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Lipopolysaccharide-specific memory B cell responses to an attenuated live cholera vaccine are associated with protection against *Vibrio cholerae* infection

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ABSTRACT

Background: The single-dose live attenuated vaccine CVD 103-HgR protects against experimental *Vibrio cholerae* infection in cholera-naïve adults for at least 6 months after vaccination. While vaccine-induced vibriocidal seroconversion is associated with protection, vibriocidal titers decline rapidly from their peak 1–2 weeks after vaccination. Although vaccine-induced memory B cells (MBCs) might mediate sustained protection in individuals without detectable circulating antibodies, it is unknown whether oral cholera vaccination induces a MBC response.

Methods: In a study that enrolled North American adults, we measured lipopolysaccharide (LPS)- and cholera toxin (CtxB)-specific MBC responses to PXVX0200 (derived from the CVD 103-HgR strain) and assessed stool volumes following experimental *Vibrio cholerae* infection. We then evaluated the association between vaccine-induced MBC responses and protection against cholera.

Results: There was a significant increase in % CT-specific IgG, % LPS-specific IgG, and % LPS-specific IgA MBCs which persisted 180 days after vaccination as well as a significant association between vaccine-induced increase in % LPS-specific IgA MBCs and lower post-challenge stool volume ($r = -0.56$, $p < 0.001$).

Discussion: Oral cholera vaccination induces antigen-specific MBC responses, and the anamnestic LPS-specific responses may contribute to long-term protection and provide correlates of the duration of vaccine-induced protection.

Clinical trials registration: NCT01895855.

1. Background

The oral single-dose live attenuated vaccine CVD 103-HgR provides protection against experimental infection with *Vibrio cholerae* in cholera-naïve North American adults for at least 6 months after vaccination [1,2]. However, data on the long-term protection of the CVD 103-HgR vaccine do not exist since no study has assessed protection beyond 6 months post-vaccination. Consequently, clinical studies evaluating the long-term protection provided by CVD 103-HgR or identifying long-lasting immunologic correlates of protection following vaccination would address a key knowledge gap surrounding the properties of this vaccine.

Subsequent to the completion of two successful challenge studies with CVD-103 HgR [1,2], PaxVax, Inc. acquired a worldwide, exclusive license to the CVD 103-HgR strain in 2009 with the aim of redeveloping the vaccine. The PaxVax research name for the vaccine, prepared from new CVD 103-HgR master and working cell banks, was PXVX0200 (now named Vaxchora®).

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In 2013, the results of a challenge study in cholera-naïve volunteers demonstrated that serum vibriocidal response 10 days after vaccination with PXVX0200 was strongly associated with protection for volunteers who were challenged either 10 days or 90 days after vaccination [3,4]. However, after peaking on Day 10 post-vaccination, vibriocidal titers dropped over 10-fold between Day 10 and Day 90. This longitudinal pattern suggests that the vibriocidal response to vaccine, while useful as a correlate of protection when assessed soon after vaccination, may not be a good measure for assessing the duration of vaccine-derived immunity. Part of the explanation for this limitation may be that vibriocidal seroconversion signals an immune response that mediates long-term protection against *V. cholerae* at the mucosal surface.

Assessment of anamnestic responses may offer a promising alternative approach for quantifying duration of protection. In particular, circulating memory B cells (MBCs) that are specific for *V. cholerae* antigens may mediate long-term protective immunity against *V. cholerae* following either infection or vaccination. It has been demonstrated in endemic Bangladesh that *V. cholerae* infection induces a long-lasting MBC response [5,6], and that the presence of LPS-specific IgG MBCs at the time of exposure to cholera is strongly predictive of protection in household contacts of patients with cholera, even among individuals who lack circulating vibriocidal antibodies [7]. Similarly, data from a study in cholera-naïve North American volunteers demonstrate that experimental *V. cholerae* infection primes a significant anamnestic IgA response to LPS that is detectable 6 months after infection [8].

While the existing data suggest that memory B cell responses may contribute to long-term immunologic memory and protection following infection with *V. cholerae*, the extent of the MBC response that results from oral cholera vaccination and the contribution of this response to protection against cholera are still unknown. To address this key knowledge gap, we characterized MBC responses following vaccination with the single-dose live attenuated oral cholera vaccine PXVX0200 and compared them to MBC responses following experimental infection in subjects who received placebo at baseline. We then measured the association between MBC responses and protection against challenge administered 90 days after vaccination.

2. Methods

2.1. Description of study cohort

This study was conducted in a subset of cholera-naïve subjects who participated in a well-characterized vaccine challenge trial. The study design, efficacy results, and safety data from the challenge trial were first described by Chen et al., who showed that for the outcome of moderate to severe diarrhea, PXVX0200 had a protective efficacy of 90% at 10 days after vaccination and 79% at 90 days after vaccination [3].

