Published in final edited form as:
*Expert Rev Anti Infect Ther*. 2012 April ; 10(4): 435–444. doi:10.1586/eri.12.23.

# Immune responses to cholera in children

**Daniel T Leung**[1,2,3,*], **Fahima Chowdhury**[1], **Stephen B Calderwood**[2,3,4], **Firdausi Qadri**[1], and **Edward T Ryan**[2,3,5]

[1]Centre for Vaccine Sciences, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

[2]Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, USA

[3]Department of Medicine, Harvard Medical School, Boston, MA, USA

[4]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA, USA

[5]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, USA

## Abstract

Cholera is a severe acute dehydrating diarrheal disease caused by *Vibrio cholerae* O1 or O139 infection, and is associated with significant mortality and morbidity globally. Although young children bear a high burden of the disease, currently available oral vaccines give a lower efficacy and shorter duration of protection in this group than in adults. According to the studies of natural infection, young children achieve comparable systemic anti-*V. cholerae* antigen-specific antibody, gut-homing antibody-secreting cell and memory B-cell responses as adults. Studies on innate and cell-mediated immune responses are lacking in children, and may offer important insights into differences in vaccine efficacy. The impact of host factors such as malnutrition, genetics and coinfection with other pathogens also remains to be fully defined.

### Keywords

children; cholera; memory B cells; mucosal immunity; oral cholera vaccines

Cholera is an acute dehydrating diarrheal disease caused by the Gram-negative bacterium *Vibrio cholerae*. Despite suboptimal surveillance and reporting, it is estimated that globally 2–3 million people have cholera each year, and more than 100,000 die from this infection annually [1,2]. Cholera is a disease of poverty, made worse in endemic urban areas by the overcrowding of informal housing settlements or slums [3]. The disease is endemic in over



*Author for correspondence: Tel.: +1 617 726 3812, Fax: +1 617 726 7416, dleung@partners.org.



**Financial & competing interests disclosure**

This work was supported by grants from the International Centre for Diarrhoeal Disease Research, NIH, including the National Institute of Allergy & Infectious Diseases (AI058935 to SB Calderwood and ET Ryan, U01 AI077883 to ET Ryan); an American Recovery and Reinvestment Act Post-doctoral Fellowship in Global Infectious Diseases (TW005572 to DT Leung) from the Fogarty International Center, a Harvard Initiative for Global Health Post-doctoral Fellowship in Global Infectious Diseases (DT Leung), and a Post-doctoral Fellowship in Tropical Infectious Diseases from the American Society for Tropical Medicine & Hygiene/Burroughs Wellcome Fund (DT Leung). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

50 countries; however, it also appears in epidemic form, both in endemic areas and in regions made susceptible by civil unrest and natural disasters, such as following the earthquake in Haiti in 2010 [4]. Although epidemic cholera is seen in all age groups in endemic areas, young children have a higher burden of disease [5–7].

The majority of cases of epidemic cholera are caused by infection with toxigenic strains of the O1 and O139 serogroups of *V. cholerae*. The O139 serogroup is differentiated from the O1 by the O antigen of the lipopolysaccharide (LPS). Although *V. cholerae* O139 has epidemic potential, for unknown reasons it has largely disappeared as a cause of cholera. The O1 serogroup is further divided into two biotypes: El Tor and classical. Although the classical biotype was the causative agent of earlier pandemics for which we have microbiologic data, the current seventh pandemic, which began in 1961, is now caused by the El Tor biotype.

In humans, ingestion of water or food contaminated with *V. cholerae* results in colonization of the small intestine, an attachment facilitated by the toxin coregulated pilus, a fimbrial protein involved in the formation of microcolonies [8]. Subsequent elaboration of cholera toxin (CT), and the action of the toxin on intestinal epithelial cells, causes the secretion of large amounts of chloride, sodium and water via activation of adenylate cyclase and increases in intracellular cyclic AMP [9]. Important factors affecting immune responses in *V. cholerae* infections are listed in BOX 1. In both adults and children, severity of infection can vary from asymptomatic or mild-to-moderate infection to death within hours of onset of massive diarrhea. Vomiting combined with purging of large volumes of stools resembling rice water can result in rapid dehydration and death due to hypovolemic shock [10]. In children, cholera can be complicated by severe hypoglycemia [11] and concomitant pneumonia [12]. The mainstay of treatment of patients with cholera is rapid fluid and electrolyte replacement, optimally in the form of oral rehydration solution containing salts, sugar and water, if the patient is able, including initiation of this at home upon onset of symptoms [10]. If available, the use of rice-based oral rehydration solution for the management of diarrhea owing to cholera having been shown to decrease volume of stools and is indicated for those 6 months and older [13]. Intravenous administration of isotonic fluids is often necessary for those who are severely dehydrated or unable to tolerate oral therapy. Antibiotic use is beneficial in severe cholera, shortening the duration of illness and thus lessening the amount of fluid replacement needed in both adults and children [14–16]. With prompt and appropriate treatment, the mortality rate of cholera is <1%, although higher mortality rates are common in complex emergencies and during the initial phase of an epidemic [17].

In areas of the world endemic for cholera, although all age groups are susceptible, the largest burden of symptomatic cholera is often among children [5]. In a study of household contacts of patients with *V. cholerae* O1 infection in an urban area of Bangladesh, those who were ≤5 years of age had a significantly higher risk of acquiring infection than older family members in a 21-day observational period [18]. Despite the susceptibility and high burden of disease among young children in endemic areas, currently available cholera vaccines have shown lower protective efficacy and a shorter duration of protection in children under 5 years of age compared with older individuals [19]. Currently available cholera vaccines include two killed, whole-cell oral cholera vaccines (OCVs), both of which are WHO prequalified. One contains killed whole cells of a number of strains of classical and El Tor *V. cholerae* O1, supplemented with 1 mg/dose of recombinant CT B subunit (WC-rBS; Dukoral, Crucell, Sweden); the other is bivalent, containing killed strains of classical and El Tor *V. cholerae* O1 as well as O139, without CtxB supplementation (WC; Shanchol, Shantha Biotechnics, India). In children aged 2–5 years, the WC-rBS vaccine is administered as three doses orally at least 1 week apart. The WC-rBS vaccine is not recommended for children under 2 years

of age. The WC vaccine is approved for children ≥1 year old, and given as two doses orally at least 14 days apart.

A recent review of studies of current OCVs and their predecessors demonstrated that vaccine efficacy in the first 2 years after vaccination was 66% in those >5 years old, but only 38% for children <5 years old [20]. A recently published report of the 3-year follow-up to a large field study of Shanchol in Kolkata, India, showed that while the overall efficacy was 66%, efficacy in those <5 years old was only 43%, with no significant protection in the third year of follow-up for this age group [21]. This is despite: the fact that after symptomatic infection, young children and older persons appear to achieve a similar reduction of at least 60–70% in disease lasting at least 3 years after an initial episode compared with controls [22]; that in volunteer studies, previous cholera is associated with 90–100% protection against subsequent challenge for the 3 years of the evaluation period [23]; that population-based field studies suggest that previous infection with classical cholera provides protection against subsequent disease lasting 6–10 years; and that previous infection with El Tor cholera provides protection against subsequent disease lasting 3–6 years [24]. It is important to note that protection against cholera following wild-type symptomatic disease appears to be similar among older individuals and young children. The reasons behind the differences between protection from natural infection and vaccination, and differences in vaccine efficacy between age groups, have yet to be determined, and such information could significantly contribute to an improved cholera vaccine or immunization strategy optimally effective in young children.

