Immune Responses to the O-Specific Polysaccharide Antigen in Children Who Received a Killed Oral Cholera Vaccine Compared to Responses following Natural Cholera Infection in Bangladesh

Daniel T. Leung,a,b,c Taher Uddin,a Peng Xu,d Amena Akhtar,a Russell A. Johnson,a Mohammad Arif Rahman,a Mohammad Murshed Alam,a Meagan Kelly Bufano,b Grace Eckhoff,b Ying Wu-Freeeman,b Yanan Yu,b Tania Sultana,a,b Farhana Khanam,a Amit Saha,a Fahima Chowdhury,a Ashraf I. Khan,a Michelle C. Charles,b,c Regina C. LaRocque,b,c Jason B. Harris,b,e Stephen B. Calderwood,b,c,d Pavol Kovalc,ag Edward T. Ryan,b,c,g

Centre for Vaccine Sciences, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh; Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts, USA; Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA; NIDDK, LBC, National Institutes of Health, Bethesda, Maryland, USA; Department of Pediatrics, Harvard Medical School, Boston, Massachusetts, USA; Department of Microbiology and Immunology, Harvard Medical School, Boston, Massachusetts, USA; Department of Infectious and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts, USA

Current oral cholera vaccines induce lower levels of protective efficacy and shorter durations of protection in young children than in adults. Immunity against cholera is serogroup specific, and immune responses to Vibrio cholerae lipopolysaccharide (LPS), the antigen that mediates serogroup-specific responses, are associated with protection against disease. Despite this, responses against V. cholerae O-specific polysaccharide (OSP), a key component of the LPS responsible for specificity, have not been characterized in children. Here, we report a comparison of polysaccharide antibody responses in children from a region in Bangladesh where cholera is endemic, including infants (6 to 23 months, n = 15), young children (24 to 59 months, n = 14), and older children (5 to 15 years, n = 23) who received two doses of a killed oral cholera vaccine 14 days apart. We found that infants and young children receiving the vaccine did not mount an IgG, IgA, or IgM antibody response to V. cholerae OSP or LPS, whereas older children showed significant responses. In comparison to the vaccinees, young children with wild-type V. cholerae O1 Ogawa infection did mount significant antibody responses against OSP and LPS. We also demonstrated that OSP responses correlated with age in vaccinees, but not in cholera patients, reflecting the ability of even young children with wild-type cholera to develop OSP responses. These differences might contribute to the lower efficacy of protection rendered by vaccination than by wild-type disease in young children and suggest that efforts to improve lipopolysaccharide-specific responses might be critical for achieving optimal cholera vaccine efficacy in this younger age group.

Cholera is a diarrheal disease caused by Vibrio cholerae O1 or O139 infection (1). A disease mostly affecting those in impoverished living conditions, cholera is endemic in over 50 countries, causing an estimated 100,000 or more deaths annually (2, 3). Although cholera is seen in all age groups, in areas where cholera is endemic, children under 5 years of age often bear a large burden of disease (4).

Improved access to clean water and sanitation in resource-poor settings is key to the control of cholera; however, despite marked efforts toward such a goal, the global burden of cholera has continued to rise, with increasing numbers of countries reporting outbreaks and epidemics, especially over the last 3 years (5, 6). The use of oral cholera vaccines (OCVs) is now advocated for achieving optimal cholera vaccine efficacy in this younger age group. The vibriocidal response, an indirect correlate of protection against cholera, which assesses complement-dependent antibody killing, has been shown to be largely comprised of IgM targeting LPS (12, 13). We have recently demonstrated that LPS-specific antibody and memory B cell responses are correlated with

Received 23 January 2013 Returned for modification 1 March 2013 Accepted 8 March 2013 Published ahead of print 20 March 2013 Address correspondence to Daniel T. Leung, dleung@partners.org. D.T.L., T.U., and P.X. are co-first authors. P.K., F.Q., and E.T.R. are co-senior authors. Copyright © 2013, American Society for Microbiology. All Rights Reserved. doi:10.1128/CVI.00035-13
protection against cholera in household contacts of patients with cholera (14). We have also shown that in children, two doses of WC-rBS vaccine induce significantly lower plasma LPS antibody levels and memory B cell levels than wild-type infection (15). Thus, we hypothesized that differences in V. cholerae polysaccharide-specific humoral responses partly account for differences in the durations of protection provided by infection and vaccination. We believe that these differences might be most pronounced in young children due to their inability to mount robust responses to T cell-independent antigens such as LPS and OSP.

