

Critical Needs in Advancing *Shigella* Vaccines for Global Health

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New O-antigen-based vaccines against *Shigella* are in clinical development. Historical efficacy studies identify serum O-antigen immunoglobulin G as a correlate of protection, leading to the suggestion that accelerated licensure could be achieved using the *Shigella*-controlled human infection model (CHIM). We discuss the role of the *Shigella* CHIM alongside critical imperatives needed for accelerated licensure and uptake of *Shigella* vaccines: (1) development of an international standard serum and harmonized enzyme-linked immunosorbent assay; (2) demonstration of field efficacy in young children in low- and middle-income countries; and (3) early engagement with regulators and policy makers.

Shigella is a major cause of diarrhea and dysentery disproportionately affecting children in low- and middle-income countries (LMICs) and responsible for 164 300 deaths annually [1]. A World Health Organization (WHO) expert consultation on *Shigella* vaccine preferred

product characteristics concluded the following: “the primary goal is to develop a safe, effective and affordable vaccine to reduce mortality and morbidity due to dysentery and diarrhea caused by *Shigella* in children under 5 years of age in LMICs” [2]. A vaccine would also benefit travelers. *Shigella* species express different O-antigens. A vaccine will need to protect against *Shigella sonnei* and *Shigella flexneri* 2a, 3a, and 6 to cover the most prevalent globally circulating strains [3].

Over 20 years ago, a National Institutes of Health (NIH) glycoconjugate vaccine of *S sonnei* O-antigen conjugated to recombinant exotoxin of *Pseudomonas aeruginosa* (rEPA) conferred 74% protection against *S sonnei* shigellosis in Israeli military [4], protection associating with serum immunoglobulin G (IgG) to O-antigen. The vaccine also protected children aged 3 to 4 years but not children under 3 years [5]. Loss of protection associated with decreasing serum O-antigen IgG seen with decreasing age. These findings indicate that serum O-antigen IgG is associated with protection, which suggests that a vaccine inducing high IgG levels among LMIC children may confer protection. We define this association as a correlate of protection, in which the attribute, serum O-antigen IgG, is statistically associated with the endpoint, protection against shigellosis, without the association necessarily being causal [6].

Shigella sonnei-rEPA used random coupling for conjugation resulting in a

heterogenous lattice structure. Variable amounts of free polysaccharide may have interfered with the immunologic response, as seen in mice where admixing free *Streptococcus pneumoniae* type 4 polysaccharide with *S pneumoniae* type 4 polysaccharide-protein conjugate decreased polysaccharide antibody responses [7]. Subsequent vaccine candidates used specific coupling to conjugate O-antigen to carrier protein producing a sun-type structure. This structure with shorter O-antigens induced greater immunogenicity in mice compared with first-generation vaccine [8]. Short saccharides and chemically controlled coupling through a single point on the saccharide improves reproducibility and vaccine characterization. Selective chemistries also avoid modification of the saccharide chain.

Enhanced immunogenicity with reduced saccharide length and site-specific conjugation is seen with dextran conjugates [9]. Pneumococcal saccharide-protein conjugates using synthetic short-chain oligosaccharides and site-specific chemistries are immunogenic and protective in mice [10, 11]. Hence, improved design of *Shigella* conjugates could increase immunogenicity, overcoming lack of protection in young children. A *S flexneri* 2a bioconjugate developed by Limmatech (GSK), with O-antigen covalently coupled in sun-type format to rEPA, was immunogenic in US adults [12], and it protected against moderate to severe shigellosis/dysentery

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in a *Shigella*-controlled human infection model (CHIM) study [13], providing further support for serum O-antigen IgG as a correlate of protection. The quadrivalent vaccine is being tested in Kenya (ClinicalTrials.gov NCT04056117).

The GSK Vaccines Institute for Global Health (GVGH) developed a *Shigella* outer membrane vesicle (OMV) vaccine, the vesicles serving as a delivery vehicle for O-antigen. An *S sonnei* OMV candidate was well tolerated and immunogenic in French, United Kingdom (UK) [14], and Kenyan adults [15], but it failed to protect against shigellosis in a CHIM study [16]. A vaccine consisting of *S flexneri* 2a synthetic O-antigen conjugated to tetanus toxoid induced significantly higher O-antigen IgG levels in Israeli adults than an NIH *S flexneri* 2a O-antigen-rEPA vaccine [17]. It is unknown how immunogenic these new O-antigen-based candidates must be in LMIC young children to confer protection. It is crucial to evaluate immunogenicity in this population, defined as infants and children from 6 to 36 months in LMICs.

By accepting serum O-antigen IgG as a correlate of protection against shigellosis [18], an analysis of immunologic data from field efficacy and CHIM studies can help to identify protective thresholds of O-antigen IgG. Additional information can be obtained from observational studies in target pediatric populations, which examine the association between pre-exposure and/or early acute-stage levels of serum O-antigen IgG with levels after culture-proven shigellosis. Paired sera are required from children before and after exposure to *Shigella*. Possible protection against shigellosis through other mechanisms including mucosal IgA and *Shigella* protein antibodies presents a confounder to this approach. Efforts are underway to confirm serum IgG as a correlate of protection and establish protective thresholds [18], but they are hampered by use of different enzyme-linked immunosorbent assay (ELISA) protocols and reference reagents.

