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 1 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 underestimate the true burden of Shigella (5, 6). Moreover, military personnel and other travelers from high-resource countries who visit these endemic settings are 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-
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 Shigellosis infections in humans confer immunity following oral inoculation, it was reasoned that live oral vaccines could produce similar results. Semiinal 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 reactivogenic 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, 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 oligosaccharyltrasferase 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.

REFERENCES

1. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black RE. 2015. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet 385:430–440. http://dx.doi.org/10.1016/S0140-6736(14)61698-8.

2. Lee LA, Franzel L, Atwell J, Datta SD, Friberg IK, Goldie SJ, Reef SE, Schwalle N, Simons E, Strebel PM, Sweet S, Suraratdecha C, Tam Y, Vynnucy E, Walker N, Walker DG, Hansen PM. 2013. The estimated mortality impact of vaccinations forecast to be administered during 2011-2020 in 73 countries supported by the Gavi Alliance. Vaccine 31(Suppl 2):B61–B72. http://dx.doi.org/10.1016/j.vaccine.2012.11.035.

3. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchal K, Saha D, Zhao Z, Gu D, Lin JS, Gormley R, Gambillara Fonck V, Clarkson KA, Weerts HE, Duplessis C, Castellano A, Paolino A, Akhter S, Bodhidatta L, Gratz J, Haque R, Hatt V, McCormick BJ, McGrath M, Olorotegui MP, Samie A, Shakoor S, Mondal D, Lima IF, Hariraju D, Rayamajhi BH, Qureshi S, Kabir F, Yori PP, Mumafami B, Amour C, Carreon JD, Richard SA, Lang D, Bessong P, Mduma E, Hassan JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA, Antonio M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acacio S, Biswas K, O’Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Herrington D, Jernigan DB, Robins-Browne RM, Levine MM. 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, aetiology-based study. Lancet 383:1–67. http://dx.doi.org/10.1016/S0140-6736(13)60197-0.

4. Panchalingam S, Kalam A, Aziz F, Qureshi S, Ramamurthy T, Roberts BH, Saha D, Sow SO, Group S, Sow D, Tamboura B, Taniuchi M, Tentam SM, Toema D, Wu Y, Zaidi A, Nataro JP, Kotloff KL, Levine MM, Houpert ER. 2016. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. Lancet 388:1291–1303. http://dx.doi.org/10.1016/S0140-6736(16)31529-X.

5. The HC, Rabaa MA, Thanh DP, De Lappe N, Cornmich M, Valcans M, Howden BP, Wangchuk S, Bodhidatta L, Mason CJ, Nguyen TN, Thuy DV, Thompson CN, Lan NP, Vinh PV, Thanh TH, Turner P, Sar P, Thwaites G, Thompson RT, Holt KE, Baker S. 2016. South Asia as a reservoir for the global spread of ciprofloxacin-resistant Shigella sonnei: a cross-sectional study. PLoS Med 13:e1002055. http://dx.doi.org/10.1371/journal.pmed.1002055.

6. Barry EM, Pasetti MF, Szttein MB, Fasano A, Kotloff KL, Levine MM. 2013. Progress and pitfalls in Shigella vaccine research. Nat Rev Gastroenterol Hepatol 10:245–255. http://dx.doi.org/10.1038/nrgastro.2013.12.

7. Levine MM, Kotloff KL, Barry EM, Pasetti MF, Szttein MB. 2007. Clinical trials of Shigella vaccines: two steps forward and one step back on a long, hard road. Nat Rev Microbiol 5:540–553. http://dx.doi.org/10.1038/nrmicro1662.

8. Herrington DA, Van de Verg L, Formal SB, Hale TL, Tall BD, Cryz SJ, Tramont EC, Levine MM. 1990. Studies in volunteers to evaluate candidate Shigella vaccines: further experience with a bivalent Salmonella typhi-Shigella sonnei vaccine and protection conferred by previous Shigella sonnei disease. Vaccine 8:353–357. http://dx.doi.org/10.1016/S0264-410X(98)00094-3.

