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Kinetics of antibody-secreting cell and fecal IgA responses after oral cholera vaccination in different age groups in a cholera endemic country

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ABSTRACT

Immune responses to oral enteric vaccines in children and infants may be influenced by factors such as age, previous priming with related microorganisms and breast feeding. In this study, we aimed to determine optimal time points to assess immune responses to oral enteric vaccines in different clinical specimens. This was done by investigating antibody secreting cell (ASC) and fecal antibody responses on different days after vaccination using the licensed oral cholera vaccine Dukoral, containing cholera toxin B-subunit (rCTB) and inactivated *Vibrio cholerae* bacteria, as a model vaccine.

Two vaccine doses were given 2 weeks apart to infants (6–11 months), young children (12–18 months), toddlers (19 months–5 years) and adults in a cholera endemic country (Bangladesh). IgA ASC responses, as determined by the antibodies in lymphocyte supernatant (ALS) assay, plasma IgA and IgG responses and secretory IgA (SIgA) responses in extracts of fecal samples were evaluated 4/5 and 7 days after each vaccination.

After the first vaccine dose, anti-CTB ALS IgA responses in adults and toddlers were high and comparable on day 5 and 7, while responses were low and infrequent in young children. After the second dose, highest ALS responses were detected on day 5 among the time points studied in all age groups and the responses declined until day 7. In contrast, plasma IgA and IgG anti-CTB responses were high both on day 5 and 7 after the second dose. Fecal SIgA responses in young children and infants were highest on day 7 after the second dose.

Our results suggest that ASC/ALS responses to two doses of the oral cholera vaccine Dukoral and related oral vaccines should be analyzed earlier than previously recommended (day 7) at all ages. Fecal antibody responses should preferably be analyzed later than ASC/ALS responses to detect the highest antibody responses.

1. Introduction

Enteric infections are important global health problems and are principal causes of morbidity and mortality in children and infants in low- and middle-income countries [1]. Since mucosal IgA antibodies are important for protection against most enteric infections it is essential to measure such responses in both adults and infants using accurate and sensitive assays suitable for clinical vaccine trials [2]. Some of the most widely used methods for evaluation of mucosal immune responses induced by oral vaccines include analysis of specific IgA antibodies in intestinal lavage or fecal specimens [3–5] as well as assessment of specific antibodies produced by intestine-derived antibody-secreting cells (ASCs), which transiently migrate through peripheral blood in response to oral vaccination [6,7] and can be detected either by ELISPOT or by the antibodies in lymphocyte supernatant (ALS) assay [8,9]. These techniques are often initially used in clinical trials of healthy adults

Abbreviations: AEs, adverse events; ALS, antibodies in lymphocyte supernatant; ASC, antibody-secreting cell; CFs, colonization factors; CT, cholera toxin; CTB, cholera toxin B-subunit; ETEC, enterotoxigenic *Escherichia coli*; LT, heat labile toxin; LTB, heat labile toxin B-subunit; MP, membrane preparation; PBMCs, peripheral blood mononuclear cells; RF, responder frequencies; SIgA, secretory IgA.

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in non-endemic countries, who are most likely naïve in relation to vaccine related microorganisms and antigens. However, immune responses to oral vaccines may be very different in infants and children in endemic countries due to e.g. natural exposure to infections, age-related differences in immune function, breast feeding and nutritional status [2,10]. Hence, optimization of immunoassays in these target populations should be undertaken, including identification of optimal time points for sampling of clinical specimens, before use in clinical vaccine trials.

Recent studies in adult Swedish volunteers have clearly demonstrated that repeated immunization with the licensed oral cholera vaccine Dukoral as well as a novel oral inactivated multivalent enterotoxigenic *Escherichia coli* (ETEC) vaccine (ETVAX) induce more rapid and transient ALS responses to toxin and bacterial protein antigens than previously appreciated [11–13]. These data suggest that such responses may have been underestimated in several previous studies of oral vaccines in both endemic and nonendemic countries [5,14–21]. However, it is still unclear how age and natural exposure to cholera and ETEC infections may influence the kinetics of ASC responses to oral vaccines.

Peripheral ASC responses induced by oral vaccination are known to reflect intestinal immunity, as measured by analysis of antibodies in fecal or lavage samples or intestinal tissue [3,5,21]. In clinical trials of mucosal vaccines, it is often more demanding to collect, store and/or extract intestinal compared to blood samples, and many studies of mucosal immunity are therefore mainly based on proxy markers such as ASC assessment [7]. However, we and others have shown that it is possible to use fecal samples to more directly measure the induction of vaccine specific IgA antibodies in adults and children and that such analyses may replace or complement ASC assessments [3,5,12,22–24]. Determination of fecal immune responses is particularly attractive in young children and infants since only small blood volumes can be collected from these age groups and venipuncture is preferably completely avoided. However, fecal specimens from infants in endemic areas may be contaminated by breast milk containing specific IgA antibodies against enteric pathogens which may obliterate vaccine immune responses.

