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REVIEW

Serum IgG antibodies to *Shigella* lipopolysaccharide antigens – a correlate of protection against shigellosis

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ABSTRACT

Shigella is a leading cause of diarrhea among children globally and of diarrheal deaths among children under 5 years of age in low- and middle-income countries. To date, no licensed *Shigella* vaccine exists. We review evidence that serum IgG antibodies to *Shigella* LPS represent a good correlate of protection against shigellosis; this could support the process of development and evaluation of *Shigella* vaccine candidates.

Case-control and cohort studies conducted among Israeli soldiers serving under field conditions showed significant serotype-specific inverse associations between pre-exposure serum IgG antibodies to *Shigella* LPS and shigellosis incidence. The same serum IgG fraction showed a dose–response relationship with the protective efficacy attained by vaccine candidates tested in phase III trials of young adults and children aged 1–4 years and in Controlled Human Infection Model studies and exhibited mechanistic protective capabilities. Identifying a threshold level of these antibodies associated with protection can promote the development of an efficacious vaccine for infants and young children.

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Introduction

Shigella is associated with significant disease burden globally which is hyperendemic in low and middle-income countries (LMICs). More than 250 million cases of shigellosis are estimated to occur annually in these settings,[1] and over 212,000 deaths. *Shigella* is responsible for over 63,000 deaths annually among children younger than 5 years old, occurring mostly in LMICs.[1] *Shigella* infection is associated with impaired linear growth and malnutrition.[1,2,3]

Shigellosis is also common in high-income countries, with incidence estimated as 1.5–2 million cases annually,[1,4] occurring mostly among toddlers living in crowded communities and day-care centers.[5,6,7] Additional high-risk groups are travelers from developed countries to endemic countries and soldiers serving under field conditions in endemic regions.[8,9]

There are four species of *Shigella*. *S. dysenteriae* (group A, 15 serotypes); *S. flexneri* (group B, 14 serotypes), *S. boydii* (group C, 19 serotypes), and *S. sonnei* (group D, 1 serotype) differing by the configuration of the O-antigenic polysaccharide within the lipopolysaccharide (LPS) of genus *Shigella*.

S. flexneri and *S. sonnei* serotypes are responsible for around 90% of cases of shigellosis globally.[10,11,12] All 14 *S. flexneri* serotypes, *except S. flexneri* 6, share a common backbone of tetrasaccharide repeats that contain three rhamnose residues and one N-acetylglucosamine. *S. flexneri* 6 has d-galactose as the third sugar of the tetrasaccharide and N-acetylgalactosamine as the terminal residue. The O-antigen repeat of *S. sonnei* single serotype is a disaccharide (FucNAc, 2-acetamido-4-amino-2,4,6-trideoxy-d-galactose; AltUA, 2-amino-2-deoxy-l-altruronic acid) between *S flexneri* 2a and 3a and other *S flexneri* (except serotype 6) suggests that serotypes 2a, 3a, and 6 could confer immunity to all 14 *S flexneri* serotypes[13,14]

The LPS of *Shigella* spp. is a virulence factor with endotoxic activities of the lipid A component of the molecule and the ability of the polysaccharide chain–the core and the O-antigenic polysaccharide–to confer resistance to host defense mechanisms such as opsonization, phagocytosis, and intracellular killing.[15,16]

Shigella mutants lacking core and O antigenic polysaccharides, known as rough LPS strains, are avirulent being susceptible to host defensive mechanisms and defective in intracellular motility and cell-to-cell spread capabilities.[17] In LMICs, *S. flexneri* accounts for most cases.[10,11,18] In high-income countries, *S. sonnei* is responsible for the vast majority of shigellosis.[7,12,19] An emerging global increase in antimicrobial resistance of *Shigella* narrows the antibiotic treatment options, especially in young children.[12,20,21]

The prevention of shigellosis should include enhancement of universal access to safe drinking water, improved sanitation infrastructure, and personal and food hygiene. However, achieving these goals will take several decades. The low inoculum required to cause shigellosis (100–1000 organisms)[22] facilitates transmission of the disease, and explains the frequent failure of adequate sanitary and hygienic measures to prevent shigellosis,[12] even in high-income countries.[19,23] Therefore, the development of an efficacious *Shigella* vaccine is highly desirable and particularly needed for young children

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residing in LMICs and other regions with high disease burden.