An international standard reference serum and harmonized ELISA are required for comparability between studies. The standard serum should cover each serotype within multivalent vaccine formulations and be generated from vaccinees. Such a serum and ELISA will underpin clinical development of all O-antigen-based *Shigella* vaccines [19]. In 2019, a pilot *Shigella* ELISA collaborative study by the National Institute of Biological Standards and Controls (NIBSC), UK, was a first step towards ELISA harmonization. In 2020, a decision was made to harmonize a *Shigella* ELISA protocol at the Walter Reed Army Institute for Research (WRAIR) and develop a first international standard serum at NIBSC with the goal of submitting it to the WHO Expert Committee on Biological Standardization for approval and adoption.

Shigella CHIMs are conducted in adults in high-income countries (HICs). Therefore, such studies could potentially be used to license new-generation *Shigella* vaccines for travelers [20]. There is precedent, with CHIM studies used to license the Vaxchora cholera vaccine [21] for travelers. As a correlate of protection, serum O-antigen IgG could permit immunobridging from a CHIM study to an immunogenicity study in infants/young children, providing a quicker licensure route than a phase 3 efficacy study [20]. A CHIM supported WHO prequalification of TypBar-TCV typhoid conjugate vaccine for children [22, 23].

However, a typhoid conjugate vaccine was efficacious in young LMIC children 20 years ago [24]. Efficacy has yet to be shown for a *Shigella* vaccine in this population. It is not known whether serum O-antigen IgG is a correlate of protection in LMIC children, nor whether a threshold of protection determined in HICs will be applicable in these children. Therefore, efficacy needs to be demonstrated in this population and can only be achieved with a phase 3 field study. Such a study could confirm correlate of protection status and protective titers for serum O-antigen IgG in this population,

enabling subsequent immunobridging and accelerating licensure for other *Shigella* vaccines.

Nevertheless, CHIM studies could assist licensure for children through licensure for travelers, by facilitating earlier use, awareness, and generation of safety data for a *Shigella* vaccine. In addition, CHIM studies may identify serum IgG thresholds of protection, although the artificial nature of infection and low numbers of participants present limitations. Finally, if CHIM for *S flexneri* 3a and 6 become available, they may confirm that *Shigella* vaccines can protect against these serotypes. The low incidence of shigellosis due to these serotypes means it is difficult in a field setting to demonstrate efficacy against them. Moreover, after a successful phase 3 efficacy study in LMIC infants and children, an established correlate and threshold of protection could leverage a large phase 2 study as basis of licensure for subsequent *Shigella* vaccines. If this is acceptable to the regulators, the strategy could accelerate licensure, which reduces the cost of late-stage clinical development.

In conclusion, an international standard serum and harmonized ELISA are needed to advance new-generation global health *Shigella* vaccines, allowing comparability between studies, confirming serum O-antigen IgG as a correlate of protection, and determining thresholds of protection. Currently, almost all data informing correlates and thresholds of protection are from HIC adults and older children. A field-efficacy study among LMIC infants and young children will be critical to advance a first vaccine to licensure, WHO policy recommendation, and prequalification, which are prerequisites for Gavi-supported introduction.

Proactive scenario planning of regulatory strategies by vaccine developers and planning of data to be collected in late-stage product development to support future policy recommendation will help mitigate delays to postlicensure implementation. Early and parallel engagement with LMIC national regulatory

authorities and policy makers, as well as global policy and financing bodies, such as WHO and GAVI, is critical before designing pivotal phase 3 efficacy studies. This is the proposed approach of the authors as *Shigella* vaccine candidates advance to phase 3 clinical evaluation.