The main aim of this study was to potentially improve assessment of immune responses to mucosal vaccines against enteric infections by identifying optimal sampling time points for determination of ALS responses after oral vaccination in different age groups in an endemic area. The study was performed to prepare for subsequent Phase I/II trials of oral vaccines, e.g. the ETEC vaccine ETVAX, containing a heat labile toxin B-subunit/cholera toxin B-subunit (LTB/CTB) hybrid toxoid and inactivated bacteria overexpressing common ETEC colonization factors (CFs). The licensed cholera vaccine Dukoral, containing CTB and killed *Vibrio cholerae* bacteria, was used as a model vaccine; two consecutive doses were given to adults, toddlers, young children and infants in Bangladesh. The feasibility to measure vaccine specific secretory IgA (SIgA) antibody responses in fecal samples collected at different time points from young children and infants was also investigated.

2. Materials and methods

2.1. Study design and vaccinations

The study was conducted in Mirpur, Dhaka, Bangladesh. Healthy adults (18–45 years, n = 40), toddlers (19 months to 5 years, n = 20), young children (12–18 months, n = 20) and infants (6–11 months, n = 10) were recruited to the study. The study physician assessed the general health of the subjects at enrollment. Subjects with a history of gastrointestinal disorders, diarrheal illness during the last two weeks, febrile illness in the preceding week or antibiotic treatment within one week prior to enrollment were excluded. Subjects who had been vaccinated with any cholera or ETEC vaccines previously were also excluded.

Participants received two oral doses of Dukoral® (Crucell, Sweden) 14 days apart. The vaccine consists of 1.25×10^{11} killed *V. cholerae* bacteria and 1 mg of rCTB. A full dose of the vaccine was suspended in 150 ml (adults), 75 ml (toddlers) or 15 ml (young children and infants) carbonate buffer (Recip, Sweden). Participants were not allowed to eat or drink one hour before and after vaccination.

The study was approved by the Research Review and Ethical Review Committees of the International Review Board of the icddr, b (International Centre for Diarrhoeal Disease Research, Bangladesh) and informed written consent was obtained from each participant and for children from their parent/guardian before enrollment.

2.2. Specimen collection

From each participant, heparinized venous blood and fecal specimens were collected at three different time points; before immunization (day 0) and at two additional time points (day 4 or 5 and day 7), either after the first or the second vaccine dose (Table 1).

2.3. Evaluation of immune responses

Mucosal immune responses were evaluated by measuring vaccine specific antibodies in secretions from intestine-derived ASCs using the ALS assay and by analysis of SIgA antibodies in fecal extracts. Systemic immune responses were determined as vaccine specific IgA and IgG antibodies in plasma.

Peripheral blood mononuclear cells (PBMCs) and plasma were separated by density-gradient centrifugation on Ficoll-Isopaque (Pharmacia, Sweden). Plasma was stored at −20 °C. For the ALS assay, PBMCs were cultured at 10^7 cells/ml as described [25] and supernatants were collected after 48 h of incubation and stored at −70 °C. Fecal extracts were prepared as described and stored at −70 °C [3].

IgA antibodies in ALS samples, SIgA antibodies in fecal extracts and IgA and IgG antibodies plasma samples were analyzed by ELISA using plates coated with GM1 ganglioside (0.3 nmol/ml) plus CTB (1 μg/ml for ALS and fecal samples and 0.5 μg/ml for plasma) or a membrane preparation (MP) from *V. cholerae* bacteria (3 μg/ml) [11,12,22]. The MP was purified from a *V. cholerae* O1 strain cultured in AKI medium followed by sonication and precipitation with 40% saturated ammonium sulphate [9,26]. The preparation contained 7.9 mg/ml protein and 6 mg/ml *V. cholerae* O1 LPS. Endpoint titers were determined as the reciprocal interpolated dilutions giving an absorbance of 0.2 (ALS, faeces) or 0.4 (plasma) above background at 492 nm. The total SIgA level of each fecal sample was also determined by ELISA, as described [3]. Fecal

Table 1
Sampling schedule in different age groups.

Study groups	Before vaccination	After dose 1	After dose 2
Adults			
A	Day 0	Days 4 + 7	–
B	Day 0	Days 5 + 7	–
C	Day 0	–	Days 4 + 7
D	Day 0	–	Days 5 + 7
Toddlers and young children			
B	Day 0	Days 5 + 7	–
D	Day 0	–	Days 5 + 7
Infants			
D	Day 0	–	Days 5 + 7