Evaluation of immune responses to LPS might be complicated by the presence of trace amounts of contaminating membrane proteins, even in highly purified LPS preparations (16). Thus, we purified the O-specific polysaccharide-core oligosaccharide fragment (OSCs) of the outer membrane of V. cholerae O1 LPS and conjugated it to bovine serum albumin (BSA) to facilitate immunological analyses of the sugar component of LPS (17). We have previously shown that immune responses target the OSP moiety in this construct (16), and henceforth we refer to this antigen as OSP. Furthermore, in adults with severe cholera, we also recently demonstrated that the OSP response contributes substantially to the observed vibriocidal antibody response, and we identified plasma antibody and mucosal responses against OSP and LPS (16). The responses of young children to OSP, however, have not been characterized. Therefore, the aim of this study was to compare the antibody responses to polysaccharide antigens in children with V. cholerae O1-induced cholera and children who received an oral cholera vaccine.

MATERIALS AND METHODS

Study design and subject enrollment. We analyzed stored plasma samples collected from studies of the WC-rBS vaccine in children from Mipur, an urban area in Dhaka, Bangladesh (15, 18, 19). The studies were approved by the Research Review and Ethics Review Committees of the ICDDR,B, Dhaka, Bangladesh. We assessed responses in the plasma of these vaccinees, including infants (age 6 to 23 months), young children (age 24 to 59 months), and older children (age 5 to 15 years). We compared responses to those of stored plasma samples collected from children, matched by age group, who were hospitalized with severe acute watery diarrhea and whose stool cultures were positive for V. cholerae O1 Ogawa (20). Blood samples from cholera patients were obtained on days 2 and 7 following presentation. Due to ethical limitations, we were unable to obtain plasma samples from children less than 2 years of age with severe dehydrating cholera.

In the vaccine studies, following a process of obtaining informed consent from parents/guardians, we enrolled children who were administered two doses of WC-rBS 14 days apart. Children were excluded if they had suffered from any diarrheal illness in the prior 2 weeks or had suffered any febrile illness or received antibiotics in the previous week. We also used standard microbiological culture techniques to screen subjects for bacterial enteric pathogens in stool (21), and we excluded subjects if the culture was positive. We collected blood samples from the subjects prior to the first dose of vaccine (day 0) and 7 days after intake of the second dose of vaccine (i.e., 21 days after the first dose). For the antibody assays described below, we used plasma that was stored at −20°C after separation from heparinized blood by Ficoll-Isopaque (Pharmacia, Piscataway, NJ). Studies including cholera patients were approved by the Research Review and Ethics Review Committees of the ICDDR,B, Dhaka, Bangladesh, and the Institutional Review Board of the Massachusetts General Hospital, Boston, MA.

Vibriocidal antibody assay. We measured vibriocidal antibody responses in plasma using guinea pig complement and V. cholerae O1 Ogawa (X-25049) as the target organism, as described previously (22). We defined the vibriocidal titer as the reciprocal of the highest plasma dilution resulting in >50% reduction of the optical density associated with V. cholerae growth compared to growth in control wells without plasma.

