ability to invade intestinal epithelial cells⁸¹ and transferred into it the xylose–rhamnose region of *E. coli* (which diminishes the ability of *Shigella* to propagate in the lamina propria even if epithelial invasion occurs)⁸². In Phase I safety and Phase II immunogenicity clinical trials in adults and children in the United States^{71,72}, the *S. flexneri* 2a mutant-hybrid vaccine was as well tolerated as Mel’s SmD vaccines and conferred partial (albeit non-significant) protection against experimental challenge in adult volunteers⁴²; by comparison, SmD *S. flexneri* 2a conferred significant protection⁴².

Building on the pioneering work of Mel and Istrati, researchers worldwide have used recombinant DNA technology and knowledge of the molecular pathogenesis of shigellosis to create modern live oral *Shigella* vaccine candidates that can overcome the drawbacks of the early strains. Progress has been steady but painstakingly slow, as unexpected obstacles have been encountered.

The importance of the parent strain

One lesson that has been learned in developing attenuated *Shigella* vaccines is the importance of starting with a wild-type parent strain of known virulence, ideally as assessed in volunteer studies. The paradigm is the array of derivatives of wild-type *S. flexneri* 2a strain 2457T, a parent strain known to be virulent in North American volunteers and to cause diarrhoea, fever and dysentery with an infection rate that increases with escalating doses^{25,49,50} (TABLE 2). Moreover, an initial clinical episode of 2457T-induced shigellosis stimulates immunity that confers ~64–75% protection against illness upon subsequent re-challenge with this strain^{25,42}. As the baseline virulence of the parent strain is well documented, the degree of attenuation achieved by putative attenuating mutations can be quantified (TABLES 2,3).

It is difficult, however, to draw conclusions about the ability of specific mutations to cause attenuation when they are introduced into wild-type parent strains that were either not tested clinically beforehand or, if tested, were found to be only minimally pathogenic in humans. Two illustrative examples are wild-type *S. flexneri* Y strain SF1 and a Shiga-toxin-deleted derivative of wild-type *S. dysenteriae* 1 strain SC595 (which retains

Phase I trial

Initial evaluation of the safety and immunogenicity of a vaccine in small groups of healthy subjects who, in a step-wise manner, receive increasing dosage levels of the vaccine. For live vaccines, vaccine shedding is also monitored. If the Phase I study is conducted under physical containment (such as in a research isolation ward), one can preliminarily assess the propensity of the live vaccine to be transmitted by including several placebo recipients as contact controls.

Phase II trial

Expanded (usually placebo controlled) studies of the safety, clinical acceptability and immunogenicity of a vaccine in larger numbers (often tens or hundreds) of subjects who exemplify the target age and other characteristics for the use of the vaccine. Phase II trials aim to identify the optimal dosage level, formulation and immunization regimen of the vaccine to be used in the target groups, thereby paving the way for a pivotal Phase III efficacy trial.

Table 2 | **Response to wild-type *Shigella flexneri* 2a**

Challenge	Adverse clinical outcomes			IgA anti-O-antigen ASC		Refs
	Diarrhoea	Dysentery	Fever	% Responders	Geometric mean*	
Challenge no. 1	12/14 (86%)	10/14 (71%)	10/14 (71%)	100%	143	50
Challenge no. 2	11/12 (92%)	10/12 (83%)	10/12 (83%)	92%	239	25

This Table shows the consistency of the clinical and immunological response in North American adult volunteers following challenge with 10⁹ colony-forming units (CFU) of wild-type *Shigella flexneri* 2a strain 2457T administered with NaHCO₃ buffer. *Per 10⁶ PBMC. ASC, antibody-secreting cells; PBMC, peripheral blood mononuclear cells.

Table 3 | **The role of the ShET1 and ShET2 enterotoxins**

Strain	Adverse clinical outcomes						IgA anti-O-antigen ASC	
	Diarrhoea		Dysentery		Fever		% Responders	Geometric mean*
	10 ⁸	10 ⁹	10 ⁸	10 ⁹	10 ⁸	10 ⁹	10 ⁹	N/A
<i>ΔguaBA</i> (CVD 1204)	2/7 (29%)	4/7 (57%)	0/7	0/7	0/7	4/7 (57%)	100%	445
<i>ΔguaBA, Δset, Δsen</i> (CVD 1208)	0/7	0/7	0/7	0/7	0/7	1/7 (14%)	86%	62