No licensed vaccine against *Shigella* is currently available, but several vaccine candidates have been tested in clinical trials, including oral live attenuated and parenteral sub-unit lipopolysaccharide (LPS)-based vaccines.[24,25] A first generation of conjugate *Shigella* vaccines reached evaluation in field efficacy trials[26,27] and a second generation of *Shigella* conjugates and other subunit vaccines are currently in clinical trials.[28,29,30,31] This naturally raises questions regarding the best correlate of protection against shigellosis that can support the clinical development of these vaccine candidates.

Herein, we reviewed the evolving evidence on the role of serum immunoglobulin G (IgG) antibodies against *Shigella* LPS as a correlate of protection against shigellosis.

Epidemiological observational studies and challenge studies in humans and primates showed that *Shigella* infection confers serotype-specific immunity, indicating that the O-specific polysaccharide is the protective antigen. The conferred protection is around 70% and is of short duration, around 2 years or less, and is probably attained after consecutive exposures to *Shigella* O-SP antigens.[7,32,33,34,35]. These observations imply that a good Shigella candidate vaccine should induce an immune response directed to O-SP, similar or greater in magnitude than that induced by natural infection. In view of the most common Shigella serotypes isolated globally and assuming that the cross-protection among S flexneri serotypes in guinea pigs[13]can be replicated in humans, it has been proposed that a quadrivalent vaccine containing *S. sonnei* and *S. flexneri* 2a, 3a, and 6 O-antigen could provide overall coverage for up to 88% of *Shigella* strains.[12,14]

Identification of correlates of protection is important for vaccine development and evaluation. Adapting previous definitions for correlates of protection[36] to shigellosis, we propose a set of conditions that such correlate should fulfill.

A necessary, but insufficient condition is that natural *Shigella* infection triggers an increase in the level of such immunological marker. Another necessary condition is that this immunological marker is associated with protection against shigellosis caused by the homologous *Shigella* serotype, under natural conditions of exposure and by a *Shigella* candidate vaccine, either in field efficacy trials or in human challenge studies, while demonstrating functional capabilities.

Evidence accumulated over the last four decades indicates that serum immunoglobulin G (IgG) antibody level to *Shigella* LPS fulfills all these conditions and can be defined as an immunological correlate of protection against shigellosis. Data supporting this statement are presented in the following sections.

Serum IgG antibodies are induced by natural *shigella* infection

In the early 1980s, ELISA was initially employed to measure immunoglobulins IgG, IgM and IgA to *Shigella* LPS in convalescent serum samples of culture-proven cases of *S. sonnei* and *S. dysenteriae* type 1 (Shiga) shigellosis in Sweden and Vietnam, respectively, and in healthy Vietnamese and Swedish controls. The sensitivity and specificity of the three distinct immunoglobulins to homologous LPS were determined by cross-sectional comparisons.[37,38] These studies also suggested that IgG antibodies to *Shigella* LPS acquired throughout the lifespan could also result from cross-reactivity with LPSs of other Enterobacteriaceae, as exemplified by the presence of IgG anti-*S. dysenteriae* type I (Shiga) among Swedish volunteers, though this serotype was not isolated in Sweden since the beginning of the 20th century[38,39] .

ELISA was further used together with passive haemagglutination to measure anti-LPS response in paired sera obtained at acute and convalescent phases from young adults involved in 10 outbreaks of shigellosis occurring in Israeli military field units during the 1980s.[40] Both assays were sensitive and specific in detecting significant antibody responses to homologous *Shigella* LPS in symptomatic and asymptomatic persons exposed to *Shigella* during these outbreaks. The kinetics of the various immunoglobulins, examined by ELISA over a 10-week period following the onset of disease, showed peak levels of IgA at 2 weeks after onset of symptoms, and a decline to baseline levels within 2.5 months. In contrast, serum IgG levels peaked at 3–4 weeks, and declined thereafter: at the late convalescent stage, IgG levels were half of those measured at early convalescence, still about twice higher than the baseline titers. Although the IgM levels showed a pattern similar to that of IgA, their elevation at the early convalescent stage was less pronounced.[40]

The use of ELISA enabled quantifying the serum IgG anti-LPS fraction separately, avoiding overexpression of the pentavalent IgM fraction in the passive haemagglutination assay.