9. Kotloff KL, Losonsky GA, Nataro JP, Wasserman SS, Hale TL, Taylor DN, Newland JW, Sadof JC, Formal SB, Levine MM. 1995. Evaluation of the safety, immunogenicity, and efficacy in healthy adults of four doses of oral hybrid Escherichia coli-Shigella flexneri 2a vaccine strain EcSf2a-2. Vaccine 13:495–502. http://dx.doi.org/10.1016/0264-410X(94)00011-B.

10. Cohen D, Green MS, Block C, Rouach T, Ofek I. 1988. Serum antibodies to lipopolysaccharide and natural immunity to shigellosis in an Israeli military population. J Infect Dis 157:1068–1071. http://dx.doi.org/10.1093/infdis/157.5.1068.

11. Ferreccio C, Prado V, Ojeda A, Cayayo M, Abrego P, Guers L, Levine MM. 1991. Epidemiologic patterns of acute diarrhea and endemic Shigella infections in children in a poor periurban setting in Santiago, Chile. Am J Epidemiol 134:614–627.

12. Cohen D, Ashkenazi S, Green MS, Gadlevich M, Robin G, Slepson R, Yavorzi M, Orr N, Block C, Ashkenazi I, Shemer J, Taylor DN, Hale TL, Sadof JC, Pavliakova D, Schnerson R, Robbins JB. 1997. Double-blind vaccine-controlled randomised efficacy trial of an investigational Shigella sonnei conjugate vaccine in young adults. Lancet 349:155–159. http://dx.doi.org/10.1016/S0140-6736(96)90825-1.

13. Elbasha EP, Kaminiska G, Di Paolo C, Porter CK, Gutierrez RL, Clarkson KA, Weerts HE, Duplessis C, Castellano A, Alaimo C, Paolino K, Gormley R, Gambillara Fonck V. 2016. Safety and immunogenicity of a candidate conjugate vaccine against Shigella flexneri 2a administered to healthy adults: a single-blind, randomized phase 1 study. Clin Vaccine Immunol 23:908–917. http://dx.doi.org/10.1128/CVI.00224-16.

14. Cohen D, Green MS, Block C, Slepson R, Ofek I. 1991. Prospective study of the association between serum antibodies to lipopolysaccharide O antigen and the attack rate of shigellosis. J Clin Microbiol 29:386–389.

15. Toapanta FR, Simon JK, Barry EM, Pasetti MF, Levine MM, Szttein MB. 2014. Gut-homing conventional plasmablasts and CD27+ plasmablasts elicited after a short time of exposure to an oral live-attenuated Shigella vaccine candidate in humans. Front Immunol 5:374. http://dx.doi.org/10.3389/fimmu.2014.00374.

16. Sinha A, Dey A, Saletti G, Samanta P, Chakraborty PS, Bhattacharya MK, Ghosh S, Ramamurthy T, Kim JO, Yang JS, Kim DW, Czerkinsky C, Nandy RK. 2016. Circulating gut-homing (α(β)7+) plasmablast responses against Shigella surface protein antigens among hospitalized patients with diarrhea. Clin Vaccine Immunol 23:610–617. http://dx.doi.org/10.1128/CVI.00205-16.

17. Clements JD, Freytag LA, Szttein MB. 2016. Parenteral vaccination can be an effective means of inducing protective mucosal responses. Clin Vaccine Immunol 23:438–441. http://dx.doi.org/10.1128/CVI.00214-16.

18. Kantele A, Pakkanen SH, Kuttunen R, Kantele JM. 2013. Head-to-head comparison of humoral immune responses to Vi capsular polysaccharide and Salmonella Typhi Ty21a typhoid vaccines—a randomized trial. PLoS One 8:e60583. http://dx.doi.org/10.1371/journal.pone.0060583.