This Table shows the consistency of the clinical and immunological response in North American adult volunteers to ingestion of 10⁸ or 10⁹ colony-forming units (CFU) of two deletion mutants of wild-type *S. flexneri* 2a strain 2457T with NaHCO₃ buffer⁸⁸. *Per 10⁶ PBMC. ASC, antibody-secreting cells; PBMC, peripheral blood mononuclear cells; ShET, *Shigella* enterotoxin.

epithelial cell invasiveness and causes keratoconjunctivitis in guinea pigs). In the case of the *S. flexneri* Y strain SF1, an *aroD* mutation was introduced, resulting in attenuated putative vaccine candidate SFL124 (REF. 83). Subsequently, however, when the wild-type *S. flexneri* Y parent strain SF1 was fed to North American volunteers, it was only minimally pathogenic⁸⁴. So, it was not possible to attribute the clinical behaviour of the vaccine strain to the introduced mutation. By contrast, when an *aroD* deletion was introduced into the well-characterized wild-type *S. flexneri* 2a strain 2457T, resulting in vaccine strain SFL1070, it was clearly demonstrated that this mutation afforded measurable attenuation⁸⁵. Similarly, *S. dysenteriae* 1 strain SC595 caused only low rates of mild diarrhoeal illness when administered to North American volunteers (except in one subject who developed dysentery)⁶⁰. SC595 did, however, elicit robust antibody and CMI responses following ingestion of as few as 300 CFU⁶⁰. Not surprisingly, SC599, a derivative of SC595 that contains deletions in *icsA* (also known as *virG*; this deletion diminishes the intracellular and intercellular spread of *Shigella*), *ent* and *fep* (which encode proteins involved in iron chelation) was well tolerated in Phase I safety trials⁶³. However, SC599 was only minimally excreted and induced only modest anti-O-antigen ASC responses and few anti-O-antigen seroconversions⁶³.

Selection of attenuating mutations

The phenotypic consequences of introducing different mutations into wild-type *S. flexneri* 2a strains have been evaluated in Phase I clinical trials. These mutations include *aroD* and *aroA* (which encode enzymes in the aromatic amino-acid biosynthesis pathway; mutation confers auxotrophy for substrates not found in sufficient concentration in mammalian tissues)^{85,86}, *virG*^{28,87}, *iuc* (which encodes aerobactin)²⁸, *guaBA* (which encodes enzymes involved in guanine-nucleotide biosynthesis)^{87,88}, *set* (which encodes ShET1) and *sen* (which encodes ShET2)^{87,88}.

The experience with strain SC602, a derivative of wild-type strain Pasteur Institute *S. flexneri* 2a 494 with mutations in *virG* and *iuc*, is instructive^{28,89}. When fed to North American volunteers, dosages $\geq 10^6$ CFU were unacceptably reactogenic, with most subjects developing diarrhoea, fever or severe constitutional symptoms, although the vaccine strain was heavily

shed and highly immunogenic²⁸. By contrast, at a dosage level of 10⁴ CFU, adverse clinical reactions were uncommon and mild, yet the induced immune response remained moderately robust²⁸. Eight weeks after vaccinees received a single 10⁴ CFU dose of SC602, 7 vaccinees (and 7 unvaccinated control volunteers) participated in an experimental challenge study and ingested 10³ CFU of virulent *S. flexneri* 2a strain 2457T²⁸. Diarrhoea was observed in 86% of the controls and in 43% of the vaccinees ($p=0.27$)²⁸. However, the 3 vaccinees that developed diarrhoea had significantly milder illness than the controls. Four of the 7 controls (57%) developed dysentery, 6 (86%) had fevers (mean temperature 39°C) and 6 (86%) manifested severe shigellosis. By contrast, none of the vaccinees had dysentery, fever or severe illness ($p=0.0027$)²⁸.

This hallmark study showed that in the experimental challenge model even a single dose of an engineered vaccine strain could confer significant protection against severe shigellosis. However, it also demonstrated the difficulty of finding a proper balance between clinical acceptability and immunogenicity in adult volunteers in developed countries. Indeed, additional studies with SC602 in 34 community volunteers showed that 10⁴ CFU caused mild diarrhoea in 5 volunteers (15%) and fever in 4 volunteers (12%)⁸⁹.

