AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical and Vaccine Immunology

COMMENTARY

Shigella Vaccine Development: Finding the Path of Least Resistance

Wilbur H. Chen,[a,c] Karen L. Kotloff[a,b,c]

Departments of Medicine[a] and Pediatrics[b] and Center for Vaccine Development,[c] University of Maryland School of Medicine, Baltimore, Maryland, USA

***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 preexposure 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

Accepted manuscript posted online 5 October 2016

Citation Chen WH, Kotloff KL. 2016. *Shigella* vaccine development: finding the path of least resistance. Clin Vaccine Immunol 23:904–907. doi:10.1128/CVI.00444-16.

Editor: D. L. Burns, Food and Drug Administration

Address correspondence to Wilbur H. Chen, wchen@medicine.umaryland.edu.

For the article discussed, see doi:10.1128/CVI.00224-16.

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* 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)61698-6.
2. **Lee LA, Franzel L, Atwell J, Datta SD, Friberg IK, Goldie SJ, Reef SE, Schwalbe N, Simons E, Strebel PM, Sweet S, Suraratdecha C, Tam Y, Vynnycky 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, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, Faruque AS, Zaidi AK, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R, Oundo 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, Sommerfelt H, 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, case-control study. Lancet **382:**209–222. http://dx.doi.org/10.1016/S0140-6736(13)60844-2.
4. **Platts-Mills JA, Babji S, Bodhidatta L, Gratz J, Haque R, Havt A, McCormick BJ, McGrath M, Olortegui MP, Samie A, Shakoor S, Mondal D, Lima IF, Hariraju D, Rayamajhi BB, Qureshi S, Kabir F, Yori PP, Mufamadi B, Amour C, Carreon JD, Richard SA, Lang D, Bessong P, Mduma E, Ahmed T, Lima AA, Mason CJ, Zaidi AK, Bhutta ZA, Kosek M, Guerrant RL, Gottlieb M, Miller M, Kang G, Houpt ER, Investigators M-EN.** 2015. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). Lancet Glob Health **3:**e564–e575. http://dx.doi.org/10.1016/S2214-109X(15)00151-5.
5. **Lindsay B, Saha D, Sanogo D, Das SK, Omore R, Farag TH, Nasrin D, Li S, Panchalingam S, Levine MM, Kotloff K, Nataro JP, Magder L, Hungerford L, Faruque AS, Oundo J, Hossain MA, Adeyemi M, Stine OC.** 2015. Association between Shigella infection and diarrhea varies based on location and age of children. Am J Trop Med Hyg **93:**918–924. http://dx.doi.org/10.4269/ajtmh.14-0319.
6. **Liu J, Platts-Mills JA, Juma J, Kabir F, Nkeze J, Okoi C, Operario DJ, Uddin J, Ahmed S, Alonso PL, Antonio M, Becker SM, Blackwelder WC, Breiman RF, Faruque AS, Fields B, Gratz J, Haque R, Hossain A, Hossain MJ, Jarju S, Qamar F, Iqbal NT, Kwambana B, Moandomando I, McMurray TL, Ochieng C, Ochieng JB, Ochieng M, Onyango C, Panchalingam S, Kalam A, Aziz F, Qureshi S, Ramamurthy T, Roberts JH, Saha D, Sow SO, Stroup S, Sur D, Tamboura B, Taniuchi M, Tennant SM, Toema D, Wu Y, Zaidi A, Nataro JP, Kotloff KL, Levine MM, Houpt 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/S0140-6737(16)31529-X.
7. **The HC, Rabaa MA, Thanh DP, De Lappe N, Cormican M, Valcanis 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.
8. **Barry EM, Pasetti MF, Sztein 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.
9. **Levine MM, Kotloff KL, Barry EM, Pasetti MF, Sztein 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.
10. **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/10.1016/0264-410X(90)90094-3.
11. **Kotloff KL, Losonsky GA, Nataro JP, Wasserman SS, Hale TL, Taylor DN, Newland JW, Sadoff JC, Formal SB, Levine MM.** 1995. Evaluation of the safety, immunogenicity, and efficacy in healthy adults of four doses of live 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.
12. **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.
13. **Ferreccio C, Prado V, Ojeda A, Cayyazo 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.
14. **Cohen D, Ashkenazi S, Green MS, Gdalevich M, Robin G, Slepon R, Yavzori M, Orr N, Block C, Ashkenazi I, Shemer J, Taylor DN, Hale TL, Sadoff JC, Pavliakova D, Schneerson 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)06255-1.
15. **Riddle MS, Kaminski RW, 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 bioconjugate vaccine against *Shigella flexneri* 2a administered to healthy adults: a single-blind, randomized phase I study. Clin Vaccine Immunol **23:**908–917. http://dx.doi.org/10.1128/CVI.00224-16.
16. **Cohen D, Green MS, Block C, Slepon 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.
17. **Toapanta FR, Simon JK, Barry EM, Pasetti MF, Levine MM, Kotloff KL, Sztein 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.
18. **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 ($\alpha_4\beta7^+$) 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.
19. **Clements JD, Freytag LC.** 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.
20. **Kantele A, Pakkanen SH, Karttunen 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.