The pattern of the IgG subclass response induced by natural *Shigella* infection was species-dependent. IgG2 was the dominant subclass produced in response to *S. flexneri* 2a shigellosis whereas IgG1 and IgG2 were the main components in the response to *S. sonnei* shigellosis. There was also a small but significant rise in IgG3 following infection with both *S. sonnei* and *S. flexneri* 2a[41]

Shigella LPS extracted by the hot phenol-water method[42] was used to coat the ELISA 96-well plates in all seroepidemiological and vaccine immunogenicity studies.

Serum IgG antibodies to *shigella* LPS are associated with reduced risk of homologous disease under natural exposure

Seroepidemiological studies carried out among Israeli soldiers serving under field conditions and highly exposed to *Shigella* showed that pre-existent serum IgG antibodies to *S. sonnei* or to *S. flexneri* 2a LPS were strongly associated with resistance to homologous *Shigella* infection.[43,44]

These studies were conducted in the late 1980s and early 1990s, when shigellosis was rampant among Israeli young recruits serving under field conditions in which *Shigella* species with a very low infectious dose could have been easily transmitted feco-orally by all means: person-to-person, infested fomites, flyborne, foodborne and waterborne.[8,45] Under these conditions of heavy natural exposure to *Shigella*, soldiers with "low" IgG titers to *S. sonnei* LPS at baseline were 5.5-fold ($p = .0001$) more likely to develop

S. sonnei shigellosis than soldiers with "high" titers. Similar analysis in *S. flexneri* 2a outbreaks showed odds ratios of 4.3 for ELISA IgG titers to *S. flexneri* 2a LPS. These numbers correspond to 82% and 77% reduction in the risk of shigellosis caused by the homologous serotypes in persons with high baseline serum IgG antibody against *S. sonnei*.

There was no indication that "high" baseline antibody titers to *S. sonnei* LPS specific antigen were associated with protection against disease caused by *S. flexneri* 2a and vice versa.[43] This supports the notion that natural immunity conferred by pre-existing IgG anti-LPS was serotype specific.

The attack rate of *S. sonnei* and *S. flexneri* 2a shigellosis, strictly defined as visits to the clinic due to diarrhea plus a positive stool culture for these organisms, was higher among individuals with shorter rather than longer prior exposure to field conditions (3.3% vs. 0.05%, p = .05). The proportion of individuals with serum antibodies to *S. sonnei* or *S. flexneri* 2a LPS at the beginning of the follow-up period was significantly lower among those who were within 0–6 months of previous military service under field conditions than among those who served 7–15 months under similar conditions (57.0% vs 72.3%, p = .001).[46] Repetitive exposures and natural boosters under field conditions appeared to induce rapidly increased levels of serum IgG anti-*Shigella* LPS antibodies and acquired immunity to shigellosis.[46]

Analogically, repetitive exposures to *Shigella* LPS and to cross-reacting antigens, albeit during a longer period, might explain the acquisition of IgG anti-LPS antibodies in an age-related manner. Similar to the situation described for capsulated pathogens, such as *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, and *Neisseria meningitidis*, the level of "natural" anti-*Shigella* LPS is inversely correlated with the age-specific incidence of shigellosis. Shigellosis rarely occurs below the age of 6 months, peaks at ages 1 to 4 years, and declines in older children and adults.[47,48] In Israel, for example, the age-specific annual incidences of culture-proven *S. sonnei* shigellosis (surveillance including more than 35,000 *S. sonnei* isolates since 1998) were 1.2, 5.9, 7.5, 5,5 and 4.0 per 1000 at ages 1, 2, 3, 4 and 5 years, respectively; and dropping to 0.9, 0.2, 0.3, 0.2, 0.06, 0.07 and 0.06 per 1000 for age groups 5–14, 15–24, 25–34, 35–44, 45–54, 55–64 and 65 and older, respectively. Geometric mean titers of IgG to *S. sonnei* LPS antibodies, measured in sera of 1096, 0 to 19-year-old individuals, followed a mirror image curve, with the highest GMT in the first 3 months of life (IgG of maternal origin), and the lowest levels between 4 and 12 months and in the second year of life, and gradually increasing IgG levels acquired after natural symptomatic or asymptomatic exposures to *S. sonnei* organisms (V. Asato et al. unpublished).

