

Shigella Vaccine Development: Finding the Path of Least Resistance

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Shigella spp. represent the second most common etiologic pathogen causing childhood diarrhea in developing countries. There are no licensed *Shigella* vaccines, and progress for such vaccines has been limited. In this issue of *Clinical and Vaccine Immunology*, Riddle and colleagues (M. S. Riddle, R. W. Kaminski, C. Di Paolo, C. K. Porter, R. L. Gutierrez, et al., *Clin Vaccine Immunol* 23:908–917, 2016, <http://dx.doi.org/10.1128/CVI.00224-16>) report results from a phase I study of a parenterally administered monovalent O-polysaccharide “bioconjugate” directed against *Shigella flexneri* 2a. Ultimately, the goal is to develop a broad-spectrum *Shigella* vaccine to address this public health concern. A parenteral *Shigella* vaccine capable of eliciting protection in children of developing countries would be an important tool to reach this goal.

Diarrheal disease causes 1 in 11 deaths worldwide, comprising the second most common cause of mortality in children <5 years of age, most of which occur in developing countries (1). Introduction of a rotavirus vaccine into Gavi-eligible countries will ensure continued progress in diminishing this burden (2); however, prevention and treatment of other important pathogens remain elusive. The Global Enteric Multicenter Study (GEMS) identified *Shigella* as one of the top two etiologic agents among toddlers and young children with moderate to severe diarrheal disease (MSD) seeking health care in seven developing countries in sub-Saharan Africa and South Asia (3). Similarly, a multisite birth cohort study (MAL-ED) conducted in eight countries in South America, Africa, and Asia identified *Shigella* as an important cause of moderate and severe diarrhea and dysentery in the community during the second year of life (4). Reanalysis of the GEMS data using molecular diagnostic tools suggested that these culture-based burden estimates substantially underrepresent the true burden of *Shigella* (5, 6). Moreover, military personnel and other travelers from high-resource countries who visit these endemic settings are also at risk for acquiring *Shigella* infections and disseminating them upon returning home. The global spread of multidrug-resistant *Shigella* has been traced to the wide dissemination of single clones (7), and this ease of transmission threatens the ability to treat shigellosis with antibiotics that are effective, readily available, and affordable. Because of the limitations of current efforts to control the spread of *Shigella*, development of a *Shigella* vaccine is considered a public health priority. Nonetheless, development of a *Shigella* vaccine has been an arduous undertaking (8, 9), and no candidate is currently commercially available.

There is general agreement that a safe and effective *Shigella* vaccine must contain several features. Considerable evidence for serotype-specific immunity suggests that the O-specific polysaccharide of *Shigella* is an essential protective antigen (10–13). The efficacy of a parenteral *Shigella sonnei* O-specific polysaccharide conjugate vaccine in preventing *S. sonnei* disease among Israeli soldiers (albeit an immunologically primed population) lent further credence to this concept (14). In this issue of *Clinical and Vaccine Immunology*, Riddle and colleagues investigated a monovalent O-polysaccharide-based “bioconjugate” vaccine against *Shigella flexneri* 2a (15). While there is no firm correlate of immunity, there appears to be a strong association between the level of serum lipopolysaccharide (LPS)-specific IgG antibodies preexpo-

sure and protection against serotype-specific infection (12, 16). The vigorous anti-LPS responses induced by the bioconjugate vaccine in this trial are thus very encouraging, with 14- and 16-fold increases in IgA and IgG, respectively, compared to baseline and seroconversion in 92 to 100% of subjects. Moreover, there was evidence of functional (bactericidal) antibody responses in the majority of subjects, and the vaccine was well-tolerated.