Detection of cholera antigen-specific plasma antibody levels. Plasma LPS- and OSP-specific IgA, IgG, and IgM antibody responses in stored plasma were measured using a standardized enzyme-linked immunosorbent assay (ELISA) technique as described previously (22). Briefly, we prepared V. cholerae O1 Ogawa LPS and OSP:BSA conjugates as described previously (17). We coated 96-well polystyrene plates (Nunc F) with either V. cholerae O1 Ogawa LPS (2.5 μg/ml) dissolved in phosphate-buffered saline (PBS) or Ogawa OSP:BSA (1 μg of the conjugated product/ml) dissolved in carbonate buffer (pH 9.6). We added 100 μl of plasma (diluted 1:40 in 0.1% bovine serum albumin in phosphate-buffered saline-0.05% Tween) per well. After incubation, we added horseradish peroxidase-conjugated secondary antibodies to human IgA, IgG, and IgM (Jackson ImmunoResearch, West Grove, PA) at a 1:1,000 dilution, followed by 1 mg/ml ortho-phenylenediamine (Sigma, St. Louis, MO) in 0.1 M sodium citrate buffer (pH 4.5) and 0.01% hydrogen peroxide for development. We determined the optical density at 450 nm by kinetic measurement of plates for 5 min at 14-s intervals and obtained values of milli-absorbance units/min. We normalized data to ELISA units by calculating the ratio of the optical density of the test sample to that of a standard of pooled convalescent-phase sera (prepared from previously infected cholera patients) included on each plate (23).

Statistical analyses. We assessed differences in immune responses between the two groups with a Mann-Whitney U test. We used Spearman’s correlation to test for the associations between vibriocidal and plasma antibody responses and between age and plasma antibody responses. All P values were two-tailed, with a value of ≤0.05 considered the threshold for statistical significance. We performed analyses using GraphPad Prism, version 5.0 (GraphPad Software, Inc., La Jolla, CA) and SPSS, version 17.0 (SPSS Inc., Chicago, IL).

RESULTS

Study population. We measured antibody responses in 15 infants (median age, 12 months; interquartile range [IQR], 9 months), 14 young children (median age, 37 months; IQR, 19 months), and 23 older children (median age, 10 years; IQR, 6 years) who received two doses of OCV (Table 1). Approximately 95% of infants were breastfed either exclusively or with complementary food. For comparison, we evaluated the responses of 14 young children
median age, 44 months; IQR, 22 months) and 24 older children (median age, 8 years; IQR, 5 years) hospitalized at the ICDDR,B with *V. cholerae* O1 Ogawa infection. All patients were treated with intravenous and oral fluid replacement and azithromycin. There were no differences in gender or blood group between age group-matched vaccinees and patients.

**Plasma vibriocidal antibody response.** We assessed vibriocidal antibody responses in vaccinees at day 0 (preimmune) and 7 days after the second dose and in patients at days 2 (acute) and 7 (early convalescence) of illness. In children receiving vaccine, we found that OSP and LPS antibody responses of all isotypes correlated significantly with the vibriocidal titers at day 7 after vaccination, with responses targeting OSP (Fig. 1A, C, and E) having higher correlation coefficient values than responses targeting LPS (Fig. 2A, C, and E) for all isotypes. Similarly, in infected children, the vibriocidal titers correlated with both anti-LPS (Fig. 2B, D, and F) and anti-OSP (Fig. 1B, D, and F) responses at day 7, although with lower correlation coefficients than those seen in vaccinees. In patients, we did not see differences in correlation coefficients between LPS and OSP as we did in vaccinees.

**Plasma *V. cholerae* polysaccharide antibody responses.** We measured antibody responses to the *V. cholerae* polysaccharide antigens LPS and OSP in child vaccinees and patients (Fig. 3). Cholera patients of both age groups and older child vaccinees

---

**FIG 1** Correlations between day 7 plasma vibriocidal antibody responses and day 7 plasma OSP antibody responses in children receiving two doses of an oral cholera vaccine (A, C, and E) and children with wild-type infection with *V. cholerae* O1 Ogawa (B, D, and F). The Spearman correlation coefficient (r) is shown.
developed significant IgA and IgG responses to both OSP and LPS by day 7. In comparison, infant vaccinees did not mount OSP or LPS responses for any isotype, and young child vaccinees mounted significant responses to only LPS IgA and near-significant responses to OSP IgA but did not mount significant IgM or IgG responses to OSP or LPS. Compared to patients, both young children and older children given vaccine had lower antibody responses to LPS and OSP, especially IgA responses. We found that infants also had significantly lower baseline (prevaccine) levels of OSP and LPS IgM and IgG than young children and older children.