## Immune responses to natural infection & vaccination in children

### Innate immune responses

The innate immune response is involved in the initial defense against pathogens, including the triggering of an adaptive immune response. Young children, especially infants, have limited exposures to antigens and therefore limited ability to rely on rapidly deployed anamnestic immune responses; thus, compared to older children and adults, innate immune responses may be especially critical in young children for controlling infection during the initial exposure. Although *V. cholerae* is a noninvasive pathogen, and historically most investigations have focused on the adaptive immune responses, recent studies have shown that the innate immune response is upregulated in cholera [25–27].

Studies of patients with severe acute cholera have demonstrated that blood levels of mediators of the innate immune response, including leukotriene B4, lactoferrin, myeloperoxidase and nitric oxide, are elevated at the initial phase of infection in both children and adults compared with age-group-matched healthy controls [25,28]. Increases of such mediators in stool are also present.

Whole-genome microarray screening of duodenal biopsies from adults acutely infected with *V. cholerae* O1 shows that the majority of upregulated genes encode for proteins that are part of the innate response [27]. Histopathological studies of duodenal biopsies demonstrate that neutrophils infiltrate the mucosa, followed by an increase in degranulated mast cells and eosinophils during convalescence [26,29]. Other mediators of the innate response, including cytokines such as TNF-α and IL-1b, as well as bactericidal proteins, including lactoferrin, myeloperoxidase and defensins are also elevated [26,30]. Furthermore, recent studies suggest that the long palate, lung and nasal epithelium clone 1 protein expressed in Paneth cells also plays a role in modulating the innate response to *V. cholerae* LPS [31].

Studies of duodenal biopsies in children with cholera have not yet been performed. However, rectal biopsies from children with acute cholera show similar findings to those of

adults, including an increase in neutrophils at onset of disease followed by an increase in mast cells at early convalescence [26]. These findings are associated with elevated expression of myeloperoxidase, lactoferrin and nitric oxide by immunohistochemistry, all of which persist up to 30 days longer than that seen in adults [25].

Many questions remain unanswered regarding the innate response in young children with *V. cholerae* infection. For instance, as innate immune responses, their signaling pathways and associated cytokine responses mature throughout early childhood [32,33], differences between young children and adults remain to be characterized in the context of *V. cholerae* infection and vaccination. Furthermore, the role of micronutrient supplementation on innate responses to *V. cholerae*, as has been demonstrated with zinc in enterotoxigenic *Escherichia coli* (ETEC) infection [34], needs to be evaluated.

## Systemic antibody & memory B-cell responses

Evaluations of humoral immunity to *V. cholerae* infection have largely involved assessing systemic antibody responses, and studies in children with cholera and receiving cholera vaccines have also mostly focused on the vibriocidal antibody and antitoxin antibodies (although mucosal immune responses have been assessed in smaller cohorts, as described below).

**Vibriocidal antibody responses—**The serum vibriocidal antibody response is perhaps the most-studied immunologic marker of cholera infection. The antibodies are bactericidal in the presence of complement, and most of their activity is of the IgM isotype directed against LPS [35–37]. Titers increase with both symptomatic and asymptomatic infection [38], but levels wane quickly and fall to baseline levels within 9–12 months [39,40]. In endemic settings, the percentage of a population with detectable vibriocidal titers increases with increasing age, and 40–80% of individuals have detectable vibriocidal antibodies by 15 years of age [41,42]. Although higher titers have been associated with protection against *V. cholerae* O1 infection and disease [18,38,42,43], there is no threshold at which protection is complete [44].

A study of patients infected with *V. cholerae* O1 showed that children ≤12 years had a lower acute phase (day 2) vibriocidal antibody titer, but achieved a higher vibriocidal response to infection than those >12 years of age [45]. Similarly, a study of children hospitalized for *V. cholerae* O1 Ogawa infection showed that while young children (2–5 years of age) had significantly lower acute-phase titers than adults (18–60 years of age), young children had higher fold-increases in vibriocidal antibodies compared with adults, resulting in comparable peak titers during convalescence [46]. As such, it appears that young children are able to mount vibriodical responses following wild-type cholera infection that are comparable to those induced in older children and adults.

The few studies with age-group-specific comparisons of vibriocidal responses after oral vaccination have shown much heterogeneity in results depending on the type of vaccine and criteria of response. Interlaboratory variability also limits comparison across studies, although fold increase in response to infection or vaccination can be used as the important outcome. Overall, studies of immunogenicity as measured by fold change or responder frequency of vibriocidal titer show that young children have comparable immune responses to vaccination as older children and adults. A study of WC-BS, a predecessor of the current killed, whole-cell OCV WC-rBS, in Bangladesh, showed that in general, children aged 2–5 years had similar responder frequencies and fold changes as older children and adults [47,48], a response that was comparable regardless of whether or not a recombinant B subunit was included in the vaccine. Notably, young children, but not adults, had incremental increases in responder frequency with subsequent vaccine doses. These findings

were also seen in a study of Shanchol vaccine, in which young children (both 2–5 years and 12–23 months of age) had similar responder rates as adults [49]. The live attenuated El Tor Inaba *V. cholerae* O1 OCV (Peru-15) also elicited comparable vibriocidal response rates in young children aged 2–5 years and infants aged 9–24 months as adults, although infants achieved the lowest rates [50].

Akin to what is seen following natural infection, studies of both live and killed formulations of cholera vaccines have demonstrated lower 'seroconversion' rates [51–53], and lower fold-increases [54], in children with higher baseline or acute-phase titers, largely reflecting a higher baseline/acute-phase level in endemic zones where exposure to *V. cholerae* is often repetitive.

## Antigen-specific antibody responses

**Immunoglobulin A:** Secretory IgA (sIgA) is thought to be an important marker of mucosal humoral immunity. On the intestinal surface, sIgA is the predominant immunoglobulin, existing as a dimeric form produced in the mucosa that can neutralize intraluminal pathogens [55]. Systemically, IgA levels increase progressively in childhood, from 1% of adult levels in the newborn to 20% at 1 year, 50% at 5 years and 75% by 16 years of age [56]. In a study in Bangladesh among household contacts, including children, observed for 21 days after identification of the index case of cholera, levels of serum IgA specific to all three *V. cholerae* antigens examined were associated with protection against subsequent *V. cholerae* O1 infection during follow-up [18].

In children infected with *V. cholerae* O1 Ogawa, levels of serum IgA against LPS and CTB rise, with peak levels (at day 7), similar to that achieved in older children and adults [46]. By day 30 of convalescence, levels of IgA are still above baseline levels, although adults maintain LPS IgA levels that are significantly higher than those of younger children. In a study that included both O1 and O139 infections, patients >12 years had higher levels of homologous LPS IgA than those ≤12 years at both acute and convalescent phases of infection, whereas younger patients had higher CTB IgA responses, including those found in feces [45]. This may be due to younger children having more recent exposures to ETEC [57], a common childhood pathogen whose heat-labile enterotoxin is immunologically crossreactive with CT [58].

Most studies of serum IgA responses after vaccination have focused on the antitoxin response. Approximately 80% of children in Bangladesh aged 2–5 years achieved a more than or equal to twofold increase in CT IgA after two doses of a WC-rBS vaccine given 2 weeks apart [59], and a similar responder frequency rate was found in children <2 years of age given a WC-rBS vaccine in a subsequent study [60]. Antitoxin responses are not believed to confer protection against cholera [61], and indeed, the most recently licensed OCV, Shanchol, is not supplemented with CtxB.