Invaluable lessons have also been learnt from clinical trials with the series of attenuated derivatives of wild-type *S. flexneri* 2a strain 2457T that was developed by the University of Maryland Center for Vaccine Development (CVD), as the results have directed iterations in vaccine design. CVD 1203 is a derivative of 2457T that harbours deletions in *aroA* and *virG*⁹⁰. In a Phase I clinical trial in 10 North American adults, CVD 1203 was well tolerated at a dose of 10⁶ CFU (a dosage level at which SC602 was extremely reactogenic)⁸⁶. However, when administered at doses of 10⁸ CFU or 10⁹ CFU⁸⁶, CVD 1203 induced unacceptable reactogenicity accompanied by strong immune responses⁸⁶. Significant increases in the concentrations of tumour-necrosis factor- α (TNF- α) were observed in serum and stool, suggesting that this cytokine might be related to the reactogenicity observed at high doses⁸⁶. Although the potent immune responses to CVD 1203 were encouraging, the reactogenicity dictated that alternative mutations should be evaluated to achieve a satisfactory degree of attenuation.

Community volunteers
Community volunteers is a term commonly used to refer to volunteers in a defined population that participate in clinical trials.

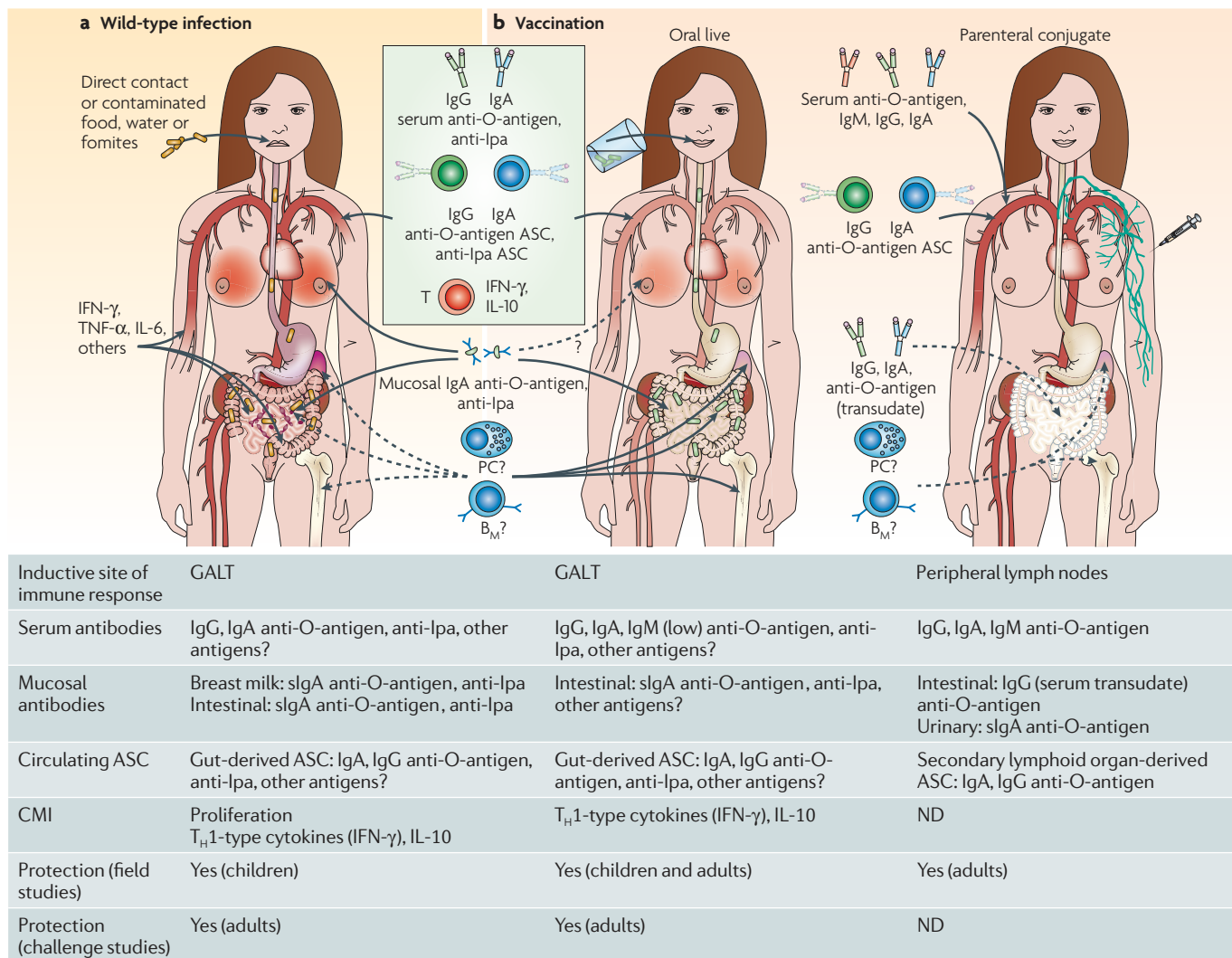


Figure 3 | Immunity to *Shigella*. **a** | Immune responses to wild-type *Shigella* infection. The specific immune responses that mediate protection against shigellosis remain controversial. The strong serum IgG and IgA antibody responses to *Shigella* O-antigen induced by wild-type infection correlate with protection data from epidemiological⁶ and seroepidemiological studies^{111,131} as well as non-human primate challenges³¹. Gut-derived IgA anti-O-antigen antibody-secreting cell (ASC) responses are also thought to have a significant role in protection. By contrast, the role of antibody responses to *Shigella* protein antigens (such as invasion plasmid antigen (lpa) proteins, VirG and others) and of cell-mediated immunity (CMI) in contributing to protection remain a matter of speculation. **b** | Immune responses to vaccines. The immune responses generated by types of *Shigella* vaccines (live oral and parenteral conjugate) that have been shown to confer protection are depicted. Responses elicited by live oral vaccines include IgA and IgG anti-O-antigen and anti-lpa ASC detected among peripheral blood mononuclear cells (PBMC), serum IgA and IgG anti-O-antigen and anti-lpa, as well as IgA anti-O-antigen in stool, intestinal fluid and urine^{25,49,50,60,86}. Interferon- γ (IFN- γ) responses to *Shigella* antigens have also been observed in volunteers immunized with *Shigella flexneri* 2a strain CVD 1207 (REF. 87), *Shigella sonnei* strain WRSS1 (REF. 94) and SFL1070 (REF. 85). The contribution of CMI and of antibodies to *Shigella* proteins (for example, lpa and VirG) elicited by live oral vaccines in preventing