Notes

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References

- GBD. 2017 Mortality Collaborators Global, regional, and national age-sex-specific mortality and life expectancy, 1950-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **2018**; 392:1684–735.
- WHO. 2020 WHO Product Development for Vaccines Advisory Committee (PDVAC). Virtual Consultation 3: Full Value of Vaccine Assessment (FVVA) for *Shigella* vaccines. 18 May 2020. Available at: https://cdn-auth-cms.who.int/media/docs/default-source/immunization/pdvac/pdvac_shigella_fvva_minutes_final.pdf. Accessed 20 February 2021.
- Livio S, Strockbine NA, Panchalingam S, et al. *Shigella* isolates from the global enteric multicenter study inform vaccine development. *Clin Infect Dis* **2014**; 59:933–41.
- Cohen D, Ashkenazi S, Green MS, et al. Double-blind vaccine-controlled randomised efficacy trial of an investigational *Shigella sonnei* conjugate vaccine in young adults. *Lancet* **1997**; 349:155–9.
- Passwell JH, Ashkenazi S, Banet-Levi Y, et al.; Israeli *Shigella* Study Group. Age-related efficacy of *Shigella* O-specific polysaccharide conjugates in 1-4-year-old Israeli children. *Vaccine* **2010**; 28:2231–5.
- World Health Organization. Correlates of vaccine-induced protection: methods and implications. Available at: https://apps.who.int/iris/bitstream/handle/10665/84288/WHO_IVB_13.01_eng.pdf;sequence=1. Accessed 21 February 2021.
- Peeters CC, Tenbergen-Meekes AM, Poolman JT, Zegers BJ, Rijkers GT. Immunogenicity of a *Streptococcus pneumoniae* type 4 polysaccharide-protein conjugate vaccine is decreased by admixture of high doses of free saccharide. *Vaccine* **1992**; 10:833–40.
- Robbins JB, Kubler-Kielb J, Vinogradov E, et al. Synthesis, characterization, and immunogenicity in mice of *Shigella sonnei* O-specific oligosaccharide-core-protein conjugates. *Proc Natl Acad Sci U S A* **2009**; 106:7974–8.
- Fernandez C, Sverremark E. Immune responses to bacterial polysaccharides: terminal epitopes are more immunogenic than internal structures. *Cell Immunol* **1994**; 153:67–78.
- Alonso de Velasco E, Verheul AE, Veeneman GH, et al. Protein-conjugated synthetic di- and trisaccharides of pneumococcal type 17F exhibit a different immunogenicity and antigenicity than tetrasaccharide. *Vaccine* **1993**; 11:1429–36.
- Jansen WT, Hogenboom S, Thijssen MJ, et al. Synthetic 6B di-, tri-, and tetrasaccharide-protein conjugates contain pneumococcal type 6A and 6B common and 6B-specific epitopes that elicit protective antibodies in mice. *Infect Immun* **2001**; 69:787–93.
- Riddle MS, Kaminski RW, Di Paolo C, et al. Safety and immunogenicity of a candidate bioconjugate vaccine against *Shigella flexneri* 2a administered to healthy adults: a single-blind, randomized phase i study. *Clin Vaccine Immunol* **2016**; 23:908–17.
- Talaat KR, Alaimo C, Martin P, et al. Human challenge study with a *Shigella* bioconjugate vaccine: analyses of clinical efficacy and correlate of protection. *EBioMedicine* **2021**; 66:103310.
- Launay O, Lewis DJM, Anemona A, et al. Safety profile and immunologic responses of a novel vaccine against *Shigella sonnei* administered intramuscularly, intradermally and intranasally: results from two parallel randomized phase 1 clinical studies in healthy adult volunteers in Europe. *EBioMedicine* **2017**; 22:164–72.
- Obiero CW, Ndiaye AGW, Sciré AS, et al. A phase 2a randomized study to evaluate the safety and immunogenicity of the 1790 GAHB generalized modules for membrane antigen vaccine against *Shigella sonnei* administered intramuscularly to adults from a shigellosis-endemic country. *Front Immunol* **2017**; 8:1884.
- Frenck RW Jr, Conti V, Ferruzzi P, et al. Efficacy, safety, and immunogenicity of the *Shigella sonnei* 1790GAHB GMMA candidate vaccine: results from a phase 2b randomized,

- placebo-controlled challenge study in adults. *EClinicalMedicine* **2021**; 39:101076.
17. Cohen D, Atsmon J, Artaud C, et al. Safety and immunogenicity of a synthetic carbohydrate conjugate vaccine against *Shigella flexneri* 2a in healthy adult volunteers: a phase 1, dose-escalating, single-blind, randomised, placebo-controlled study. *Lancet Infect Dis* **2021**; 21:546–58.
 18. Cohen D, Meron-Sudai S, Bialik A, et al. Serum IgG antibodies to *Shigella* lipopolysaccharide antigens - a correlate of protection against shigellosis. *Hum Vaccin Immunother* **2019**; 15:1401–8.
 19. Kaminski RW, Pasetti MF, Aguilar AO, et al. Consensus report on *Shigella* controlled human infection model: immunological assays. *Clin Infect Dis* **2019**; 69:596–601.
 20. Giersing BK, Porter CK, Kotloff K, Neels P, Cravioto A, MacLennan CA. How can controlled human infection models accelerate clinical development and policy pathways for vaccines against *Shigella*? *Vaccine* **2019**; 37:4778–83.
 21. Chen WH, Cohen MB, Kirkpatrick BD, et al. Single-dose live oral cholera vaccine CVD 103-HgR protects against human experimental infection with *Vibrio cholerae* O1 El Tor. *Clin Infect Dis* **2016**; 62:1329–35.
 22. Jin C, Gibani MM, Moore M, et al. Efficacy and immunogenicity of a Vi-tetanus toxoid conjugate vaccine in the prevention of typhoid fever using a controlled human infection model of *Salmonella* Typhi: a randomised controlled, phase 2b trial. *Lancet* **2017**; 390: 2472–80.
 23. World Health Organization. Typhoid vaccine prequalified. Available at: <https://www.who.int/medicines/news/2017/WHOprequalifies-breakthrough-typhoid-vaccine/en/>. Accessed 6 June 2020.
 24. Lin FY, Ho VA, Khiem HB, et al. The efficacy of a *Salmonella* typhi Vi conjugate vaccine in two-to-five-year-old children. *N Engl J Med* **2001**; 344:1263–9.