In children vaccinated with WC-rBS, we found statistically significant correlations between age and OSP antibody responses for all isotypes (Fig. 4A, C, and E). Significance was maintained even if infants were excluded. Age was also correlated with LPS antibody responses in vaccinees, although with slightly lower $r$ values ($r = 0.55$, $0.39$, and $0.46$, respectively, for IgG, IgM, and IgA, all $P < 0.005$; data not shown). Conversely, in children with wild-type infections, age did not correlate with the antibody response of any isotype to OSP (Fig. 4B, D, and F) or LPS ($r = 0.04$, $0.22$, and $0.25$, respectively, for IgG, IgM, and IgA, all $P > 0.10$; data not shown).

**DISCUSSION**

Although children bear a large burden of cholera globally (4), vaccination of young children with OCVs provides lower levels of
protective efficacy and shorter durations of protection than vaccination of adults (9, 10, 24, 25). This is despite the fact that the duration of protection after natural infection seems to be similar in young children and adults (26). The mechanisms behind these observations are not well understood. We have previously shown that immune responses, including memory B cell responses, to *V. cholerae* LPS are associated with immunity against cholera (14, 27) and that in children memory B cell responses targeting LPS are induced and more sustained by natural infection than by vaccination (15). In this report, we describe the antibody response of child vaccinees and patients against OSP, the component of LPS that defines serogroup specificity and perhaps is an important target of immune responses that mediate protection.

The vibriocidal antibody is a commonly used immunological marker for *V. cholerae* infection but likely an indirect and surrogate marker of protection, given that there is no titer threshold at which protection is complete (28). We have previously shown that in adult cholera patients antibody responses against OSP are com-

![FIG 3](image-url)
parable to those against LPS and that vibriocidal responses predominantly target OSP (16). In our current study, we showed that in children OSP-specific responses following vaccination closely correlate to vibriocidal antibody responses. Furthermore, we demonstrated that the OSP-specific antibody responses after vaccination have slightly higher correlation coefficients with the vibriocidal response than do LPS responses. In wild-type infections, although the vibriocidal responses also correlated with OSP and LPS antibody responses, we interestingly found that high OSP and LPS responses occurred only in patients with high vibriocidal responses, but that the opposite was not true—three children with high vibriocidal titers did not have correspondingly high LPS or OSP responses. The reasons for these observations are unclear. It is possible that the vibriocidal responses in these patients might target other *V. cholerae* antigens.

Polysaccharides are T cell-independent antigens that elicit poor immune responses in young children, especially infants (29). We have previously reported that infants (6 to 18 months of age) given 2 doses of WC-rBS vaccine achieve poor LPS IgA responses despite prominent induction of antibodies against CtxB (19). We have also shown that in children given Peru-15, a live attenuated oral cholera vaccine, those 2 to 5 years of age achieved higher LPS IgA response rates than those <2 years of age (30); both of these response rates were lower than those seen in adults (31). We have recently demonstrated, in children given Shanchol, a bivalent whole-cell (bi-WC) OCV without supplemental CtxB, that while...
LPS IgA seroconversion rates in young children (both 12 to 23 months and 2 to 5 years of age) were similar to those of adults, absolute titers were approximately 10-fold lower in infants (12 to 23 months) than in adults (32). In the present study, we have shown that groups of infants (6 to 23 months of age) and young children (24 to 59 months) given 2 doses of an oral WC-rBS vaccine did not mount significant IgG, IgM, or IgA antibody responses against OSP, whereas older children did. In marked distinction, young children with wild-type cholera developed prominent IgG, IgM, and IgA anti-OSP responses. These responses were comparable to those seen in older children receiving vaccines. These data suggest that young children have the potential to mount significant responses against polysaccharide antigens during wild-type disease but that such responses do not follow oral vaccination, even in an area of cholera endemity where children are presumed to be exposed to antigen at very young ages. This finding is consistent with earlier reported studies demonstrating a lack of detectable rises in LPS-specific memory B cell responses even in adults receiving the vaccine (33), suggesting that the WC-rBS vaccine might not be a good inducer of polysaccharide memory at any age. Importantly, our study did not include the youngest children (ages less than 24 months) with wild-type cholera; thus, the response in infants after infection has not yet been studied. We hypothesize that the presence of cholera holotoxin (CT) and/or other antigens in wild-type infections, compared toCtxB in the current vaccine, might contribute to enhancing the OSP responses in patients with wild-type cholera versus vaccinees.