clinical *Shigella* infection remains uncertain. In contrast to the broad immune responses elicited by live oral vaccines, parenteral conjugate vaccines elicit a narrower response, characterized predominantly by serum IgG antibodies to *Shigella* O-antigen. Wild-type *Shigella* or live oral vaccines are likely to generate long- and short-lived plasma cells (PC) and memory B cells (B_M) that reside in the bone marrow, spleen and gut-associated lymphoid tissue (GALT). By contrast, PC and B_M elicited by conjugate vaccines are likely to be confined to the bone marrow and spleen. The relative magnitudes of immune responses are depicted by gradients of colour at the various effector sites. IL, interleukin; ND, no data; TNF- α , tumour-necrosis factor- α .

Strain CVD 1207 harbours deletion mutations in *guaBA*⁹¹, *virG*⁹⁰, *set*³⁴ and *sen*³⁶. When volunteers ingested escalating single doses of up to 10¹⁰ CFU of CVD 1207 (REF. 87), this live vaccine strain was remarkably well tolerated. No subjects manifested adverse reactions

through the 10⁸ CFU dosage level⁸⁷, and even at 10⁹ and 10¹⁰ CFU only a few volunteers experienced mild diarrhoea⁸⁷. The conclusion from the Phase I clinical trial of CVD 1207 was that the mutations definitively attenuated the 2457T parent strain, resulting in a vaccine that was

well tolerated by North American volunteers at high dosage levels yet remained immunogenic. However, it was hypothesized that strain CVD 1207 could have been hyperattenuated⁸⁷.

CVD 1204 and CVD 1208, isogenic derivatives of wild-type strain 2457T, both carry deletions in *guaBA*^{88,91}, and CVD 1208 also has deletions in *sen* and *set*. These strains differ from isogenic CVD 1207 by having an intact *virG* gene. This design permitted researchers to assess the impact of inactivating ShET1 and ShET2 (TABLE 3). A randomized, double-blind clinical trial was performed in adult North Americans who ingested a single dose containing 10⁷, 10⁸ or 10⁹ CFU of either strain⁸⁸. Objective adverse reactions were observed in 35% of CVD 1204 recipients but only in 4% of CVD 1208 recipients ($p=0.02$)⁸⁸. Immune responses to *S. flexneri* 2a O-antigen (ASCs, serum IgG or faecal IgA) were observed in 86%, 43% and 100% of CVD 1208 recipients, respectively. The enterotoxin-negative strain CVD 1208 was considered a highly attractive vaccine candidate that reflects the desired balance of clinical acceptability and robust immunogenicity (TABLE 3). As this strain was constructed using culture media that contained animal products, it was reconstructed using soy-based media for regulatory purposes, resulting in strain CVD 1208S. In a Phase I clinical trial, CVD 1208S demonstrated the same clinical acceptability, shedding pattern and immunogenicity as CVD 1208 (K.L.K., unpublished data). Thus, CVD 1208S, like CVD 1208, exhibits the desirable balance of low reactogenicity and robust immunogenicity.