Immunity against cholera is serogroup specific, as previous infection with *V. cholerae* O1 does not provide protection against O139 and vice versa [62]. Serogrouping reflects antigenic differences within the O-specific polysaccharide of LPS; unfortunately, few studies have examined IgA responses to LPS following vaccination (and none have yet evaluated O-specific polysaccharide responses), although the studies available suggest that young children may have lower anti-LPS responses than adults. In Bangladeshi children given Peru-15, a live attenuated Inaba vaccine, 54% of 2–5-year-old children achieved a more than or equal to twofold increase in homologous LPS IgA, while only 34% of those <2 years of age achieved such increases [50], both of which were lower than the responses seen in adults (88%) receiving the same vaccination in an earlier study [63]. Studies of the killed WC vaccine, also among Bangladeshi subjects, showed that while young children (2–5 years

and 12–23 months) achieved comparable rates of response (defined as more than or equal to twofold increase in titer), the peak geometric mean titer decreased with age (LPS IgA against O1 Inaba, geometric mean titer of 171 in adults, 37 in those aged 2–5 years and 13 in those aged 12–23 months) [49]. LPS is a T-cell-independent antigen and despite the similar responder rates, the lower magnitude of the absolute antibody response may be a reflection of poorer humoral responses to T-cell-independent antigens in very young children [64].

**Immunoglobulin G:** Significant systemic IgG responses to *V. cholerae* antigens are detected following both natural infection and vaccination. In adults, elevations in serum IgG to CTB are detectable for at least 270 days following both natural infection [39] and two doses of WC-rBS vaccination [65]. However, studies of household contacts of cholera patients have not found levels of plasma antigen-specific IgG on exposure to be predictive of protection against subsequent cholera [39].

Children with *V. cholerae* infection also mount significant increases in plasma IgG against CTB and LPS by day 7 of infection [45,46]. Notably, baseline levels of plasma CTB IgG are significantly lower in adults than children, likely reflecting more recent exposure to ETEC in children. During *V. cholerae* O1 Ogawa infection, all age groups achieve similar magnitudes of CTB- and LPS-specific plasma IgG at convalescence [46], while a study including patients infected with *V. cholerae* O1 or O139 showed that adults mounted higher LPS IgG levels at convalescence, while children mounted higher CTB IgG levels [45]. In adults, systemic antibody responses in multiple subclasses of IgG have been demonstrated against both CT and LPS [66]. Such subclass studies have not been performed in children with cholera.

Antitoxin IgG responses have been demonstrated in children receiving OCV. In studies using a predecessor to the WC-rBS vaccine, WC-BS, which did not include recombinant CtxB but instead included CtxB isolated from cholera holotoxin (and therefore may have included residual amounts of the immunoadjuvant cholera holotoxin), children aged 2–5 years achieved a 2.6-fold rise in CT IgG compared with those receiving placebo, while those >15 years had a 4.7-fold rise [48]. A more recent study has shown that approximately 55% of Bangladeshi children aged 2–5 years receiving two doses of WC-rBS achieved a more than or equal to twofold increase in serum IgG antibody to CTB [59].

**Immunoglobulin M:** Antigen-specific IgM is the first antibody isotype to rise in the serum after exposure to antigen, and plays an important role in subsequent affinity maturation and isotype switching, giving way to other antibody isotypes such as high-affinity IgG [67]. IgM exists in pentameric form, giving it the ability to crosslink antigens and making it a strong activator of the complement system. On the mucosal surface, IgM is excreted by intestinal epithelia, contributing to the luminal defenses [68]. In newborns, IgM-producing plasma cells predominate in the mucosa, and secretory IgM is found in breast milk. Infants with selective IgM deficiency have an increased incidence of viral, Gram-negative bacterial, and polysaccharide-containing bacterial infections [69]. A study of African children showed that those with acute watery diarrhea had a tenfold greater intestinal IgM output in whole-gut lavage than controls [70].

In adults with *V. cholerae* O1 infection, IgM responses against LPS are elevated by day 7 after onset of illness, and remain persistently elevated above baseline for at least 30 days, the last period examined [71]. This finding is not unexpected, as the vibriocidal antibody is mostly composed of IgM directed against LPS [35]. Levels of IgM to CTB do not appear to change with cholera infection. Similarly, adults vaccinated with Peru-15 also produced low levels of serum IgM to CTB [63].

No studies are available characterizing IgM responses in children with cholera. Such investigations are needed given the importance of IgM antibody in humoral immunity against T-cell-independent antigens such as LPS [72], and its role in long-term immunity involving memory B cells (MBCs) [71].

**MBCs in cholera—**MBCs are found in the circulation after natural infection and vaccination and are thought to play a critical role in mediating long-term protective immunity by facilitating rapid anamnestic antibody responses upon re-exposure to antigen [73]. In adults hospitalized with natural infection, both IgA and IgG MBCs against *V. cholerae* antigens are detectable by day 30 after infection [39]. In fact, IgG MBC responses to T-cell-dependent antigens CTB and TcpA (a major pilus colonization factor of *V. cholerae*) persist for up to 1 year, longer than any other known marker of cholera immunity [39]. Despite the lack of increase in systemic IgM responses against CTB, IgM MBCs against LPS and CTB have also been described up to 30 days following acute infection with *V. cholerae* O1 [71].

Younger children with *V. cholerae* O1 Ogawa infection mount comparable CTB- and LPS-specific MBC responses by day 30 after infection as older children and adults [46], and there is a trend for younger children to mount higher levels of CTB IgG MBC responses than older children, likely reflecting more recent exposure to the crossreactive heat-labile toxin antigen of ETEC. Evaluation of the MBC responses in children with longer follow-up is needed.

In adults receiving WC-rBS vaccine, MBC responses to CTB and LPS are significantly shorter in duration and lower in magnitude than those in adults recovering from cholera [65]. These differences may partially account for the difference in duration of protection between vaccination and natural infection. The evaluation of MBC in children receiving cholera vaccination remains to be reported, and comparisons between vaccinated children and adults may uncover the role that MBCs play in determining the duration of vaccine protection. As of yet, there has also been no evaluation of memory responses targeting O-specific polysaccharide in children and adults, despite the fact that immunity to *V. cholerae* is serogroup specific.

## Mucosal-specific adaptive responses

As *V. cholerae* is a noninvasive enteric pathogen, antigen-specific immune responses at the mucosal surface are believed to play a major role in protective immunity.

**Secretory IgA—**Secretory IgA is the predominant immunoglobulin at the mucosal surface, and may play a significant role in protection against cholera. In adults, antitoxin sIgA responses are detected in intestinal fluid, breast milk and saliva after both cholera infection and vaccination [74]. Anamnestic responses also likely contribute to protection, as evidenced by a rapid rise in intestinal lavage antitoxin IgA by day 3 in adults receiving a second course of immunization 15 months after primary vaccination [75], and by day 8 in previously infected adults given a repeated administration of *V. cholerae* O1 [23], both of which are the earliest times examined. However, such responses are unlikely to be the main mediators of immunity to cholera, as adults infected with *V. cholerae* O1 produce elevations in duodenal IgA against CTB and LPS up to day 30, but levels decrease to baseline by day 180, a shorter period of persistence than seen in circulating IgA [76].