In the challenge trial, 197 study volunteers were randomized to receive PXVX0200 or placebo in a 1:1 ratio. Subsets of volunteers were randomly selected to be challenged at either 10 days or 90 days after vaccination: 35 vaccinees and 33 placebo recipients were challenged at Day 10, and 33 vaccinees and 33 placebo recipients were challenged at Day 90. Each challenged participant was administered 1×10^5 CFU of virulent *V. cholerae* O1 El Tor Inaba strain N16961 – a heterologous biotype but homologous serotype to the classical Inaba strain from which PXVX0200 is derived. Sixty-three additional subjects – 27 vaccine recipients and 36 placebo recipients – were randomized but not challenged.

Since our study is focused upon the role of memory B cells in long-term immunity, one part of the dataset for our analysis is comprised of the 66 subjects who participated in the 90-day challenge. In this cohort, we looked at the association between memory B cell response and protection against moderate/severe diarrhea. We also evaluated MBC responses at Day 180 in 22 of the 27 vaccine recipients who were not challenged with *V. cholerae*. Finally, we assessed the MBC response 170 days after challenge in 26 of the 33 individuals who received placebo on Day 0 followed by *V. cholerae* challenge on Day 10.

This trial was approved by Institutional Review Boards at the three enrollment centers (Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD; Cincinnati Children's Hospital Medical Center, Cincinnati, OH; and Vaccine Testing Center, University of Vermont College of Medicine, Burlington, VT). Informed consent was obtained for all trial participants. The registration number of the trial at clinicaltrials.gov is NCT01895855.

2.2. Vaccine

Lyophilized PXVX0200 sachets stored at -20 °C were reconstituted in a sodium bicarbonate buffer that was made using 100 mL of bottled water and single-dose buffer sachets containing 2.5 g $NaHCO_3$, 1.5 g ascorbic acid, and 0.2 g lactose. At reconstitution, the single-dose PXVX0200 solution contained 5×10^8 CFU. One batch of vaccine, Lot No. P701.550-8WA02, was used in the study. The manufacturer of the vaccine is PaxVax, Inc (San Diego, CA, USA).

2.3. Measurement of memory B cell responses

Standard operating protocols for shipping, processing and testing of samples of blood and peripheral blood mononuclear cell (PBMC) were established and followed throughout the clinical trial.

Blood samples for measurements of antigen-specific MBC levels for the 66 subjects in the 90-day challenge cohort were drawn on Day 0 just prior to receipt of vaccine or placebo and on Day 90 prior to challenge. Blood samples for the 22 unchallenged vaccinees and for the 26 placebo subjects in the 10-day challenge cohort were drawn on Days 0 and 180. Blood samples were shipped overnight to PaxVax where peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient separation and cryopreserved in liquid nitrogen until ready for testing.

An enzyme-linked immunospot (ELISPOT) assay was used to measure MBC frequencies and an assay protocol was developed and optimized based on previous studies [9–12]. Cryopreserved PBMC were thawed then stimulated in culture medium containing R848 (InvivoGen VacciGrade, 1 μg/mL) and human recombinant IL-2 (Peprotech, 10 ng/mL) under conditions optimized for proliferation and differentiation of $CD19^+/CD27^+$ MBC. PBMC (1×10^6/mL) were stimulated for 5 days at 37 °C in 5% CO_2 in upright 25 cm^2 tissue culture flasks (Corning). Pre- and post-treatment PBMC samples from each subject were batch-tested in the same experiment.

Culture medium for all experiments consisted of RPMI-1640 with HEPES and glutamine (Mediatech) which was further supplemented with 10% fetal bovine serum (Hyclone), 1X MEM non-essential amino acids (Invitrogen), 1 mM sodium pyruvate (Corning), 50 μg/mL gentamicin (Amresco) and 2 mM GlutaMax (Invitrogen).

On the day prior to testing for MBC, 96-well ELISPOT plates (Millipore MIPS4150) were coated overnight at 4 °C with antigen or capture antibody. Coating antigen consisted of LPS from *V. cholera*, Inaba 569B serotype (University of Maryland Center for Vaccine Development; 50 μg/mL) or cholera toxin B (CtxB) (List Biological Laboratories; 15 μg/mL). Assay wells receiving CtxB were first pre-coated with GM1 ganglioside (Enzo Life Sciences; 3 nM) for 3 h at room temperature. To detect total IgG- or IgA-secreting MBC, wells were coated with monoclonal anti-human

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