Higher levels of serum IgG antibodies to *Shigella* LPS were measured in subpopulations of lower socioeconomic level or in transition with an increased risk of exposure to *Shigella* or cross-reacting enteric bacteria earlier in life.[49,50] *Shigella* LPS antibodies were also found to increase in an age-related manner among other populations living in highly endemic regions and correlated with a decreased risk for shigellosis[51,52] and an abrogated response to a potent oral live attenuated *Shigella* vaccine.[53]

Serum IgG antibodies to *shigella* LPS are associated with reduced risk of disease in efficacy field vaccine studies

In view of the findings of the observational studies mentioned above and the successful experience with the development of the pioneering *H. influenza* type b conjugate vaccine, injectable glycoconjugates incorporating detoxified LPS from *S. flexneri* 2a, *S. sonnei* and *Shigella dysenteriae* type 1 (Shiga), linked to carrier proteins, were developed at the National Institutes of Health, USA[39,54] by John Robbins and Rachel Schneerson. Drs. Robbins and Schneerson hypothesized that a critical level of IgG anti-LPS strongly induced in serum by a conjugate vaccine exudes onto the epithelium of the intestine, and in combination with complement, could result in bacteriolysis of the inoculum of shigellae reaching the mucosa.[4,39].

O-specific polysaccharides of *S. sonnei* and of *S. flexneri* 2a obtained by acid hydrolysis and further purification from the LPS of the same serotypes extracted by the hot-water phenol method were bound to Pseudomonas aeruginosa recombinant exoprotein A (rEPA). These conjugates showed a good safety profile and indeed induced high levels of serum IgG anti-homologous LPS in phase I and phase II clinical trials conducted among healthy adult volunteers[55,56] In a phase II clinical trial performed among young adults in Israel, 66 and 64 participants were vaccinated with *S. sonnei*-rEPA and *S. flexneri*-rEPA vaccines, respectively; of them, 17 and 16 participants, received a second dose of the same conjugate 6 weeks after the first dose, and 62 controls received hepatitis B vaccine.[56] Fourteen days after the first injection, 90% of *S. sonnei*-rEPA recipients and 73–77% of *S. flexneri*-rEPA recipients had a fourfold or greater increase in serum IgG and IgA anti-LPS levels, while none of the control groups had significant antibody increase to either LPS. The second dose given at day 42 did not boost antibody levels.[56] At 4 years after vaccination, 50% of vaccinees still had fourfold or higher titers, as compared to pre-immunization IgG antibody levels. Serum IgG antibody level was the highest and most sustained class of LPS antibodies.

The levels of serum IgG and IgA anti-LPS elicited by *S. flexneri* type 2a-rEPA and *S. sonnei*-rEPA conjugate vaccines were similar to or even higher than those of Israeli soldiers following natural infection with those pathogens.[40,55] The persistence of elevated IgG and IgA anti-*Shigella* LPS induced by these conjugates was of longer duration than that following shigellosis.[40] The IgG subclass response in sera of volunteers receiving *S. flexneri* 2a-*r*EPA or *S. sonnei* -*r*EPA vaccine was similar in pattern to the response elicited by natural infection. In both cases, IgG2 was the dominant subclass produced in response to *S. flexneri* 2a O-SP whereas IgG1 and IgG2 were the main components in the response to *S. sonnei* LPS O-SP[41,57]