21. **Livio S, Strockbine NA, Panchalingam 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 LY, O'Reilly CE, Mintz ED, Biswas K, Cohen D, Farag TH, 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. Infect Immun **67:**782–788.
23. **Mel DM, Terzin 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 D, Gangarosa EJ, Radovanovic ML, Arsic BL, Litvinjenko S.** 1971. Studies on vaccination against bacillary dysentery. 6. Protection of children by oral immunization with streptomycin-dependent Shigella strains. Bull World Health Organ **45:**457–464.
25. **Mel DM, Arsic BL, Radovanovic ML, Litvinjenko SA.** 1974. Live oral Shigella vaccine: vaccination 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:**704–707.
27. **DeLaine BC, Wu T, Grassel CL, Shimanovich A, Pasetti MF, Levine MM, Barry EM.** 2016. Characterization of a multicomponent live, attenuated Shigella flexneri vaccine. Pathog Dis **74:**fw034. http://dx.doi.org/10.1093/femspd/ftw034.
28. **Kotloff KL, Pasetti MF, Barry EM, Nataro JP, Wasserman SS, Sztein MB, Picking WD, Levine MM.** 2004. Deletion in the Shigella enterotoxin genes further attenuates Shigella flexneri 2a bearing guanine auxotrophy in a phase I trial of CVD 1204 and CVD 1208. J Infect Dis **190:**1745–1754. http://dx.doi.org/10.1086/424680.
29. **Kotloff KL, Simon JK, Pasetti MF, Sztein 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 Deltasen Deltaset Shigella flexneri 2a vaccine grown on animal-free media. Hum Vaccin **3:**268–275. http://dx.doi.org/10.4161/hv.4746.
30. **Kotloff KL, Taylor DN, Sztein MB, Wasserman SS, Losonsky GA, Nataro JP, Venkatesan M, Hartman A, Picking WD, Katz DE, Campbell JD, Levine MM, Hale TL.** 2002. Phase I evaluation of delta virG Shigella sonnei live, attenuated, oral vaccine strain WRSS1 in healthy adults. Infect Immun **70:**2016–2021. http://dx.doi.org/10.1128/IAI.70.4.2016-2021.2002.
31. **Coster TS, 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 strain SC602. Infect Immun **67:**3437–3443.
32. **Chakraborty S, Harro C, DeNearing B, Bream J, Bauers N, Dally L, Flores J, Van de Verg L, Sack DA, Walker R.** 2016. Evaluation of the safety, tolerability, and immunogenicity of an oral, inactivated whole-cell Shigella flexneri 2a vaccine in healthy adult subjects. Clin Vaccine Immunol **23:**315–325. http://dx.doi.org/10.1128/CVI.00608-15.
33. **Rahman KM, Arifeen SE, Zaman K, Rahman M, Raqib R, Yunus M, Begum N, Islam MS, Sohel BM, Rahman M, Venkatesan M, Hale TL, Isenbarger DW, Sansonetti PJ, Black RE, Baqui AH.** 2011. Safety, dose, immunogenicity, and transmissibility of an oral live attenuated Shigella flexneri 2a vaccine candidate (SC602) among healthy adults and school children in Matlab, Bangladesh. Vaccine **29:**1347–1354. http://dx.doi.org/10.1016/j.vaccine.2010.10.035.
34. **Pitisuttithum P, Islam D, Chamnanchanunt S, Ruamsap N, Khantapura P, Kaewkungwal J, Kittitrakul C, Luvira V, Dhitavat J, Venkatesan MM, Mason CJ, Bodhidatta L.** 2016. Clinical trial of an oral live Shigella sonnei vaccine candidate, WRSS1, in Thai adults. Clin Vaccine Immunol **23:**564–575. http://dx.doi.org/10.1128/CVI.00665-15.