Investigators at WRAIR constructed strain WRSS1 by deleting *virG* in the wild-type *S. sonnei* Moseley strain⁹². Although the virulence of this *S. sonnei* strain had not been demonstrated in volunteers, it was selected because its form I invasiveness plasmid (required for expression of O-antigen by this serotype) was remarkably stable, in contrast with the form I plasmid of most wild-type *S. sonnei* strains^{24,93}. In a Phase I trial in North American volunteers given a single dose of up to 10⁶ CFU, low-grade fever or mild diarrhoea was recorded in 6 of 27 (22%) WRSS1 vaccinees⁹⁴. Strong IgA anti-O-antigen ASC responses were observed in recipients of all dosage levels⁹⁴. Moderate IFN- γ responses were also observed in some volunteers⁹⁴.

Safety and immunogenicity trials of WRSS1 were next performed in Israel in community adults who ingested 10³, 10⁴ or 10⁵ CFU⁹⁵. At the two lower dosage levels, the vaccine was well tolerated with the exception of 1 of 30 subjects (3%) who developed moderate diarrhoea, and 5 (17%) who experienced mild diarrhoea. By contrast, at the 10⁵ CFU dosage level, 2 of 15 subjects (13%) developed fever and 4 (27%) experienced moderate diarrhoea⁹⁵. The 10⁴ CFU dosage level provided the better balance of immunogenicity and clinical acceptability, as all subjects manifested IgA anti-O-antigen ASC responses and 73% of the vaccinees showed more than 50 IgA anti-O-antigen ASCs per 10⁶ PBMC⁹⁵.

So, certain attenuating mutations introduced into wild-type strains in recent years have generated promising vaccine candidates. Some modifications, for example deletion of the genes that encode ShET1 and ShET2, might work well in combination with an array of other attenuating mutations. If commercial and intellectual property considerations do not create barriers, it would be of interest to 'mix and match' novel combinations of attenuating mutations that have not previously been used in the same strains. For example, in their latest generation of live vaccine candidates, WRAIR investigators have deleted the ShET-encoding genes in strains that carry a *virG* deletion as the primary attenuating mutation (M. Venketasan, personal communication). Similarly, it would be instructive to directly compare the clinical acceptability and immunogenicity of two or more candidate live vaccine strains of the same serotype in randomized, double-blind clinical trials. Last, it has generally been proposed that once attenuating mutations have been documented in one serotype, they can be introduced into other epidemiologically relevant serotypes. However, if the commercial considerations can be managed, another track towards licensure of a broadly protective vaccine would be to combine promising strains of several different serotypes that have been attenuated by distinct mutations.

Hybrid live vector *Shigella* vaccines

As an alternative strategy to construct live *Shigella* vaccines, several research groups have attempted to express *Shigella* O- and other (Ipa) antigens in well-tolerated live vectors such as normal flora, rough *E. coli* or highly attenuated *Salmonella enterica* serovar Typhi. The genes that allow synthesis of group- and type-specific O-antigens of *S. flexneri* 2a were introduced into an *E. coli* O8 strain, resulting in the vaccine strain PGAI 42-1-15. This hybrid *E. coli* expressing *S. flexneri* 2a O-antigen was well tolerated when given to adult US volunteers and was excreted for several days²⁷. In two separate large experimental challenge studies, volunteers given two (first study) or three (second study) doses of vaccine one month apart were challenged 4–8 weeks later with 10⁴ CFU (first study) or 10² CFU (second study) of virulent *S. flexneri* 2a strain 2457T²⁷. The rates of shigellosis were similar in vaccinees and controls²⁷. It was unclear why this live vaccine failed to protect. However, one hypothesis is that to be protective, an *E. coli* live vector strain expressing *Shigella* O-antigens must also exhibit the capacity to invade epithelial cells²⁷. Accordingly, investigators at WRAIR constructed two *E. coli* live vector strains expressing *S. flexneri* 2a O-antigen that manifested epithelial cell invasiveness.

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 strain, EcSf2a-1, that could invade epithelial cells⁴⁹. At a dose of 10⁹ CFU, this strain was unacceptably reactogenic as 4 of 13 (31%) vaccinees developed fever, diarrhoea or dysentery.