A double-blind randomized vaccine-controlled phase III trial assessed the efficacy of a single injection of the *S. sonnei*-rEPA vaccine among 1446 military recruits from seven separate field sites (cohorts) in Israel at high risk of exposure to *Shigella* spp. During the trial, culture-proven *S. sonnei* shigellosis occurred in three cohorts, 70–155 days after vaccination

and in one cohort, as early as 1–17 days after vaccination. In the first three cohorts, the attack rate of shigellosis was 2.2% in recipients of *S. sonnei*-rEPA vaccine compared with 8.6% in controls (protective efficacy 74% [95% CI 28–100], p = .006). *S. sonnei*-rEPA also showed significant protection against shigellosis in the cohort in which cases occurred 1–17 days after vaccination (43% [95% CI 4–82], p = .039).[26] Baseline level serum IgG and IgA antibodies to *S. sonnei* LPS were similar in recipients of *S. sonnei*-rEPA vaccine and the controls. Pre-vaccination and post-vaccination ELISA measurements of antibody to *S. sonnei* LPS among recipients of *S. sonnei*-rEPA vaccine showed that the vaccinees who developed *S. sonnei* shigellosis had significantly lower serum IgG responses to the homologous lipopolysaccharide than those who did not. The higher serum IgG antibody response induced by the *S. sonnei*-rEPA conjugate vaccine in volunteers who did not develop shigellosis (p = .014) supports the association between serum antibody titer and protection against homologous disease.[26]

Following the favorable findings on the immunogenicity and efficacy of the *S. sonnei*-rEPA conjugate vaccine in young adults, a series of age-descending phase II randomized controlled clinical trials were conducted with *S. sonnei* and *S. flexneri* 2a conjugates in Israel among healthy children aged 4–7 years[58] and 1–4 years.[59] These trials demonstrated a good safety profile of these vaccines among children.

Before vaccination, the geometric mean levels of IgG and IgM levels were similar among children who received the conjugates vaccines and the control group. Both *Shigella* conjugates induced homologous serum IgM, IgA, and IgG LPS antibodies; however, the highest and most sustained rise was in IgG.[58]The fold increases in geometric mean concentrations of anti-LPS serum antibody were similar in all age groups studied, and 2 years after vaccination the geometric mean concentrations of IgG anti-LPS were significantly higher than the pre-immunization levels. Both pre-immunization and post-immunization concentrations of IgG anti-LPS differed by age, being higher in adults than in children. The geometric mean of homologous IgG anti-LPS levels at 4 weeks after a second injection of the *S. sonnei* conjugate vaccine was 48.0, 8.0 and 2.9 in adults, children aged 4–7 years and 1–4 years, respectively, and for *S. flexneri* 2a IgG anti-LPS, 113.0, 48.0 and 40.1, respectively.[58,59,60]

Based on these findings, a double-blinded, randomized and vaccine-controlled phase III trial of *S. sonnei* and *S. flexneri* 2a O-SP-rEPA conjugates was conducted in 2799 healthy 1 to 4-year-old children in Israel.[27] The investigational vaccines were administered to children in 2 intramuscular injections, 6-weeks apart. Sera taken from a random sample of 10% of the participants were tested for serum IgG anti-LPS. The numbers for *S. flexneri* 2a isolates were too few to enable an efficacy analysis of *S. flexneri* 2a O-SP-rEPA in this trial The overall efficacy of the *S. sonnei* conjugate against culture-proven *S. sonnei* shigellosis was 27.5%; however, stratification by age-groups showed an age-dependent efficacy for recipients of the *S. sonnei* conjugate vaccine: 3.8% for the 1–2 year olds, 35.5% for the 2–3 year olds and 71.1% (p = .043) for the 3–4 year olds. Similarly, an age-related increase in vaccine-induced serum antibody levels was found; among recipients of the *S. sonnei* vaccine, levels of IgG against-LPS of *S. sonnei* antibody were 1.40 ELISA units (EU), 3.71 EU and 6.38 EU in children aged 1–2, >2–3 and >3–4 years, respectively. The corresponding values for the *S. flexneri* 2a LPS IgG antibodies among *S. flexneri* 2a O-SP-rEPA vaccinees were 18.98 EU, 29.96 EU and 43.86 EU.[27] Clearly, the efficacy estimates in this trial paralleled the age-related immunogenicity of the *S. sonnei* conjugate. The "dose-response" relationship identified indicates that a critical level of serum IgG anti-O-SP antibodies confers immunity to shigellosis. Specifically, the threshold level associated with 71% protective efficacy in children aged 3–4 years in this study could serve as a reference when assessing the immunogenicity of the new generation of conjugates in infants and toddlers, and for prediction of their efficacy in this target population.