We also demonstrated that increasing age is a significant correlate of the magnitude of OSP and LPS antibody response in WC-rBS vaccinees. This correlation was not seen in patients, a finding that largely reflects the ability of even young children with wild-type cholera to develop significant OSP and LPS responses. Our findings are consistent with studies of unconjugated polysaccharide-containing vaccines, including those against Salmonella Typhi, Neisseria meningitides, Haemophilus influenzae, and Streptococcus pneumoniae, which demonstrate a relationship of age with polysaccharide antibody responses and vaccine efficacy (34). Maturation of the T-independent response to polysaccharide antigens likely occurs between 6 months and 2 years of age (35, 36), with marked variability between antigens (36, 37). Furthermore, total levels of IgG2, the predominant IgG subclass in responses to polysaccharide antigens (38, 39) and the subclass with the highest fold increase among LPS IgG in adults with cholera (40), do not reach adult levels in children until they are 8 to 10 years of age (41, 42).

The development of protein-conjugate polysaccharide vaccines, which take advantage of a T-dependent response, has resulted in effective vaccines against encapsulated bacteria in infants and young children (43) and improved immunogenicity and efficacy in animal models of cholera (44–46). Nevertheless, currently available conjugated polysaccharide vaccines used in infants require booster doses at older ages due to waning antibody levels. However, there is evidence that in countries of high *H. influenzae* endemcity, vaccinated infants have persistence of high polysaccharide-specific antibody levels compared to infants from high-income countries with low endemcity (47, 48), possibly due to the priming effect and repeated antigenic exposures.

OSP-protein conjugate vaccines are under development for a number of diarrheal diseases (49–51). A *Shigella* conjugate vaccine has been shown to elicit significant levels of LPS IgG in children as young as 1 year (52), although this did not translate into protective efficacy for those younger than the age of 3 years (51). We have shown, in the neonatal mouse model, that transcutaneous administration of a neoglycoconjugate vaccine containing a hexasaccharide of *V. cholerae* OSP was effective in the induction of LPS and vibriocidal antibodies in orally primed mice (44). We have also shown, in children as young as 3 years, that wild-type infections induce detectable LPS-specific memory B cell responses (20). Likewise, in our current study, we have demonstrated that natural infection also induces significant levels of OSP-specific antibodies in young children. These findings suggest that improving antipolysaccharide responses might be a pertinent step in achieving optimal cholera vaccine efficacy in young children. Further studies into the immunogenicity and efficacy in young children of conjugated vaccines or other immunization strategies targeting OSP of *V. cholerae* might be warranted.

**ACKNOWLEDGMENTS**

This work was supported by the ICDDR,B and its donors, which provide unrestricted support to ICDDR,B for its operations and research. Current donors providing unrestricted support include the Australian Agency for International Development (AusAID), the government of the People’s Republic of Bangladesh, the Canadian International Development Agency (CIDA), the Swedish International Development Cooperation Agency (SIDA), and the Department for International Development, United Kingdom (DFID). This study was also supported by grants from the Intramural Research Program of the National Institutes of Health, NIDDK, and extramural grants from the National Institutes of Health, including the National Institute of Allergy & Infectious Diseases (U01 AI058935 [to S.R.C. and E.T.R.], R03 AI063079 [to F.Q.], U01 AI077883 [to E.T.R.], K08 AI089721 [to R.C.C.], and K08AI100923 [to D.T.L.]) and the Fogarty International Center, Training Grant in Vaccine Development and Public Health (TW005572 [to T.U., M.M.A., F.K., and F.Q.]), a Career Development Award (K01 TW07409 [to J.B.H.] and TW07144 [to R.C.L.]), and a Fogarty International Clinical Research Scholars award (R24 TW007988 [to R.A.J. and T.U.]), as well as by the Swedish International Development Cooperation Agency (to F.Q.), a Physician Scientist Early Career Award from the Howard Hughes Medical Institute (to R.C.L.), a Thrasher Research Fund Early Career Award (to D.T.L.), and a postdoctoral fellowship in tropical infectious diseases from the American Society for Tropical Medicine & Hygiene—Burroughs Wellcome Fund (to D.T.L.).