However, at lower, better-tolerated dosage levels, the vaccine failed to protect vaccinees against experimental challenge with *S. flexneri* 2a⁴⁹. In an attempt to diminish reactogenicity, an *aroD* deletion was introduced, resulting in the further derivative strain EcSf2a-2 (REF. 96). At high dosage levels, this strain, which retained the ability to invade epithelial cells, also caused adverse reactions but was strongly immunogenic. However, despite robust immunogenicity, the vaccine conferred only 36% protection against illness (fever, diarrhoea or dysentery) upon experimental challenge ($p=0.17$)⁵⁰. Overall, *E. coli*, with or without epithelial invasiveness, has been unsuccessful as a live vector vaccine expressing *Shigella* O-antigen.

In the 1980s, Formal and co-workers at WRAIR developed a vaccine candidate that consisted of licensed oral *Salmonella* Typhi live vaccine strain Ty21a engineered to express the O polysaccharide of *S. sonnei*⁹⁷. This live vector strain, 5076-1C, was well tolerated but exhibited lot-to-lot variability in its immunogenicity and ability to protect in challenge studies in which vaccinees and controls ingested wild-type *S. sonnei* strain 53G^{24,48,98}; however, two pilot lots did confer significant protection⁹³. Multiple hypotheses were pursued to explain the lot-to-lot variability in efficacy⁹⁹⁻¹⁰², but none were predictive and further development of 5076-1C was abandoned²⁴.

To overcome the inconsistency of 5076-1C, Kopecko and co-workers attempted to more reliably express *S. sonnei* O-antigen in Ty21a, as well as O-antigens from other serotypes¹⁰³. As *Salmonella* has a lipopolysaccharide (LPS) core that is chemically distinct from that of *Shigella*¹⁰³, *S. sonnei* O polysaccharide in *Salmonella* Typhi is expressed only as a non-core-linked surface-associated antigen¹⁰³. Clinical trials are required to assess if these new Ty21a-based constructs will offer more consistent protection.

Intestinal barrier in developing countries

Oral vaccines against polio, rotavirus and cholera have exhibited lower immunogenicity in underprivileged subjects in developing countries compared with subjects in developed countries¹⁰⁴⁻¹⁰⁷. The immunogenicity of some of these vaccines was successfully enhanced by increasing the number of vaccine organisms per dose or by administering additional doses of vaccine^{104,106}. Although *S. flexneri* 2a vaccine strain SC602 had been reactogenic in North American adults, as it conferred protection against severe disease in an experimental challenge, Phase I and II trials were undertaken in adults and children in a developing country. None of 68 Bangladeshi adults who ingested up to 10⁶ CFU of SC602 experienced adverse reactions, but neither did any vaccinee shed SC602, and the immune responses induced were meager⁶³. Next, Bangladeshi children of 8-10 years of age and 12-36 months of age ingested up to 10⁶ CFU⁶³. Although no child manifested an adverse reaction, neither did any child excrete the vaccine strain or mount a significant immune response. So, the developing country subjects manifested markedly different clinical, bacteriological and immunological responses

to SC602 compared with North American subjects. In adults in the United States, a low dose (10⁴ CFU) of SC602 was reactogenic, heavily excreted and moderately immunogenic, whereas in the children and adults in Bangladesh it was virtually inert, even in a 100-fold higher dose⁶³. One possible hypothesis to explain these divergent results is that Bangladeshis have decreased levels of accessible iron in tissues compared with subjects in developed countries, so that SC602 is more crippled in individuals in developing countries.

Does this mean that most live oral *Shigella* vaccines will be poorly immunogenic in developing country populations? The response of Vietnamese adults⁸³ and children¹⁰⁸ to the ingestion of *S. flexneri* Y strain SFL124 (an *aroD*-deletion mutant) and the efficacy of the SmD and T32 live oral *Shigella* vaccines in subjects in Yugoslavia (late 1960s and early 1970s)^{69,70} and China (1980s)⁷⁸, respectively, suggest not. A single 2×10⁹ CFU dose of SFL124 was administered to 15 Vietnamese adult volunteers, whereas another 15 subjects ingested three doses (at an interval of two days between doses)⁸³. No subjects manifested fever or diarrhoea, SFL124 was excreted by all volunteers and was immunogenic. Although few subjects excreted the strain, SFL124 was also immunogenic in Vietnamese children of 9-14 years of age and stimulated local mucosal immune responses and ASC responses to *S. flexneri* Y O- and Ipa antigens in a dose-dependent manner¹⁰⁸. The high baseline titres of serum antibodies to *S. flexneri* Y O- and Ipa antigens in the Vietnamese children indicate that the vaccine was seen as a booster rather than as a primary immunogen. It is not clear whether the difference in the immune responses to SC602 and SFL124 live oral *Shigella* vaccines in developing country populations is due to differences in the populations that participated in the clinical trials, to the inherent immunogenicity of the two strains or to disparities in the immunological assays.