Serum IgG antibodies to *shigella* LPS are associated with reduced risk of disease in vaccinees challenged with homologous virulent strain

Controlled Human Infection Model (CHIM) studies of vaccines can also serve as an important tool for identifying correlates of protection; advantages are the well-controlled quantification of the exposure to the challenge virulent organism and the possibility of examining a wide range of immunological parameters following vaccination with the candidate vaccine. However, a main limitation of CHIM studies that should be considered is the uncertainty regarding the extent by which they truly mimic natural exposure to the microorganism, host susceptibility and induction of immune responses, as in field conditions. Moreover, CHIM studies typically assess short-term protection. In a CHIM study conducted in the early 1980s, 3 doses of a live-attenuated oral *Salmonella typhi* strain Ty21a, expressing the form I O polysaccharide antigen of *S. sonnei* (1–8 X 10^9 organisms/dose) were administered to young adults who, along with unvaccinated controls, were challenged one month later with virulent *S. sonnei*. Vaccinees developed serum and local intestinal immune responses to *S. sonnei* LPS, and the presence of specific serum IgA or IgG antibody before challenge with virulent *S. sonnei* correlated with protection from shigellosis[61]

Additional CHIM studies showed that high pre-challenge serum IgG anti-LPS in volunteers vaccinated with oral live attenuated *S. flexneri* 2a SC602 and *E. coli* K12-*S. flexneri* 2a hybrid vaccines was associated with protection against dysentery or with decreased disease severity (n = 15; r = 0.52; p = .05 for decreased severity of disease) after challenge with wild-type *S. flexneri* 2a strain 2457T[62,63]

A recent CHIM study showed that flexyn2a, a candidate bioconjugate vaccine against *S. flexneri 2a*, conferred 30% (p = .11) and 50% protection (p = .01) against shigellosis and more severe diseases or dysentery, respectively. Serum IgG anti-*Sfl2a*-LPS after vaccination with 2 doses and before challenge significantly correlated with protection after challenge (p = .0061) (Vaccines of Enteric Diseases (VED) meeting 2017, Ablufeira, Portugal (Abstract 139).

Collectively, results obtained from CHIM studies confirm findings from epidemiological observational studies and field clinical trials regarding the pivotal role of the serum IgG antibodies to *Shigella* LPS in protection against shigellosis caused by the homologues serotype.

Serum IgG antibodies to *shigella* LPS have functional capabilities

We examined functional capabilities of serum IgG anti-*Shigella* LPS induced by natural *Shigella* infection and vaccination with *Shigella* conjugates, employing the serum bactericidal antibody assay (SBA).[64,65] Using a stable *S. sonnei* phase 1 strain as a target for SBA, we found a significantly higher SBA GMT in volunteers who received the *S. sonnei*-rEPA conjugate (GMT = 1407, 95% CI: 687–2884, n = 27) 3 months after vaccination, compared with young adults with culture-proven *S. sonnei* shigellosis about 3 months after disease onset (GMT = 271, 95% CI 91–810; n = 16) (Shiri-Meron Sudai et al. unpublished). A strong and significant correlation was observed between the individuals' titers of IgG anti-*S. sonnei* LPS and the SBA titers (Spearman's correlation coefficient = 0.72; p < .01).Using the thiocyanate elution assay assessing avidity,[66] we found that sera with bactericidal activity (n = 17) had a significantly higher avidity index than did sera with no bactericidal activity (n = 9) against *S. sonnei* (Avidity index: 2.3 vs 1.8, p = .048). In recent studies we found that the IgG serum response to homologous Shigella LPS in children with culture-proven *S. sonnei* and *S. flexneri* 2a shigellosis at various times after the onset of disease correlated with the B memory cell response measured by the ELISPOT method[67,68]after polyclonal stimulation of peripheral blood mononuclear cells (n = 84, Pearson correlation coefficient = 0.76, p < .01) suggesting that the magnitude of the serum IgG anti-LPS could predict the length of acquired immunity following natural infection (Shiri-Meron Sudai et al. unpublished).