**Gut-homing antibody-secreting cells—**Following antigen presentation in the gut mucosa, intestinal lymphocytes transiently migrate in the peripheral circulation before rehoming to the gut. These lymphocytes, termed antibody-secreting cells (ASCs), are

detectable in blood even before antigen-specific sIgA is detectable, peaking at 7–10 days after mucosal challenge [77]. ASCs to LPS and CTB have been demonstrated in adults infected with both serotypes of *V. cholerae* [78]. ASCs are also detected in duodenal tissue after infection, and a recent study reported that LPS-specific IgA ASCs are significantly increased up to day 180 after infection [76], despite the absence of corresponding increases in mucosal LPS IgA antibody.

Young Bangladeshi children infected with *V. cholerae* O1 Ogawa mount ASC responses to CTB and LPS that peak by day 7 after onset of illness and are comparable to the magnitude seen in older children and adults [46], although there is a trend for older age groups to have higher LPS-specific IgA ASCs, likely the reflection of a poorer mucosal response to the T-independent antigen in young children. In vaccinated adults, ASCs to LPS have been reported to be present in the circulation 7 days after vaccination [63]; however, such responses have not been evaluated in vaccinated children.

## Cell-mediated immunity

Although *V. cholerae* is a noninvasive mucosal pathogen, and pathogen-specific effector mucosal defense against *V. cholerae* is thought to be largely B-cell mediated, helper T cells likely play an important role in the development of B-cell immunity directed against protein antigens, as seen in the involvement of Th17 cells [79] and the chemokine receptor CXCR5 [80] in the generation of mucosal immune responses against CT. Adults recovering from cholera have increased frequencies of gut-homing, $CD4^+$ expressing, effector and central memory T-cells that peak at day 7 [81–83]. In these patients, *ex vivo* stimulation of cells with a *V. cholerae* O1 Ogawa membrane protein preparation results in priming of both Th1 (IFN-$\gamma$) and Th17 (IL-17) cytokine responses, and an increase in the Th1 to Th2 $CD4^+$ ratio [83]. By contrast, subjects given WC-rBS vaccine did not show these responses, although there was a trend toward an increase in IL-10 response. Furthermore, cytokines associated with a Th17 response are detectable in lamina propria samples during the acute stage of cholera using duodenal biopsy samples [83].

There are limited data on cellular immune responses in children with cholera. One study assessed aspects of cellular responses in infants aged 10–18 months given two doses of WC-rBS vaccine [84]. In these children, stimulation of post-vaccination lymphocytes *ex vivo* with a modified CTB resulted in increased $CD4^+$ blast formation and IFN-$\gamma$ production compared with responses elicited using prevaccination lymphocytes; however, in contrast with what occurs in adults, stimulation with a cholera membrane preparation did not produce any increases in children. Studies of T-cell responses in children with natural infection are needed.

## Modifiers of immune responses in children

A number of host factors modify disease severity and immune responses following infection and vaccination, including genetic polymorphisms, nutrition, micronutrient status, blood group and coinfection [85].

**Coinfection—**In regions endemic for cholera, concomitant intestinal infection with parasites and bacteria is common, especially in children. Greater than 35% of children aged ≤10 years presenting with acute *V. cholerae* infection to a hospital in India had concomitant parasitic infection [49,86]. The impact of helminthic coinfection on blunting the mucosal immune response to cholera infection and vaccination has been demonstrated in both adults and children [87,88], and there is evidence that alterations in cell-mediated immunity are responsible for differences in mucosal responses. In patients presenting with severe cholera in Bangladesh, helminth coinfection was associated with decreased fecal and serum IgA

responses to the T-dependent antigen CTB, but not to the T-independent antigen LPS [88]. Additionally, Ecuadorean children aged 13–17 years infected with *Ascaris lumbricoides* had a diminished Th1 cytokine response to vaccination with a live, attenuated OCV CVD 103-HgR compared with noninfected US controls, and this diminished response was partially reversed in a group treated with albendazole [89].

Coinfection with ETEC, an enteric bacterial pathogen commonly coendemic with *V. cholerae*, also alters the immune response to *V. cholerae*. In Bangladeshi adults and children hospitalized for diarrhea, those infected with both ETEC and *V. cholerae* (13% of patients) produced higher vibriocidal and higher antibody levels to CTB and LPS than those infected with *V. cholerae* alone [90].

**Genetic factors—**Recent studies have begun to address the role that genetics may play in the host immune response to cholera. It has long been known that patients with blood group O experience increased severity of cholera [91,92]. In a study of household contacts of index cholera patients in Bangladesh, familial segregation of susceptibility within households independent of blood group was observed, suggesting possible additional genetic contributions to cholera susceptibility and severity [18]. A family-based candidate gene association study in Dhaka, Bangladesh identified a variant in the promoter region of long palate, lung and nasal epithelium clone 1 to be associated with the disease [93]. Further exploration of genetic factors and their role in susceptibility to infection may uncover additional factors of importance affecting host immunity during cholera.

**Malnutrition & micronutrient deficiency—**Studies in animal models have demonstrated that protein deprivation results in impaired mucosal antitoxin responses [94] and severely diminished antibody responses to cholera vaccination [95]. In humans, malnutrition and intestinal infections are integrally related [96], and in Bangladesh, malnutrition is associated with up to half of deaths due to diarrhea in children under 5 years of age [97]. Low weight-for-age Z scores are associated with a 9.5 odds ratio for mortality from diarrhea [98], and in hospitalized patients with *V. cholerae* infection, individuals with protein–calorie malnutrition have increased stool losses and prolonged diarrhea [99].

Vitamin A, or retinol, and its metabolite, retinoic acid, are associated with host defense of infectious diseases, possibly through its effects on $CD4^+$ T-cell function [100]. Vitamin A supplementation has been associated with reductions in mortality and morbidity, including incidence of diarrhea, when given to nonhospitalized young children in developing countries [101]. In patients hospitalized for cholera, retinol deficiency is more common in children <12 years than in older patients [45], and is associated with an increased risk of *V. cholerae* O1 infection and symptomatic disease [18]. Unfortunately, vitamin A supplementation of oral cholera vaccination in young children produced only small increases in vibriocidal antibody responses [102], and had no effect on antitoxin antibody responses [59,102].

Zinc deficiency in children is associated with an increased risk of diarrhea, and supplementation of children with zinc in developing countries is associated with reductions in both incidence and severity of diarrhea [103]. In children with *V. cholerae* infection, zinc supplementation decreases the duration of diarrhea and stool output [104], although zinc deficiency was not associated with increased susceptibility to infection in household contacts [18]. By contrast to vitamin A supplementation, zinc supplementation in children has a differential effect on the immunogenicity of OCV, in that it increases the vibriocidal antibody response [60,102] and IFN-$\gamma$ production by $CD4^+$ T cells [84], but the antitoxin responses were lowered [59]. In ETEC, a related acute toxigenic diarrheal infection, zinc supplementation resulted in an increase of complement C3 levels and in phagocytic

functional activities [34]. The role that environmental or tropical enteropathy [105] may have on *V. cholerae* infection or vaccination and immune responses is unknown.