Another correlate of immunoprotection is the induction of gut-homing anti-LPS antibody-secreting cells which can be detected in the circulation approximately 1 week after oral immunization, challenge, or infection as an indicator of intestinal immune priming (11, 17, 18). Antibodies in lymphocyte supernatants (ALS) have also been evaluated in *Shigella* vaccine studies, as they parallel circulating antibody-secreting cell (ASC) responses (19). The *S. flexneri* 2a bioconjugate vaccine evaluated by Riddle et al. elicited antibodies in lymphocyte supernatants assays (ALS) for most subjects 7 days after the first immunization, although the significance of these responses with regard to protective immunity is not yet known. In comparing oral versus parenteral vaccination to prevent infection with an enteric pathogen, Kantele et al. examined the tissue-homing profiles of plasmablasts stimulated by live oral (Ty21a) and parenteral (Vi) typhoid vaccines (20). Although similar numbers of typhoid-specific plasmablasts were observed in the circulation following receipt of either vaccine, oral Ty21a elicited a gut mucosal homing response while the parenteral Vi vaccine demonstrated a systemic homing profile, reflecting the expected localization of the immune effector cells produced. Since both vaccines elicit protective immunity, these results suggest that typhoid immunity can be achieved by two very different mechanisms. Although homing markers were not measured in the *Shigella* bioconjugate vaccine trial, it is quite possible that a systemic

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plasmablast pattern would be observed, whereas, like typhoid, oral *Shigella* vaccines and natural infection stimulate an intestinal B cell homing profile. More extensive analyses of these ASC homing profiles, ALS responses, and protective immunity will be required to determine the significance of these responses following administration of parenteral *Shigella* vaccines.

Another essential feature of a *Shigella* vaccine would be its ability to prevent the majority of clinically significant *Shigella* illnesses, in the context of the fact that there are ~50 serotypes and subserotypes that cause human infections. GEMS data suggest that a vaccine containing *S. sonnei* and three of the 15 *S. flexneri* serotypes (2a, 3a, and 6) could provide direct protection against ~64% of *Shigella* strains causing MSD in children from developing countries (21). The observations of Noriega et al., who used a guinea pig keratoconjunctivitis model in their study, further suggest that heterologous protection among *S. flexneri* serotypes could be elicited based on shared group- and type-specific moieties on the O-antigen, (22), so that a quadrivalent vaccine containing *S. sonnei* and *S. flexneri* 2a, 3a, and 6 could provide overall coverage (direct plus cross-reactive) of up to 88% of *Shigella* strains. This forms the basis of the multivalent vaccine strategy pursued by Riddle et al. and other *Shigella* vaccine developers.

The optimal route of immunization continues to be a subject of debate. Since natural and experimental *Shigella* infections in humans confer immunity following oral inoculation, it was reasoned that live oral vaccines could produce similar results. Seminal field studies conducted in the 1960s by David Mel and colleagues provided the first clues that protection could be achieved through oral vaccination with attenuated strains of *Shigella*. These noninvasive, streptomycin-dependent *Shigella* strains induced high-level protection in adults (23) and children 2 to 8 years of age (24) during field trials in Yugoslavia. However, multiple doses of large inocula were required, immunity was short-lived (25), and the strains were genetically unstable (26). During the ensuing decades, investigators have applied molecular techniques to develop rationally engineered live *Shigella* strains as oral vaccines. Strains which retain their enteroinvasive properties are thought to be capable of stimulating broader, more vigorous immune responses that could be dose sparing compared to the streptomycin-dependent vaccines. Two approaches involving replicating, invasive vaccines are in clinical trials. One approach is based on fundamental mutations creating guanine auxotrophy (*guaBA*) and the genes encoding *Shigella* enterotoxins (27–29), and the other involves mutations in *virG* that limit cell-to-cell spread of *Shigella* in the intestinal epithelium (30, 31). A formalin-killed oral whole-cell vaccine is also under development (32). The challenge with oral vaccines has been in finding the optimal balance between reactogenicity and immunogenicity. Furthermore, vaccines which appear immunogenic but somewhat reactogenic in volunteers from high-resource settings have been well-tolerated but overattenuated when given to adults and children in developing countries (33, 34). Reviews of the pitfalls and successes of *Shigella* vaccine candidates under development have been published (8, 35–37). Nonetheless, there is hope that newer generations of these constructs can be both well-tolerated and immunogenic.