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21. Livio S, Stockbleine NA, Panchalangan S, Tennant SM, Barry EM, Marohn ME, Antonio M, Hossain A, Mandomando I, Ochieng JB, Oundo JO, Qureshi S, Ramamurthy T, Tamboura B, Adegbola RA, Hossain MJ, Saha D, Sen S, Faruque AS, Alonso PL, Breiman RF, Zaidi AK, Sur D, Sow SO, Berkeley LT, O'Reilly CE, Mintz ED, Biswas K, Cohen D, Fauci AS, Nasrin D, Wu Y, Blackwelder WC, Kotloff KL, Nataro JP, Levine MM. 2014. Shigella isolates from the global enteric multicenter study inform vaccine development. Clin Infect Dis 59:933–941. http://dx.doi.org/10.1093/cid/ciu468.

22. Noriega FR, Liao FM, Maneval DR, Ren S, Formal SB, Levine MM. 1999. Strategy for cross-protection among Shigella flexneri serotypes. In: Infect Immun 67:782–788.

23. Mel DM, Terzijn AL, Vuksic L. 1965. Studies on vaccination against bacillary dysentery. 3. Effective oral immunization against Shigella flexneri 2a in a field trial. Bull World Health Organ 32:647–655.

24. Mel, Gangarosa EJ, Radovanovic ML, Arsic BL, Litvinjenko S. 2016. Vaccine development for Shigella flexneri type 2a. In: J Infect Dis 119:704–707.

25. Mel DM, Arsic BL, Radovanovic ML, Litvinjenko SA. 1974. Live oral Shigella vaccine: vaccine schedule and the effect of booster dose. Acta Microbiol Acad Sci Hung 21:109–114.

26. Levine MM, Gangarosa EJ, Barrow WB, Morris GK, Wells JG, Weiss CF. 1975. Shigellosis in custodial institutions. IV. In vivo stability and transmissibility of oral attenuated streptomycin-dependent Shigella vaccines. J Infect Dis 131:221–226.

27. DeLaine BC, Wu T, Grassel CL, Shimanoovich A, Pasetti MF, Levine MM, Barry EM. 2016. Characterization of a multicomponent live, attenuated Shigella flexneri vaccine. Pathog Dis 74:60034. http://dx.doi.org/10.1139/femspd/ftw034.

28. Kotloff KL, Pasetti MF, Barry EM, Nataro JP, Wasserman SS, Szein MB, Picking WD, Levine MM. 2004. Deletion in the Shigella enterotoxin genes further attenuates Shigella flexneri 2a bearing guanine auxotrophy. J Infect Dis 190:1745–1754. http://dx.doi.org/10.1086/424680.

29. Kotloff KL, Simon JK, Pasetti MF, Szein MB, Wooden SL, Livio S, Nataro JP, Blackwelder WC, Barry EM, Picking W, Levine MM. 2007. Safety and immunogenicity of CVD 1208S, a live, oral DeltaguaBA Delta-sonnei vaccine candidate, WRSS1, in Thai adults. Clin Vaccine Immunol 14:379–386. http://dx.doi.org/10.1128/CVI.00608-15.

30. Coster HC, Hoge CW, VanDeVerg LL, Hartman AB, Oaks EV, Venkatesan MM, Cohen D, Robin G, Fontaine-Thompson A, Sansonetti PJ, Hale TL. 1999. Vaccination against shigellosis with attenuated Shigella flexneri 2a bearing guanine auxotrophy. J Infect Dis 179:1387–1398. http://dx.doi.org/10.1086/314759.

31. Adams DG, Weaver KA, Cocihi SL, Plikaytis BD, Zell ER, Broome CV, Wengard JD. 1993. Decline of childhood Haemophilus influenzae type b (Hib) disease in the Hib vaccine era. JAMA 269:221–226.

32. Takala AK, Esola J, Leinonen M, Kayhty H, Nissinen A, Pekkanen E, Makela PH. 1991. Reduction of oropharyngeal carriage of Haemophilus influenzae type b (Hib) in children immunized with an Hib conjugate vaccine. J Infect Dis 164:982–986. http://dx.doi.org/10.1093/infdis/164.5.982.