## Expert commentary

Current correlates of protective immunity to *V. cholerae* infection, including the vibriocidal antibody and antigen-specific antibodies, are imperfect measures that fall to baseline within months of infection, while protection against recurrent, symptomatic cholera lasts for 3–7 years. MBC responses have recently been identified in cholera patients, and these responses are long lived. The lack of detailed immunologic studies of immune responses in children with cholera reflects in part difficulties performing clinical investigations in children, as well as difficulties in obtaining the required volumes of blood for immunologic studies, although these studies are needed given the inequity of vaccine efficacy between children and adults. The recent characterization of prominent innate mucosal immune responses in adults should be extended to children, despite the limitations of obtaining intestinal biopsies in this population. Given the advances in knowledge of cell-mediated immunity and the role of helper T cells in B-cell development, analysis of T-cell responses and their contribution to the induction of protective immunity following cholera are also needed. Analysis of polysaccharide responses are also warranted since immunity to *V. cholerae* is serogroup specific. Evaluating the effect of tropical enteropathy on both susceptibility and response to *V. cholerae* infection and vaccination is also required. As cholera continues to cause a large burden of mortality and morbidity in children globally, a better understanding of anti-*V. cholerae* immunity in young children will critically contribute to the development of an improved cholera vaccine and immunization strategy.

## Five-year view

Recent advances in immunological methods have provided opportunities to look in detail at host responses to *V. cholerae* infection. In the next 5 years, we anticipate further advances in technology and methodology that will enable the evaluation of immune responses using much smaller volumes of biological specimens, thus further facilitating study of immune responses in infants and young children. Ongoing longitudinal studies of cholera patients and vaccinees will better inform our understanding of protective immunity following cholera. Progress on high-throughput techniques, including immunoproteomic and immunogenetic screening, will facilitate identification of novel antigens and factors contributing to host susceptibility. Currently available OCVs will set a benchmark for the development of future vaccines and immunization strategies, especially for children, who bear the largest burden of cholera globally.

## References

Papers of special note have been highlighted as

• of interest

1. Zuckerman JN, Rombo L, Fisch A. The true burden and risk of cholera: implications for prevention and control. Lancet Infect Dis. 2007; 7(8):521–530. [PubMed: 17584531]
2. Cholera: global surveillance summary 2008. Wkly Epidemiol Rec. 2009; 84(31):309–324. [PubMed: 19645127]
3. Penrose K, de Castro MC, Werema J, Ryan ET. Informal urban settlements and cholera risk in Dar es Salaam, Tanzania. PLoS Negl Trop Dis. 2010; 4(3):e631. [PubMed: 20300569]
4. Cholera, 2010. Wkly Epidemiol Rec. 2011; 86(31):325–339. [PubMed: 21800468]

5. Deen JL, von Seidlein L, Sur D, et al. The high burden of cholera in children: comparison of incidence from endemic areas in Asia and Africa. PLoS Negl Trop Dis. 2008; 2(2):e173. [PubMed: 18299707]
6. Agtini MD, Soeharno R, Lesmana M, et al. The burden of diarrhoea, shigellosis, and cholera in North Jakarta, Indonesia: findings from 24 months surveillance. BMC Infect Dis. 2005; 5:89. [PubMed: 16242013]
7. Glass RI, Becker S, Huq MI, et al. Endemic cholera in rural Bangladesh, 1966–1980. Am J Epidemiol. 1982; 116(6):959–970. [PubMed: 7148820]
8. Taylor RK, Miller VL, Furlong DB, Mekalanos JJ. Use of *phoA* gene fusions to identify a pilus colonization factor coordinately regulated with cholera toxin. Proc Natl Acad Sci USA. 1987; 84(9): 2833–2837. [PubMed: 2883655]
9. Sharp GW, Hynie S. Stimulation of intestinal adenyl cyclase by cholera toxin. Nature. 1971; 229(5282):266–269. [PubMed: 4323551]
10. Sack DA, Sack RB, Nair GB, Siddique AK. Cholera. Lancet. 2004; 363(9404):223–233. [PubMed: 14738797]
11. Lindenbaum J, Akbar R, Gordon RS Jr, Greenough WB 3rd, Hirschorn N, Islam MR. Cholera in children. Lancet. 1966; 1(7446):1066–1068. [PubMed: 4160844]
12. Ryan ET, Dhar U, Khan WA, et al. Mortality, morbidity, and microbiology of endemic cholera among hospitalized patients in Dhaka, Bangladesh. Am J Trop Med Hyg. 2000; 63(1–2):12–20. [PubMed: 11357989]
13. Molla AM, Ahmed SM, Greenough WB 3rd. Rice-based oral rehydration solution decreases the stool volume in acute diarrhea. Bull World Health Organ. 1985; 63(4):751–756. [PubMed: 3910298]
14. Lindenbaum J, Greenough WB, Islam MR. Antibiotic therapy of cholera in children. Bull World Health Organ. 1967; 37(4):529–538. [PubMed: 5301733]
15. Lindenbaum J, Greenough WB, Islam MR. Antibiotic therapy of cholera. Bull World Health Organ. 1967; 36(6):871–883. [PubMed: 4865453]
16. Saha D, Karim MM, Khan WA, Ahmed S, Salam MA, Bennish ML. Single-dose azithromycin for the treatment of cholera in adults. N Engl J Med. 2006; 354(23):2452–2462. [PubMed: 16760445]
17. Harris JB, Larocque RC, Charles RC, Mazumder RN, Khan AI, Bardhan PK. Cholera's western front. Lancet. 2010; 376(9757):1961–1965. [PubMed: 21112083]
18•. Harris JB, LaRocque RC, Chowdhury F, et al. Susceptibility to *Vibrio cholerae* infection in a cohort of household contacts of patients with cholera in Bangladesh. PLoS Negl Trop Dis. 2008; 2(4):e221. Identifies immune correlates of protection based on a study of household contacts. [PubMed: 18398491]
19. Cholera vaccines: WHO position paper. Wkly Epidemiol Rec. 2010; 85(13):117–128. [PubMed: 20349546]
20•. Sinclair D, Abba K, Zaman K, Qadri F, Graves PM. Oral vaccines for preventing cholera. Cochrane Database Syst Rev. 2011; 3:CD008603. Summary of differences in efficacy between age groups of current oral cholera vaccines. [PubMed: 21412922]
21. Sur D, Kanungo S, Sah B, et al. Efficacy of a low-cost, inactivated whole-cell oral cholera vaccine: results from 3 years of follow-up of a randomized, controlled trial. PLoS Negl Trop Dis. 2011; 5(10):e1289. [PubMed: 22028938]
22. Ali M, Emch M, Park JK, Yunus M, Clemens J. Natural cholera infection-derived immunity in an endemic setting. J Infect Dis. 2011; 204(6):912–918. [PubMed: 21849288]
23. Levine MM, Black RE, Clements ML, Cisneros L, Nalin DR, Young CR. Duration of infection-derived immunity to cholera. J Infect Dis. 1981; 143(6):818–820. [PubMed: 7252264]
24. Koelle K, Rodo X, Pascual M, Yunus M, Mostafa G. Refractory periods and climate forcing in cholera dynamics. Nature. 2005; 436(7051):696–700. [PubMed: 16079845]
25. Qadri F, Raqib R, Ahmed F, et al. Increased levels of inflammatory mediators in children and adults infected with *Vibrio cholerae* O1 and O139. Clin Diagn Lab Immunol. 2002; 9(2):221–229. [PubMed: 11874856]
26•. Qadri F, Bhuiyan TR, Dutta KK, et al. Acute dehydrating disease caused by *Vibrio cholerae* serogroups O1 and O139 induce increases in innate cells and inflammatory mediators at the

mucosal surface of the gut. Gut. 2004; 53(1):62–69. Report of innate immune responses as seen in mucosal biopsies from adults and children with acute cholera infection. [PubMed: 14684578]