**REFERENCES**

1. Harris JB, LaRocque RC, Qadri F, Ryan ET, Calderwood SB. 2012. Cholera. Lancet 379:2466–2476.
2. Zuckerman JN, Rombo L, Fisch A. 2007. The true burden and risk of cholera: implications for prevention and control. Lancet Infect. Dis. 7:521–530.
3. Anonymous. 2009. Cholera: global surveillance summary, 2008. Wkly. Epidemiol. Rec. 84:309–324.
4. Deen JL, von Seidlein L, Sur D, Agtini M, Lucas ME, Lopez ME, Lopez AL, Kim DR, Ali M, Clemens JD. 2008. The high burden of cholera in children: comparison of incidence from endemic areas in Asia and Africa. PLoS Negl. Trop. Dis. 2:e173. doi:10.1371/journal.pntd.0000173.
5. Anonymous. 2011. Cholera. 2010. Wkly. Epidemiol. Rec. 86:325–339.
6. Anonymous. 2012. Cholera. 2011. Wkly. Epidemiol. Rec. 87:289–304.
7. Anonymous. 2010. Cholera vaccines: WHO position paper. Wkly. Epidemiol. Rec. 85:117–128.
8. Shin S, Desai SN, Sah BK, Clemens JD. 2011. Oral vaccines against cholera. Clin. Infect. Dis. 52:1343–1349.
9. Sinclair D, Abba K, Zaman K, Qadri F, Graves PM. 2011. Oral vaccines for preventing cholera. Cochrane Database Syst. Rev. 3:CD008863. doi:10.1002/14651858.CD008863.pub2.
10. Sur D, Kanungo S, Sah B, Manna B, Ali M, Paisley AM, Niyogi SK, Park...
Majumdar AS, Ghose AC. Chowdhury F, Rahman MA, Begum YA, Khan AI, Faruque AS, Xu P, Alam MM, Kalsy A, Charles RC, Calderwood SB, Qadri F, Ryan ET, Calderwood SB, Qadri F, Harris JB.

John M, Bridges EA, Miller AO, Calderwood SB, Ryan ET. 2002. Comparison of immune B cell, antibody-secreting cell, and plasma antibody responses in young children, older children, and adults with infection caused by Vibrio cholerae O1 El Tor Ogawa in Bangladesh. Clin. Vaccine Immunol. 19:1712–1721.

Xu P, Alam MM, Kalsy A, Charles RC, Calderwood SB, Qadri F, Ryan ET, Vocak P. 2011. Simple, direct conjugation of bacterial O-SP-core vaccine in infants with cholera in Bangladesh. PLoS Negl. Trop. Dis. 5:e999. doi:10.1371/journal.pntd.0000999.

Qadri F, Wonnacott C, Albert MJ, Rossiter A, Mannor K, Begum YA, Mohi G, Salam MA, Sack RB, Swerdlow HM, Ali M, Niyogi SK, Park JK, Sarkar B, Purula AM, Donner D, Goyal NK, Bhatcharya SK, Clemens JD. 2009. Efficacy and safety of a modified killed-whole-cell oral cholera vaccine in India: an interim analysis of a cluster-randomised, double-blind, placebo-controlled trial. Lancet 374:1694–1702.

van Loon FP, Clemens JD, Chakraborty J, Roe MR, Kay BA, Sack DA, Yunus M, Ali M, Svennerholm AM, Holmgren J. 1996. Field trial of inactivated oral cholera vaccines in Bangladesh: results from 5 years of follow-up of a randomized, controlled trial. PLoS Negl. Trop. Dis. 5:e1289.