33. Taylor DN, Trofa AC, Sadoff J, Chu C, Bryla D, Shiloach J, Cohen D, Ashkenazi S, Lerman Y, Egan W, Schneer J, Robbins R, 1993. Synthesis, characterization, and clinical evaluation of conjugate vaccines composed of the O-specific polysaccharides of Shigella dysenteriae type 1, Shigella flexneri type 2a, and Shigella sonnei (Plesiomonas shigelloides) bound to bacterial toxoids. Infect Immun 61:3678–3687.

34. Cohen D, Ashkenazi S, Green M, Lerman Y, Slepon R, Robin G, Orr N, Taylor DN, Sadoff JC, Chu C, Shiloach J, Schneer J, Robbins R, 1996. Safety and immunogenicity of investigational Shigella conjugate vaccines in Israeli volunteers. Infect Immun 64:4074–4077.

35. Passwell JH, Harleve E, Ashkenazi S, Chu C, Miron D, Ramon R, Farzan N, Shiloach J, Bryla DA, Majdly F, Roberson R, Robbins JB, Schneer R 2001. Safety and immunogenicity of improved Shigella O-specific polysaccharide-protein conjugate vaccines in adults in Israel. Infect Immun 69:1351–1357. http://dx.doi.org/10.1128/IAI.69.8.1351-1357.2001.

36. Ashkenazi S, Passwell JH, Harleve E, Miron D, Dagan R, Farzan N, Ramon R, Majdly F, Bryla DA, Karpas AB, Robbins JB, Schneer R. 1999. Safety and immunogenicity of Shigella sonnei and Shigella flexneri 2a O-specific polysaccharide conjugates in children. J Infect Dis 179:1565–1568. http://dx.doi.org/10.1086/314759.

37. Passwell JH, Ashkenazi S, Harleve E, Miron D, Ramon R, Farzan N, Lerner-Geva L, Levy Y, Chu C, Shiloach J, Robbins J, Schneer R, Israel Shigella Study G. 2003. Safety and immunogenicity of Shigella sonnei-CRM9 and Shigella flexneri type 2a-REPAucc conjugate vaccines in one- to four-year-old children. Pediatr Infect Dis J 22:701–706. http://dx.doi.org/10.1097/01.inf.0000078156.03697.a5.

38. Passwell JH, Ashkenazi S, Banet-Levi Y, Ronan-Saraf R, Farzan N, Lerner-Geva L, Even-Nir H, Yerushalmi B, Chu C, Shiloach J, Robbins JB, Schneer R, Israel Shiella Study Group. 2010. Age-related efficacy of Shigella O-specific polysaccharide conjugates in 1-4-year-old Israeli children. Vaccine 28:2231–2235. http://dx.doi.org/10.1016/j.vaccine.2009.12.050.

39. Ravenscroft N, Haeuptle MA, Kowarik M, Fernandez FS, Carranza P, Brunner A, Steffen M, Wetter M, Keller S, Ruch C, Wacker M. 2016. Purification and characterization of a Shigella conjugate vaccine, produced by glycoengineering Escherichia coli. Glycobiology 26:51–62. http://dx.doi.org/10.1093/glycob/cwv077.

40. Wahid R, Simon JK, Picking W, Kotloff KL, Levine MM, Szein MB. 2013. Shigella antigen-specific B memory cells are associated with decreased disease severity in subjects challenged with wild-type Shigella flexneri 2a. Clin Immunol 148:35–43. http://dx.doi.org/10.1016/j.clim.2013.03.009.

41. Walker RI, Clifford A. 2015. Recommendations regarding the development of combined enterotoxigenic Escherichia coli and Shigella vaccines for infants. Vaccine 33:946–953. http://dx.doi.org/10.1016/j.vaccine.2014.11.048.

42. Walker RI. 2015. An assessment of enterotoxigenic Escherichia coli and Shigella vaccine candidates for infants and children. Vaccine 33:954–965. http://dx.doi.org/10.1016/j.vaccine.2014.11.049.