Alternatively, other investigators have hypothesized that the barriers facing mucosal vaccination can be overcome by intramuscular *Shigella* O-polysaccharide vaccines. Robbins and Schneerson pioneered the concept that vaccines which stimulate high levels of serum IgG antibodies are capable of conferring protective

immunity against invasive as well as mucosal pathogens by a process involving transudation of specific antibodies to the mucosal surface and killing of the inoculum in the intestine (38). They recognized that antibodies to capsular polysaccharides confer protective immunity to many infections. However, T-cell-independent antigens are poorly immunogenic in young children (who are often most at risk for disease), fail to induce IgM-to-IgG class switching, and do not elicit T-cell memory. This limitation could be overcome with covalent attachment of polysaccharide to proteins. Consequently, these investigators developed chemical conjugation chemistry methods to synthesize glycoconjugate vaccines against a variety of organisms (39) and reported their ability to prevent disease as well as pharyngeal carriage of the pathogen (40, 41). In recognizing similarities between age-related protective immunity to *Shigella* infections and immunity to other infections in which the protective antigen is capsular polysaccharide, they generated vaccine candidates by using *S. sonnei*, *S. flexneri* 2a, and *Shigella dysenteriae* type 1 O-specific polysaccharides conjugated to recombinant exotoxin A of *Pseudomonas aeruginosa* (rEPA). In clinical trials, these O-polysaccharide conjugate vaccines appeared safe and immunogenic in adults (42–44) and in children 4 to 7 years of age (45), but the antibody responses were lower for children ≤3 years of age (46, 47). A field trial among Israeli army recruits documented 74% homologous efficacy following a single dose of the *S. sonnei*-rEPA vaccine infection (14). However, when efficacy was evaluated in a phase III trial with children, protection from *S. sonnei* was 71% among children 3 to 4 years old but only 35.5% among children 2 to 3 years old, and there was no protection demonstrated among children 1 to 2 years of age (47).

In this issue of *Clinical and Vaccine Immunology*, Riddle and colleagues present results from a phase I trial of a parenterally administered *S. flexneri* 2a O-polysaccharide bioconjugate vaccine (15). As opposed to traditional purification followed by detoxification of LPS and then chemical conjugation to a protein carrier, this candidate vaccine was biosynthesized using recombinant *Escherichia coli* expressing the *Campylobacter jejuni* oligosaccharyltransferase PglB, which transfers the O-antigen repeating unit of *S. flexneri* 2a LPS to asparagine residues of the periplasmic carrier protein EPA, resulting in stable N-glycosidic linkage (48). Chemical conjugation methods have achieved considerable success but require multistep procedures, resulting in an expensive process that is prone to producing lot-to-lot heterogeneity. Bioconjugation, on the other hand, is expected to be less expensive with more uniformity of production. Immunogenicity was not improved in the alum-adjuvanted formulation, and a booster effect was not seen following a second dose, as was the case following the chemically conjugate *S. flexneri* 2a-EPA vaccines of Robbins and Schneerson (45). The ability to administer a single, unadjuvanted dose of the bioconjugate vaccine would certainly enhance its economic favorability.

Shigella vaccines that appear well-tolerated and immunogenic in high-resource settings but falter when given to young children in developing countries are a path well-trodden. Further studies will be needed to address whether the bioconjugate vaccine can elicit protective immunity among young, unprimed children living in developing countries, who bear the greatest burden disease. Fortunately, the bioconjugate *S. flexneri* 2a vaccine is off to a strong start, with the promise of clinical tolerability, strong immunogenicity, and the potential for efficient, low-cost manufacturing.

There are other hurdles that must be cleared. For one, in working toward a broad-spectrum *Shigella* vaccine, bioconjugates representing *S. flexneri* 3a and 6 and *S. sonnei* will need to be evaluated in clinical trials. The incidence of shigellosis peaks in children 2 to 4 years of age, perhaps creating a need for more enduring protection than required for other pediatric vaccines. Thus, investigation of the induction of anti-LPS B memory cell responses, which would provide functional antibody at the mucosal surface, is warranted (49). A final consideration is the recognition that enterotoxigenic *Escherichia coli* (ETEC) represents another major etiologic bacterial pathogen responsible for MSD in children of developing countries (3). There has been growing interest in the cost:benefit ratio for developing combination *Shigella* and ETEC vaccines (50, 51). Consequently, in working toward a broad-spectrum *Shigella*/ETEC vaccine, there might be consideration for these *Shigella* O-polysaccharide conjugates to be coadministered with candidate parenteral ETEC vaccines. Ultimately, we wonder whether the use of a parenteral route of administration for eliciting protection against a gastrointestinal mucosal pathogen is the path of least resistance in our collective pursuit of an effective *Shigella* vaccine.

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