27. Flach CF, Qadri F, Bhuiyan TR, et al. Broad up-regulation of innate defense factors during acute cholera. Infect Immun. 2007; 75(5):2343–2350. [PubMed: 17307946]
28. Rabbani GH, Islam S, Chowdhury AK, Mitra AK, Miller MJ, Fuchs G. Increased nitrite and nitrate concentrations in sera and urine of patients with cholera or shigellosis. Am J Gastroenterol. 2001; 96(2):467–472. [PubMed: 11232692]
29. Mathan MM, Chandy G, Mathan VI. Ultrastructural changes in the upper small intestinal mucosa in patients with cholera. Gastroenterology. 1995; 109(2):422–430. [PubMed: 7615191]
30. Shirin T, Rahman A, Danielsson A, et al. Antimicrobial peptides in the duodenum at the acute and convalescent stages in patients with diarrhea due to *Vibrio cholerae* O1 or enterotoxigenic *Escherichia coli* infection. Microbes Infect. 2011; 13(12–13):1111–1120. [PubMed: 21782033]
31. Shin OS, Uddin T, Citorik R, et al. LPLUNC1 modulates innate immune responses to *Vibrio cholerae*. J Infect Dis. 2011; 204(9):1349–1357. [PubMed: 21900486]
32. PrabhuDas M, Adkins B, Gans H, et al. Challenges in infant immunity: implications for responses to infection and vaccines. Nat Immunol. 2011; 12(3):189–194. [PubMed: 21321588]
33. Corbett NP, Blimkie D, Ho KC, et al. Ontogeny of Toll-like receptor mediated cytokine responses of human blood mononuclear cells. PLoS One. 2010; 5(11):e15041. [PubMed: 21152080]
34. Sheikh A, Shamsuzzaman S, Ahmad SM, et al. Zinc influences innate immune responses in children with enterotoxigenic *Escherichia coli*-induced diarrhea. J Nutr. 2010; 140(5):1049–1056. [PubMed: 20237063]
35. Neoh SH, Rowley D. The antigens of *Vibrio cholerae* involved in the vibriocidal action of antibody and complement. J Infect Dis. 1970; 121(5):505–513. [PubMed: 4986889]
36. Losonsky GA, Yunyongying J, Lim V, et al. Factors influencing secondary vibriocidal immune responses: relevance for understanding immunity to cholera. Infect Immun. 1996; 64(1):10–15. [PubMed: 8557325]
37. Majumdar AS, Ghose AC. Evaluation of the biological properties of different classes of human antibodies in relation to cholera. Infect Immun. 1981; 32(1):9–14. [PubMed: 7216497]
38. Mosley WH, Ahmad S, Benenson AS, Ahmed A. The relationship of vibriocidal antibody titre to susceptibility to cholera in family contacts of cholera patients. Bull World Health Organ. 1968; 38(5):777–785. [PubMed: 5303331]
39. Harris AM, Bhuiyan MS, Chowdhury F, et al. Antigen-specific memory B-cell responses to *Vibrio cholerae* O1 infection in Bangladesh. Infect Immun. 2009; 77(9):3850–3856. [PubMed: 19528207]
40. Clements ML, Levine MM, Young CR, et al. Magnitude, kinetics, and duration of vibriocidal antibody responses in North Americans after ingestion of *Vibrio cholerae*. J Infect Dis. 1982; 145(4):465–473. [PubMed: 7069227]
41. Mosley WH, Benenson AS, Barui R. A serological survey for cholear antibodies in rural east Pakistan. 1 The distribution of antibody in the control population of a cholera-vaccine field-trial area and the relation of antibody titre to the pattern of endemic cholera. Bull World Health Organ. 1968; 38(3):327–334. [PubMed: 5302327]
42. Glass RI, Svennerholm AM, Khan MR, Huda S, Huq MI, Holmgren J. Seroepidemiological studies of El Tor cholera in Bangladesh: association of serum antibody levels with protection. J Infect Dis. 1985; 151(2):236–242. [PubMed: 3968450]
43. Mosley WH, McCormack WM, Ahmed A, Chowdhury AK, Barui RK. Report of the 1966–67 cholera vaccine field trial in rural East Pakistan. 2 Results of the serological surveys in the study population–the relationship of case rate to antibody titre and an estimate of the inapparent infection rate with *Vibrio cholerae*. Bull World Health Organ. 1969; 40(2):187–197. [PubMed: 5306539]
44. Saha D, LaRocque RC, Khan AI, et al. Incomplete correlation of serum vibriocidal antibody titer with protection from *Vibrio cholerae* infection in urban Bangladesh. J Infect Dis. 2004; 189(12): 2318–2322. [PubMed: 15181581]
45•. Chowdhury F, Khan AI, Harris JB, et al. A comparison of clinical and immunologic features in children and older patients hospitalized with severe cholera in Bangladesh. Pediatr Infect Dis J.

2008; 27(11):986–992. Comparison of antibody responses between children and adults in both natural infection and Peru-15 vaccination in Bangladesh. [PubMed: 18833030]

46•. Leung DT, Rahman MA, Mohasin M, et al. A comparison of memory 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. 2011; 18(8): 1317–1325. Comparison of antibody, antibody-secreting cell and memory B-cell responses between young children, older children and adults with acute cholera. [PubMed: 21697337]

47. Clemens JD, Stanton BF, Chakraborty J, et al. B subunit-whole cell and whole cell-only oral vaccines against cholera: studies on reactogenicity and immunogenicity. J Infect Dis. 1987; 155(1):79–85. [PubMed: 3540139]

48. Sack DA, Clemens JD, Huda S, et al. Antibody responses after immunization with killed oral cholera vaccines during the 1985 vaccine field trial in Bangladesh. J Infect Dis. 1991; 164(2):407–411. [PubMed: 1856488]

49•. Saha A, Chowdhury MI, Khanam F, et al. 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. 2011; 29(46):8285–8292. Comparison of antibody responses to WC vaccine in young children, toddlers and adults in Bangladesh. [PubMed: 21907255]

50. Qadri F, Chowdhury MI, Faruque SM, et al. Peru-15, a live attenuated oral cholera vaccine, is safe and immunogenic in Bangladeshi toddlers and infants. Vaccine. 2007; 25(2):231–238. [PubMed: 16996172]

51•. Taylor DN, Cardenas V, Perez J, Puga R, Svennerholm AM. Safety, immunogenicity, and lot stability of the whole cell/recombinant B subunit (WC/rCTB) cholera vaccine in Peruvian adults and children. Am J Trop Med Hyg. 1999; 61(6):869–873. Comparison of antibody responses to WC-rBS vaccine in children and adults in Peru. [PubMed: 10674661]

52. Su-Arehawaratana P, Singharaj P, Taylor DN, et al. Safety and immunogenicity of different immunization regimens of CVD 103-HgR live oral cholera vaccine in soldiers and civilians in Thailand. J Infect Dis. 1992; 165(6):1042–1048. [PubMed: 1583321]

53. Kanungo S, Paisley A, Lopez AL, et al. Immune responses following one and two doses of the reformulated, bivalent, killed, whole-cell, oral cholera vaccine among adults and children in Kolkata, India: a randomized, placebo-controlled trial. Vaccine. 2009; 27(49):6887–6893. [PubMed: 19761838]