35. **Mani S, Wierzba T, Walker RI.** 2016. Status of vaccine research and development for Shigella. Vaccine **34:**2887–2894. http://dx.doi.org/10.1016/j.vaccine.2016.02.075.
36. **Camacho AI, Irache JM, Gamazo C.** 2013. Recent progress towards development of a Shigella vaccine. Expert Rev Vaccines **12:**43–55. http://dx.doi.org/10.1586/erv.12.135.
37. **Kaminski RW, Oaks EV.** 2009. Inactivated and subunit vaccines to prevent shigellosis. Expert Rev Vaccines **8:**1693–1704. http://dx.doi.org/10.1586/erv.09.127.
38. **Robbins JB, Schneerson R, Szu SC.** 1995. Perspective: hypothesis: serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. J Infect Dis **171:**1387–1398. http://dx.doi.org/10.1093/infdis/171.6.1387.
39. **Robbins JB, Schneerson R.** 1990. Polysaccharide-protein conjugates: a new generation of vaccines. J Infect Dis **161:**821–832. http://dx.doi.org/10.1093/infdis/161.5.821.
40. **Adams WG, Deaver KA, Cochi SL, Plikaytis BD, Zell ER, Broome CV, Wenger JD.** 1993. Decline of childhood Haemophilus influenzae type b (Hib) disease in the Hib vaccine era. JAMA **269:**221–226.
41. **Takala AK, Eskola 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.
42. **Taylor DN, Trofa AC, Sadoff J, Chu C, Bryla D, Shiloach J, Cohen D, Ashkenazi S, Lerman Y, Egan W, Schneerson R, Robbins JB.** 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.
43. **Cohen D, Ashkenazi S, Green M, Lerman Y, Slepon R, Robin G, Orr N, Taylor DN, Sadoff JC, Chu C, Shiloach J, Schneerson R, Robbins JB.** 1996. Safety and immunogenicity of investigational Shigella conjugate vaccines in Israeli volunteers. Infect Immun **64:**4074–4077.
44. **Passwell JH, Harlev E, Ashkenazi S, Chu C, Miron D, Ramon R, Farzan N, Shiloach J, Bryla DA, Majadly F, Roberson R, Robbins JB, Schneerson 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.69.3.1351-1357.2001.
45. **Ashkenazi S, Passwell JH, Harlev E, Miron D, Dagan R, Farzan N, Ramon R, Majadly F, Bryla DA, Karpas AB, Robbins JB, Schneerson 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.
46. **Passwell JH, Ashkenazi S, Harlev E, Miron D, Ramon R, Farzam N, Lerner-Geva L, Levi Y, Chu C, Shiloach J, Robbins JB, Schneerson R, Israel Shigella Study G.** 2003. Safety and immunogenicity of Shigella sonnei-CRM9 and Shigella flexneri type 2a-rEPAsucc 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.
47. **Passwell JH, Ashkenzi S, Banet-Levi Y, Ramon-Saraf R, Farzam N, Lerner-Geva L, Even-Nir H, Yerushalmi B, Chu C, Shiloach J, Robbins JB, Schneerson R, Israeli Shigella 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.
48. **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.
49. **Wahid R, Simon JK, Picking WL, Kotloff KL, Levine MM, Sztein 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.
50. **Walker RI, Clifford A.** 2015. Recommendations regarding the development of combined enterotoxigenic Eschericha coli and Shigella vaccines for infants. Vaccine **33:**946–953. http://dx.doi.org/10.1016/j.vaccine.2014.11.048.
51. **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.