Ali M, Emch M, Park JK, Yunus M, Clemens JD. 2011. Natural cholera infection-derived immunity in an endemic setting. J. Infect. Dis. 204:912–918.

Harris JB, LaRocque RC, Chowdhury F, Khan AI, Logvinenko T, Faruque AS, Ryan ET, Qadri F, Calderwood SB. 2008. Susceptibility to Vibrio cholerae infection in a cohort of household contacts of patients with cholera in Bangladesh. PLoS Negl. Trop. Dis. 2:e2221. doi:10.1371/journal.pntd.0000221.

Saha D, LaRocque RC, Khan AI, Harris JB, Begum YA, Akramuzzaman SM, Faruque AS, Ryan ET, Qadri F, Calderwood SB. 2004. Incomplete correlation of serum vibriocidal antibody titer with protection from Vibrio cholerae infection in urban Bangladesh. J. Infect. Dis. 189:2318–2322.

Clutterbuck EA, Oh S, Hamaluba M, Westcar S, Beverley PC, Pollard AJ. 2008. Serotype-specific and age-dependent generation of pneumococcal polysaccharide-specific memory B- and antibody responses to immunization with a pneumococcal conjugate vaccine. Clin. Vaccine Immunol. 15:182–193.

Qadri F, Chowdhury MI, Faruque SM, Salam MA, Ahmed T, Begum YA, Saha A, Ali Tarique A, Seidell LV, Park E, Killeen KP, Mekalanos JF, Clemens JD, Sack DA, Study Group PXY. 2007. Peru-15, a live attenuated oral cholera vaccine, is safe and immunogenic in Bangladeshi toddlers and infants. Vaccine 25:231–238.

Qadri F, Chowdhury MI, Faruque SM, Salam MA, Ahmed T, Begum YA, Saha A, Alam MS, Zaman K, Seidell LV, Park E, Killeen KP, Mekalanos JF, Clemens JD, Sack DA, Peru-15 Study Group. 2005. Randomized, controlled study of the safety and immunogenicity of Peru-15, a live attenuated oral vaccine candidate for cholera, in adult volunteers in Bangladesh. J. Infect. Dis. 192:573–589.

Saha A, Chowdhury MI, Khanam F, Bhiuyan MS, Chowdhury F, Khan IA, Clemens J, Ali M, Cravioto A, Qadri F. 2011. Safety and immunogenicity study of a killed bivalent (O1 and O139) whole-cell oral cholera vaccine Shanchol, in Bangladeshi adults and children as young as 1 year of age. Vaccine 29:8285–8292.

Alam MM, Riyadh MA, Fatema K, Rahman MA, Akhtar N, Ahmed T, Chowdhury MI, Chowdhury F, Calderwood SB, Harris JB, Ryan ET, Qadri F. 2011. Antigen-specific memory B-cell responses in Bangladeshi adults after one- or two-dose oral killed cholera vaccination and comparison with responses in patients with naturally acquired cholera. Clin. Vaccine Immunol. 18:844–850.

Cadoz M. 1998. Potential and limitations of polysaccharide vaccines in infancy. Vaccine 16:1391–1395.

Rijkers GT, Sanders EA, Breukels MA, Zegers BJ. 1998. Infant B cell responses to polysaccharide determinants. Vaccine 16:1396–1400.

Laferriere C. 2011. The immunogenicity of pneumococcal polysaccharides in infants and children: a meta-regression. Vaccine 29:6838–6847.

Bossuyt X, Borgers H, Moens L, Verbinnen B, Meyts I. 2001. Age- and serotype-dependent antibody response to pneumococcal polysaccharides. J. Allergy Clin. Immunol. 127:1079–1080; author reply 1080–1081.

Barrett DJ, Ayoob EM. 1986. IgG2 subclass restriction of antibody to pneumococcal polysaccharides. Clin. Exp. Immunol. 63:127–137.