54. Mahalanabis D, Lopez AL, Sur D, et al. A randomized, placebo-controlled trial of the bivalent killed, whole-cell, oral cholera vaccine in adults and children in a cholera endemic area in Kolkata, India. PLoS One. 2008; 3(6):e2323. [PubMed: 18523643]

55. Mantis NJ, Rol N, Corthesy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. Mucosal Immunol. 2011; 4(6):603–611. [PubMed: 21975936]

56. Stiehm ER, Fudenberg HH. Serum levels of immune globulins in health and disease: a survey. Pediatrics. 1966; 37(5):715–727. [PubMed: 4956666]

57. Chowdhury F, Rahman MA, Begum YA, et al. Impact of rapid urbanization on the rates of infection by *Vibrio cholerae* O1 and enterotoxigenic *Escherichia coli* in Dhaka, Bangladesh. PLoS Negl Trop Dis. 2011; 5(4):e999. [PubMed: 21483709]

58. Smith NW, Sack RB. Immunologic cross-reactions of enterotoxins from *Escherichia coli* and *Vibrio cholerae*. J Infect Dis. 1973; 127(2):164–170. [PubMed: 4570128]

59. Qadri F, Ahmed T, Wahed MA, et al. Suppressive effect of zinc on antibody response to cholera toxin in children given the killed, B subunit-whole cell, oral cholera vaccine. Vaccine. 2004; 22(3–4):416–421. [PubMed: 14670323]

60. Ahmed T, Svennerholm AM, Al Tarique A, Sultana GN, Qadri F. Enhanced immunogenicity of an oral inactivated cholera vaccine in infants in Bangladesh obtained by zinc supplementation and by temporary withholding breast-feeding. Vaccine. 2009; 27(9):1433–1439. [PubMed: 19146904]

61. Nandy RK, Albert MJ, Ghose AC. Serum antibacterial and antitoxin responses in clinical cholera caused by *Vibrio cholerae* O139 Bengal and evaluation of their importance in protection. Vaccine. 1996; 14(12):1137–1142. [PubMed: 8911010]

62. Qadri F, Mohi G, Hossain J, et al. Comparison of the vibriocidal antibody response in cholera due to *Vibrio cholerae* O139 Bengal with the response in cholera due to *Vibrio cholerae* O1. Clin Diagn Lab Immunol. 1995; 2(6):685–688. [PubMed: 8574829]
63. Qadri F, Chowdhury MI, Faruque SM, et al. 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. 2005; 192(4):573–579. [PubMed: 16028125]
64. Clutterbuck EA, Oh S, Hamaluba M, Westcar S, Beverley PC, Pollard AJ. Serotype-specific and age-dependent generation of pneumococcal polysaccharide-specific memory B-cell and antibody responses to immunization with a pneumococcal conjugate vaccine. Clin Vaccine Immunol. 2008; 15(2):182–193. [PubMed: 18032593]
65. Alam MM, Riyadh MA, Fatema K, et al. 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. 2011; 18(5):844–850. [PubMed: 21346055]
66. Qadri F, Ahmed F, Karim MM, et al. Lipopolysaccharide- and cholera toxin-specific subclass distribution of B-cell responses in cholera. Clin Diagn Lab Immunol. 1999; 6(6):812–818. [PubMed: 10548569]
67. Boes M. Role of natural and immune IgM antibodies in immune responses. Mol Immunol. 2000; 37(18):1141–1149. [PubMed: 11451419]
68. Brandtzaeg P. The mucosal immune system and its integration with the mammary glands. J Pediatr. 2010; 156(2 Suppl):S8–S15. [PubMed: 20105666]
69. Belgemen T, Suskan E, Dogu F, Ikinciogullari A. Selective immunoglobulin M deficiency presenting with recurrent impetigo: a case report and review of the literature. Int Arch Allergy Immunol. 2009; 149(3):283–288. [PubMed: 19218822]
70. Croft NM, Hodges M. IgM: mucosal response in acute diarrhoeal disease of infants. Scand J Gastroenterol. 2005; 40(8):965–971. [PubMed: 16165711]
71. Kendall EA, Tarique AA, Hossain A, et al. Development of immunoglobulin M memory to both a T-cell-independent and a T-cell-dependent antigen following infection with *Vibrio cholerae* O1 in Bangladesh. Infect Immun. 2010; 78(1):253–259. [PubMed: 19858296]
72. Mond JJ, Vos Q, Lees A, Snapper CM. T cell independent antigens. Curr Opin Immunol. 1995; 7(3):349–354. [PubMed: 7546399]
73. Kelly DF, Pollard AJ, Moxon ER. Immunological memory: the role of B cells in long-term protection against invasive bacterial pathogens. JAMA. 2005; 294(23):3019–3023. [PubMed: 16414950]
74. Jertborn M, Svennerholm AM, Holmgren J. Saliva, breast milk, and serum antibody responses as indirect measures of intestinal immunity after oral cholera vaccination or natural disease. J Clin Microbiol. 1986; 24(2):203–209. [PubMed: 3528211]
75. Svennerholm AM, Jertborn M, Gothefors L, Karim AM, Sack DA, Holmgren J. Mucosal antitoxic and antibacterial immunity after cholera disease and after immunization with a combined B subunit-whole cell vaccine. J Infect Dis. 1984; 149(6):884–893. [PubMed: 6736680]
76. Uddin T, Harris JB, Bhuiyan TR, et al. Mucosal immunologic responses in cholera patients in Bangladesh. Clin Vaccine Immunol. 2011; 18(3):506–512. [PubMed: 21248157]
77. Czerkinsky C, Prince SJ, Michalek SM, et al. IgA antibody-producing cells in peripheral blood after antigen ingestion: evidence for a common mucosal immune system in humans. Proc Natl Acad Sci USA. 1987; 84(8):2449–2453. [PubMed: 3470804]
78. Qadri F, Wenneras C, Albert MJ, et al. Comparison of immune responses in patients infected with *Vibrio cholerae* O139 and O1. Infect Immun. 1997; 65(9):3571–3576. [PubMed: 9284121]
79. Datta SK, Sabet M, Nguyen KP, et al. Mucosal adjuvant activity of cholera toxin requires Th17 cells and protects against inhalation anthrax. Proc Natl Acad Sci USA. 2010; 107(23):10638–10643. [PubMed: 20479237]
80. Velaga S, Herbrand H, Friedrichsen M, et al. Chemokine receptor CXCR5 supports solitary intestinal lymphoid tissue formation, B cell homing, and induction of intestinal IgA responses. J Immunol. 2009; 182(5):2610–2619. [PubMed: 19234155]