Shigella conjugate vaccines

Investigators at the **National Institute of Child Health and Human Development** (NICHD) in the United States have proposed that in subjects immune to *Shigella* small amounts of serum IgG transude onto the gut surface, where they can neutralize or otherwise inactivate inocula of wild-type *Shigella* organisms shortly after ingestion^{109,110} (FIG. 3). On the basis of this concept, NICHD investigators have developed parenteral conjugate vaccines that consist of O polysaccharides derived from the LPS of relevant *Shigella* serotypes covalently linked to a carrier protein (*Pseudomonas aeruginosa* exotoxin A (PsA) or CRM9-mutant diphtheria toxin). Data supporting the concept that serum IgG anti-O-antigen antibodies correlate with (and might mediate) protection come from the seroepidemiological studies of Cohen and colleagues^{111,112,131}. Baseline serum specimens were obtained from Israeli military conscripts upon deployment to training bases where they were exposed to a high risk of shigellosis^{111,112,131}. The rates of *S. sonnei* and *S. flexneri* 2a disease were significantly lower in individuals who had elevated non-IgM anti-O-antigen titres at baseline. Additional supporting data come from the studies of Black *et al.*, who showed

that North American volunteers with elevated serum IgA and IgG anti-*S. sonnei*-O-antigen antibodies following oral immunization with attenuated *Salmonella* Typhi expressing *S. sonnei* O-antigen had lower infection rates when challenged with virulent *S. sonnei* in experimental challenge studies⁹³.

A parenteral prototype NICHHD vaccine that consists of *S. sonnei* O polysaccharide conjugated to PsA was shown to be well tolerated and highly immunogenic in stimulating serum IgG O-antigen antibodies in young Israeli soldiers^{113,114}. In a randomized, controlled, double-blind Phase III efficacy trial involving several hundred Israeli soldiers, a single dose of the *S. sonnei* conjugate vaccine conferred 74% protection against *S. sonnei* diarrhoea during outbreaks on army bases¹¹⁴. The efficacy of the vaccine was related to the level of conjugate-induced IgG anti-O-antigen antibody.

Phase II trials assessed the clinical acceptability and immunogenicity of *S. sonnei* (conjugated to PsA or CRM9) and *S. flexneri* 2a (conjugated to PsA) vaccines in Israeli children that were 4–7 and 1–4 years of age^{115,116}. In these paediatric studies, a two-dose immunization schedule was used, with doses spaced 6 weeks apart^{115,116}. The first injection of each vaccine stimulated a significant (9–13-fold) rise in homologous serum IgG anti-O-antigen antibodies¹¹⁶. In 1–4-year-olds, the second injection stimulated a booster response in both recipients of *S. sonnei* (2.9-fold) and *S. flexneri* 2a (1.6-fold) conjugates; the titres remained elevated 2 years after vaccination¹¹⁶.

A Phase III randomized efficacy trial of *S. sonnei* conjugate and *S. flexneri* 2a conjugate vaccines, with each serving as a control for the other, was recently completed in paediatric subjects in Israel. The results of this trial will determine whether the *S. sonnei* conjugate vaccine can protect young children. The next generation of *Shigella* conjugates, based on synthetic oligosaccharides linked to proteins, offers promise for enhanced immunogenicity and diminished production costs¹¹⁷.

Strategies to achieve broad-spectrum protection

Arguably the greatest impediment to achieving a useful *Shigella* vaccine is devising a strategy that can confer broad protection against a large number of epidemiologically relevant serotypes. There is widespread agreement that a global *Shigella* vaccine must protect against 16 serotypes and subserotypes, namely *S. dysenteriae* 1, *S. sonnei* and all 14 classical *S. flexneri* types and subserotypes (TABLE 1; BOX 1; FIG. 2). Yet a vaccine containing so many strains or conjugates would be impractical and expensive, even if it could be formulated. To resolve this dilemma with a 'pentavalent strategy', researchers at the CVD included *S. flexneri* 2a, *S. flexneri* 3a and *S. flexneri* 6 strains along with the attenuated *S. dysenteriae* 1 and *S. sonnei* strains in a multivalent vaccine, because these 3 *S. flexneri* serotypes have O-antigen group determinants that are shared by the remaining 11 *S. flexneri* serotypes and subserotypes¹¹⁸ (BOX 1). CVD investigators have demonstrated in a guinea pig model that a multivalent vaccine containing serotypes *S. flexneri* 2a and *S. flexneri* 3a provides an impressive level of cross-protection against other *S. flexneri* serotypes that share the common O-antigen epitopes¹¹⁸.