Fachra Chowdhury RM, Begum YA, Khan AI, Faruque AS, Saha NC, Baby NL, Malek MA, Kumar AR, Svennerholm AM, Pietroni M, Cravioto A, Qadri F. 2011. Impact of rapid urbanization on the rates of infection by Vibrio cholerae O1 and enterotoxigenic Escherichia coli in Dhaka. PLoS Negl. Trop. Dis. 5:e999. doi:10.1371/journal.pntd.0000999.

Qadri F, Wonnacott C, Albert MJ, Hossain J, Mannoor K, Begum YA, Mohi G, Salam MA, Sack RB, Svennerholm AM. 1997. Comparison of immune responses in patients infected with Vibrio cholerae O139 and O1. Infect. Immun. 65:3571–3576.

John M, Bridges EA, Miller AO, Calderwood SB, Ryan ET. 2002. Comparison of mucosal and systemic humoral immune responses after transcutaneous and oral immunization strategies. Vaccine 20:2720–2726.

Sur D, Lopez AL, Kanungo S, Paisley A, Manna B, Ali M, Niyogi SK, Park JK, Sarkar B, Purula AM, Donner D, Goyal NK, Bhatcharya SK, Clemens JD. 2009. Efficacy and safety of a modified killed-whole-cell oral cholera vaccine in India: an interim analysis of a cluster-randomised, double-blind, placebo-controlled trial. Lancet 374:1694–1702.
44. Tarique AA, Kalsy A, Arifuzzaman M, Rollins SM, Charles RC, Leung DT, Harris JB, Larocque RC, Sheikh A, Bhuiyan MS, Saksena R, Clements JD, Calderwood SB, Qadri F, Kovac P, Ryan ET. 2012. Transcutaneous immunization with a Vibrio cholerae O1 Ogawa synthetic hexasaccharide conjugate following oral whole-cell cholera vaccination boosts vibriocidal responses and induces protective immunity in mice. Clin. Vaccine Immunol. 19:594–602.

45. Boutonnier A, Villeneuve S, Nato F, Dassy B, Fournier JM. 2001. Preparation, immunogenicity, and protective efficacy, in a murine model, of a conjugate vaccine composed of the polysaccharide moiety of the lipopolysaccharide of Vibrio cholerae O139 bound to tetanus toxoid. Infect. Immun. 69:3488–3493.

46. Kossaczka Z, Shiloach J, Johnson V, Taylor DN, Finkelstein RA, Robbins JB, Szu SC. 2000. Vibrio cholerae O139 conjugate vaccines: synthesis and immunogenicity of V. cholerae O139 capsular polysaccharide conjugates with recombinant diphtheria toxin mutant in mice. Infect. Immun. 68:5037–5043.

47. Hutter J, Pasetti MF, Sanogo D, Tapia MD, Sow SO, Levine MM. 2012. Naturally acquired and conjugate vaccine-induced antibody to Haemophilus influenzae type b (Hib) polysaccharide in Malian children: serological assessment of the Hib immunization program in Mali. Am. J. Trop. Med. Hyg. 86:1026–1031.

48. Hoppenbrouwers K, Lagos R, Swennen B, Ethevenaux C, Knops J, Levine MM, Desmyter J. 1998. Safety and immunogenicity of an Haemophilus influenzae type b-tetanus toxoid conjugate (PRP-T) and diphtheria-tetanus-pertussis (DTP) combination vaccine administered in a dual-chamber syringe to infants in Belgium and Chile. Vaccine 16:921–927.

49. Simon R, Tennant SM, Wang JY, Schmidlein PJ, Lees A, Ernst RK, Pasetti MF, Galen JE, Levine MM. 2011. Salmonella enterica serovar enteritidis core O polysaccharide conjugated to H,g,m flagellin as a candidate vaccine for protection against invasive infection with S. enteritidis. Infect. Immun. 79:4240–4249.

50. Ahmed A, Li J, Shiloach Y, Robbins JB, Szu SC. 2006. Safety and immunogenicity of Escherichia coli O157 O-specific polysaccharide conjugate vaccine in 2-5-year-old children. J. Infect. Dis. 193:515–521.

51. 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.

52. 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, Israeli Shigella Study Group. 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.