81. Bhuiyan TR, Lundin SB, Khan AI, et al. Cholera caused by *Vibrio cholerae* O1 induces T-cell responses in the circulation. Infect Immun. 2009; 77(5):1888–1893. [PubMed: 19237532]
82. Weil AA, Arifuzzaman M, Bhuiyan TR, et al. Memory T-cell responses to *Vibrio cholerae* O1 infection. Infect Immun. 2009; 77(11):5090–5096. [PubMed: 19703973]
83. Kuchta A, Rahman T, Sennott EL, et al. *Vibrio cholerae* O1 infection induces proinflammatory $CD4^+$ T-cell responses in blood and intestinal mucosa of infected humans. Clin Vaccine Immunol. 2011; 18(8):1371–1377. [PubMed: 21697339]
84•. Ahmed T, Arifuzzaman M, Lebens M, Qadri F, Lundgren A. $CD4^+$ T-cell responses to an oral inactivated cholera vaccine in young children in a cholera endemic country and the enhancing effect of zinc supplementation. Vaccine. 2009; 28(2):422–429. Description of cell-mediated immunity in young children receiving cholera vaccine. [PubMed: 19837094]
85. Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. BMC Biol. 2010; 8:129. [PubMed: 20920375]
86. Saha DR, Rajendran K, Ramamurthy T, Nandy RK, Bhattacharya SK. Intestinal parasitism and *Vibrio cholerae* infection among diarrhoeal patients in Kolkata, India. Epidemiol Infect. 2008; 136(5):661–664. [PubMed: 17594735]
87. Cooper PJ, Chico ME, Losonsky G, et al. Albendazole treatment of children with ascariasis enhances the vibriocidal antibody response to the live attenuated oral cholera vaccine CVD 103-HgR. J Infect Dis. 2000; 182(4):1199–1206. [PubMed: 10979918]
88. Harris JB, Podolsky MJ, Bhuiyan TR, et al. Immunologic responses to *Vibrio cholerae* in patients co-infected with intestinal parasites in Bangladesh. PLoS Negl Trop Dis. 2009; 3(3):e403. [PubMed: 19333369]
89. Cooper PJ, Chico M, Sandoval C, et al. Human infection with *Ascaris lumbricoides* is associated with suppression of the interleukin-2 response to recombinant cholera toxin B subunit following vaccination with the live oral cholera vaccine CVD 103-HgR. Infect Immun. 2001; 69(3):1574–1580. [PubMed: 11179329]
90. Chowdhury F, Begum YA, Alam MM, et al. Concomitant enterotoxigenic *Escherichia coli* infection induces increased immune responses to *Vibrio cholerae* O1 antigens in patients with cholera in Bangladesh. Infect Immun. 2010; 78(5):2117–2124. [PubMed: 20176796]
91. Glass RI, Holmgren J, Haley CE, et al. Predisposition for cholera of individuals with O blood group. Possible evolutionary significance. Am J Epidemiol. 1985; 121(6):791–796. [PubMed: 4014172]
92. Harris JB, Khan AI, LaRocque RC, et al. Blood group, immunity, and risk of infection with *Vibrio cholerae* in an area of endemicity. Infect Immun. 2005; 73(11):7422–7427. [PubMed: 16239542]
93. LaRocque RC, Sabeti P, Duggal P, et al. A variant in long palate, lung and nasal epithelium clone 1 is associated with cholera in a Bangladeshi population. Genes Immun. 2009; 10(3):267–272. [PubMed: 19212328]
94. Barry WS, Pierce NF. Protein deprivation causes reversible impariment of mucosal immune response to cholera toxoid/toxin in rat gut. Nature. 1979; 281(5726):64–65. [PubMed: 551268]
95. Flo J, Roux ME, Massouh E. Deficient induction of the immune response to oral immunization with cholera toxin in malnourished rats during suckling. Infect Immun. 1994; 62(11):4948–4954. [PubMed: 7927775]
96. Guerrant RL, Oria RB, Moore SR, Oria MO, Lima AA. Malnutrition as an enteric infectious disease with long-term effects on child development. Nutr Rev. 2008; 66(9):487–505. [PubMed: 18752473]
97. Baqui AH, Black RE, Arifeen SE, Hill K, Mitra SN, al Sabir A. Causes of childhood deaths in Bangladesh: results of a nationwide verbal autopsy study. Bull World Health Organ. 1998; 76(2): 161–171. [PubMed: 9648357]
98. Black RE, Allen LH, Bhutta ZA, et al. Maternal and child undernutrition: global and regional exposures and health consequences. Lancet. 2008; 371(9608):243–260. [PubMed: 18207566]
99. Palmer DL, Koster FT, Alam AK, Islam MR. Nutritional status: a determinant of severity of diarrhea in patients with cholera. J Infect Dis. 1976; 134(1):8–14. [PubMed: 820813]
100. Pino-Lagos K, Guo Y, Brown C, et al. A retinoic acid-dependent checkpoint in the development of $CD4^+$ T cell-mediated immunity. J Exp Med. 2011; 208(9):1767–1775. [PubMed: 21859847]

101. Mayo-Wilson E, Imdad A, Herzer K, Yakoob MY, Bhutta ZA. Vitamin A supplements for preventing mortality, illness, and blindness in children aged under 5: systematic review and meta analysis. BMJ. 2011; 343:d5094. [PubMed: 21868478]

102. Albert MJ, Qadri F, Wahed MA, et al. Supplementation with zinc, but not vitamin A, improves seroconversion to vibriocidal antibody in children given an oral cholera vaccine. J Infect Dis. 2003; 187(6):909–913. [PubMed: 12660937]

103. Aggarwal R, Sentz J, Miller MA. Role of zinc administration in prevention of childhood diarrhea and respiratory illnesses: a meta-analysis. Pediatrics. 2007; 119(6):1120–1130. [PubMed: 17545379]

104. Roy SK, Hossain MJ, Khatun W, et al. Zinc supplementation in children with cholera in Bangladesh: randomised controlled trial. BMJ. 2008; 336(7638):266–268. [PubMed: 18184631]

105. Humphrey JH. Child undernutrition, tropical enteropathy, toilets, and handwashing. Lancet. 2009; 374(9694):1032–1035. [PubMed: 19766883]

**Box 1**

**Important microbial factors expressed by *Vibrio cholerae.***

- Lipopolysaccharide – T-cell-independent antigen, a serogrouping and serotyping determinant of *Vibrio cholerae* strains
- O-specific polysaccharide – T-cell-independent antigen, the determinant of serogroup and serotype
- Toxin coregulated pilus – T-cell-dependent group of antigens, involved in intestinal colonization
- Cholera toxin – T-cell-dependent antigen, causes intestinal secretion of electrolytes and water. A potent enterotoxin and immunoadjuvant. B subunit referred to as CTB or CtxB.

CTB: Cholera toxin B (CtxB).

## Key issues

- Cholera is a dehydrating diarrheal disease caused by *Vibrio cholerae* serogroups O1 and O139.
- In areas of the world endemic for cholera, children bear a large burden of infection.
- During cholera epidemics among immunologically naive populations, children and adults are equally affected by cholera.
- Current oral cholera vaccines have lower efficacy and shorter duration of protection in young children compared with adults.
- Innate immune responses occur during cholera in adults, although studies of innate immune responses to cholera have not been performed in children.
- Young children are able to mount comparable vibriocidal and toxin-specific antibody responses as adults to both infection and vaccination, but neither are sufficient predictors of protective immunity against cholera.
- Limited studies suggest that young children mount a lower magnitude of IgA responses to lipopolysaccharide than adults following vaccination.
- Anti-polysaccharide responses have not yet been evaluated during cholera, despite serogroup specificity of protection.
- Memory B cells mediate long-term protective immunity by facilitating anamnestic antibody responses, and such responses against *V. cholerae* antigens are comparable in young children and adults up to a month after infection.
- Current oral cholera vaccines have not been shown to induce memory responses comparable to those induced by infection.
- Investigations are lacking regarding long-term anti-*V. cholerae* memory responses in children following natural infection and vaccination.
- Th1 and Th17 responses may be involved in development of memory B-cell responses following cholera.
- Host factors such as helminth coinfection, blood group, enteropathy and micronutrient deficiencies affect immune responses in children with cholera.