Chowers et al.[69]used immune sera from children immunized with *S. sonnei* and *S. flexneri* 2a conjugates in the age-descending phase II studies, to examine their effect on the invasion of epithelial cells by shigellae and on the induced inflammatory response, by using an *in vitro* model of bacterial invasion into intestinal epithelial cells (Caco-2). Incubation of shigellae with post-immunization but not pre-immunization sera of children vaccinated with *S. sonnei* or *S. flexneri* 2a O-SP conjugate vaccines inhibited *in vitro* invasion of Caco-2 cells and the infection-associated increases in IL-1β and IL-8 mRNA and extracellular cytokine levels in a type-specific and dose-dependent manner. These effects were abolished by pre-treatment of these sera or of Caco-2 cells with homologous but not heterologous O-SPs. The protective effects were duplicated by IgG purified from these sera. The authors proposed that a critical level of IgG anti-O-SP could have a prophylactic as well as a curative role in shigellosis[69]

Altogether, these findings confirm the rationale of developing conjugate vaccines that induce high and persistent levels of serum IgG antibodies against *Shigella* LPS, with functional capabilities.

Conclusions and next steps

We conclude that serum IgG antibodies to *Shigella* LPS has emerged as a correlate of protection against shigellosis with mechanistic capabilities. These antibodies are elicited by natural *Shigella* infection, which confers serotype-specific immunity, albeit for limited duration; they have been associated with reduced risk of shigellosis under natural conditions of exposure, their levels increase in an age-related manner parallel with a significant decrease in the incidence of shigellosis, they have shown a dose-response relationship with the extent of protective efficacy attained by various vaccine candidates tested in phase 3 efficacy studies and in CHIM studies, and they exhibited mechanistic protective capabilities (Figure 1). In adults, the level, persistence and functionality of the serum IgG anti-*S. sonnei* LPS were greater following immunization with the detoxified *S. sonnei* O-SP- rEPA conjugate than after natural *S. sonnei* infection. The possibility is encouraging that similar outcomes may be attained also in infants and toddlers by the new generation *Shigella* conjugates, namely the *S. flexneri* 2a-rEPA bioconjugate and the synthetic carbohydrate-based *S. flexneri* 2a-tetanus toxoid 15 vaccine (S.flex2a-TT15), or the subunit Generalized Modules for Membrane Antigen (GMMA)-based 1790GAHB *S. sonnei* vaccine. If this will be the case, it is expected that these vaccines will also confer a higher and more sustained protection against shigellosis than that induced by natural infection. Moreover, it is hoped that this can be also accomplished by multivalent constructs, conjugates or GMMA platform-based vaccines delivering the O-SPs assumed to confer direct or cross-protection against almost 90% of shigellae.

Condition	Characteristic	Selected references
Always present	Elicited by *Shigella* natural infection	37, 38, 40
AND	Associated with reduced risk of shigellosis caused by the homologous *Shigella* serotype under natural exposure	43, 44, 46 Current review
AND	Associated with serotype-specific protection conferred by a candidate vaccine in field efficacy studies	26, 27
OR	Associated with serotype-specific protection conferred by a candidate vaccine in human challenge studies	61-63 Current review
AND	Has functional capabilities	69 Current review

Figure 1. Serum IgG antibodies to *Shigella* LPS – a correlate of protection against shigellosis.

Serum IgG antibodies against *Shigella* LPS emerging as a correlate of protection against shigellosis as reviewed here, were measured in various labs, by several ELISA protocols and with results expressed in different units such as end point titers, ELISA units or percent of a standard, all of them being a proxy of the IgG anti-LPS concentration. The results were consistent in indicating the association between the level of IgG anti-*Shigella* LPS and protection against shigellosis and this is a strength of these findings. Laboratories employed in-house reference sera or standards to relate individual results and control for intra and inter-assay variations without using an international standard which is a limitation of these studies. There is a clear need to harmonize internationally between the assays employed and their results as has been done in one case so far[31]and use common quantitative reference standards. This is essential towards the identification of threshold levels of serum IgG antibodies to *Shigella* LPS associated with protection against homologous disease that can guide and accelerate the development of the current promising *Shigella* vaccine candidates.

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