As Ipa and VirG proteins are plasmid-encoded virulence attributes that are expressed by all virulent *Shigella*, irrespective of serotype, it is attractive to propose basing broad protection on stimulating serological and CMI responses to these (and perhaps to other common protein) antigens (FIG. 3). However, such responses will have to be even stronger than those elicited by wild-type infection (FIG. 3). This is because NHP challenges and epidemiological data suggest that protection due to wild-type infection does not confer serogroup-heterologous cross-protection, despite eliciting serological responses to Ipa proteins⁵³.

The way forward

Identification of the correlates of protection is arguably the most crucial catalyst needed to accelerate the development of effective *Shigella* vaccines. The strongest immunizing regimens that confer protection (wild-type infection and live oral vaccines) appear to mediate protection via a confluence of immune responses. These include systemic and mucosal antibody responses to *Shigella* O- (and other) antigens, cytokines (particularly IFN- γ , secreted early as part of the innate immune response and later by specific T cells) and other CMI responses. Correlation of the panoply of effector immune responses elicited in subjects immunized with *Shigella* vaccines, exposed to wild-type *Shigella* and protected due to exposure to wild-type *Shigella* will help us identify the key immune responses that afford strong, long-lasting protection from disease. Another catalyst required to accelerate *Shigella* vaccine development is increased financial support for clinical trials (including challenge studies) to evaluate vaccine candidates and elucidate correlates of protection.

Although attractive candidate vaccines have entered clinical development, the road ahead presents barriers to be overcome. Whether the pentavalent strategy of the CVD can provide broad cross-protection in humans remains an important question. Also, we must document whether *Shigella* vaccines — live oral or parenteral conjugate — can protect vulnerable immunologically naive paediatric populations in developing countries as well as travellers from developed countries. Once vaccines have been developed that overcome the biological and epidemiological hurdles and result in licensed products, realization of the potential of vaccines as public health tools will require that yet other hurdles be surmounted, including generating political will and devising financial solutions to assure the introduction and programmatic use of vaccines in developing countries. The advent of the [Global Alliance for Vaccines and Immunisation](#) (GAVI) and its Fund, [Advanced Market Commitments](#), and the [International Finance Facility for Immunisation](#) and other innovative tools for financing the purchase of vaccines for the poorest developing countries offer tangible solutions that were unimaginable a decade ago. These innovations offer optimism that the fruits of microbiological and immunological research, such as the development of well tolerated, effective *Shigella* vaccines, can reach the neediest target populations.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

The following terms in this article are linked online to:
Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
 virG
Entrez Genome Project: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=genomeprj>
Shigella dysenteriae | *Shigella flexneri* | *Shigella sonnei*
UniProtKB: <http://ca.expasy.org/sprot>
 IFN-γ | IL-10 | TNF-α

FURTHER INFORMATION

Eileen Barry’s homepage: http://medschool.umaryland.edu/CVD/Fac_Res_Interest.asp?id=38
Karen Kotloff’s homepage: <http://medschool.umaryland.edu/facultyresearchprofile/viewprofile.aspx?id=800>
Marcela Pasetti’s homepage: <http://medschool.umaryland.edu/facultyresearchprofile/viewprofile.aspx?id=4848>
Marcelo Sztein’s homepage: http://medschool.umaryland.edu/CVD/Fac_Res_Interest.asp?id=52
Myron Levine’s homepage: <http://medschool.umaryland.edu/facultyresearchprofile/viewprofile.aspx?id=723>
Advanced Market Commitments for Vaccines: <http://www.vaccineamc.org/about/index.html>
Center for Vaccine Development: <http://medschool.umaryland.edu/CVD>
Global Alliance for Vaccines and Immunisation: <http://www.gavialliance.org>
International Finance Facility for Immunisation: <http://www.iff-immunisation.org>
National Institute of Child Health and Human Development: <http://www.nichd.nih.gov>
Walter Reed Army Institute of Research: <http://wrair-www.army.mil>
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