Safety and Immunogenicity of Escalating Dosages of a Single Oral Administration of Peru-15 pCTB, a Candidate Live, Attenuated Vaccine against Enterotoxigenic Escherichia coli and Vibrio cholerae

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Enterotoxigenic Escherichia coli (ETEC) organisms are a leading cause of infectious diarrhea in developing countries. A live, attenuated cholera strain that expresses high levels of the nontoxic B subunit of cholera toxin, which might also serve as an ETEC protective antigen, was evaluated for safety, excretion, and immunogenicity in healthy volunteers. We enrolled four inpatient dose-escalation cohorts of 15 to 16 eligible subjects to randomly (3:1) receive a single oral dose of vaccine or placebo (buffer alone), evaluating 1 × 10⁷, 1 × 10⁸, 1 × 10⁹, and 1 × 10¹⁰ CFU of the vaccine. The vaccine was well tolerated, although some subjects experienced moderate diarrhea. The serum Inaba vibriocidal antibody response appeared to display a dose-response relationship with increasing doses of vaccine, plateauing at the 10⁸-CFU dosage. The serum antitoxin (cholera toxin and heat-labile enterotoxin) antibody seroconversion rate (4-fold increase over baseline) also appeared to display a dose-response relationship. The vaccine strain was excreted in stool cultures, displaying a dose-response relationship. A single oral dose of Peru-15 pCTB at dosages up to 1 × 10¹⁰ CFU was safe and immunogenic in this first-in-human trial. These encouraging data support the ongoing clinical development of this candidate combined cholera and ETEC vaccine. (This study has been registered at ClinicalTrials.gov under registration no. NCT00654108.)

One of the most important etiologic agents causing diarrhea among travelers from industrialized countries who visit developing countries is the mucosally noninvasive bacterial pathogen enterotoxigenic Escherichia coli (ETEC) (1-3). ETEC infections are also a leading cause of serious diarrheal illness and death in infants and young children in developing countries (4). Cumulatively, ETEC is estimated to cause ~600 million total cases of diarrhea worldwide annually, including ~280 million cases and >400,000 deaths in children <5 years of age (5).

After the ingestion of contaminated food or water, ETEC organisms colonize the upper intestinal tract by a variety of antigenically distinct colonization factors (6). Once an infection is established, the bacteria secrete either a heat-labile toxin (LT), heat-stable toxin (ST), or both. ETEC strains are antigenically diverse, and the existence of many different O:K serotypes, multiple fimbrial colonization factors, and three different enterotoxin phenotypes (LT only, ST only, and LT plus ST) (7) have complicated vaccine development.

However, LT is an oligomeric protein that is structurally, functionally, and antigenically similar to the cholera toxin (CT) of Vibrio cholerae and consists of a single enzymatically active subunit (LTA) and a pentameric complex of five identical receptor binding subunits (LTB) similar to the corresponding cholera toxin subunits (CTA and CTB, respectively). Although LT and CT have many features in common, they are clearly distinct molecules with biochemical and immunologic differences that make them unique (8). However, several studies have demonstrated cross-protective immunity between CT and LT. For example, short-term protection against LT-ST-producing ETEC infections has been observed with a killed cholera vaccine in a setting endemic for cholera (9), and with Finnish travelers to Morocco (10), protection was dependent on the inclusion of CTB in the vaccine.

Peru-15 is a live, attenuated, and nonmotile V. cholerae O1 El Tor Inaba strain that has been well tolerated as a single-dose oral vaccine at dosages ranging from 1 × 10⁷ to 1 × 10⁹ CFU (11-15) and was documented to provide protection against moderate to severe cholera diarrhea in a human challenge study (16). Peru-15 pCTB, the current investigational vaccine, was created by inserting a plasmid carrying the gene for the nontoxic B subunit of cholera toxin (ctxB) into the parental Peru-15 strain, resulting in a 30-fold greater expression of CTB (17). On the basis of previous findings showing CTB to be an effective immunogen for preventing diarrhea against LT-producing ETEC (9,10), we hypothesized that Peru-15 pCTB could be developed as a vaccine for the prevention of both cholera and ETEC-related diarrheal disease. The purpose of this study (registered at ClinicalTrials.gov under registration no. NCT00654108) was to determine the safety and immunogenicity of a range of oral doses of Peru-15 pCTB in a phase 1 trial.
MATERIALS AND METHODS

Vaccine. Peru-15 pCTB consists of the live, attenuated vaccine strain Peru-15, from which the glutamine synthetase gene (glnA) was deleted, rendering the organism unable to grow in the absence of glutamine and creating a nonantibiotic selection marker. The glnA defect was complemented in trans with a plasmid (pCTB) carrying glnA and ccb, producing a balanced-lethal system for high-level expression of CTB (17).

Peru-15 pCTB organisms were grown in a fermentor using vegetable-based nutrient medium, according to current good manufacturing practices (cGMP), by Avant Immunotherapeutics, Inc. (Fall River, MA). At the end of fermentation, the broth, containing live Peru-15 pCTB, was put into 2-ml polypropylene cryovials containing 1 ml of ≥5 × 10^8 CFU/ml of vaccine organisms in a 23% glycerol solution. The vaccine vials were frozen and stored at below -65°C until use. The vaccine was prepared by thawing the vial and diluting to the desired concentration with sterile phosphate-buffered saline (PBS). The vaccine was then administered within 60 min in a final volume of 100 ml. The bicarbonate buffer solution was prepared by reconstituting the contents of a buffer sachet containing 2.5 g of NaHCO3, 1.65 g of ascorbic acid, and 25 mg of aspartame into 100 ml of water. The placebo consisted of 100 ml of the bicarbonate buffer solution. The vaccine and placebo were prepared and administered by unblinded research personnel who were otherwise not involved in the study.

Study design. Healthy adults 18 to 45 years of age were recruited at a single center (Cincinnati, OH) to participate in this first-in-human phase I trial. Four sequential dose cohorts of 15 to 16 eligible subjects were randomly assigned (3:1) to receive a single oral dose of vaccine or placebo. The first dose cohort (1 × 10^7 CFU) was divided into two groups; 4 initial subjects were randomized, dosed, and followed through day 28. In the absence of meeting a halting rule, the remaining 12 subjects were randomized and dosed. Thereafter, the 3 following dose-escalation cohorts proceeded in stepwise fashion, evaluating dosages of 1 × 10^6, 1 × 10^7, and 1 × 10^8 CFU of the vaccine.

Eligible subjects were nonpregnant healthy adults who provided informed consent and were screened for the absence of chronic medical conditions, immunodeficiencies, a history of recent foreign travel to a region endemic for cholera or ETEC, history of a prior cholera or ETEC infection (natural infection or experimental challenge), or prior receipt of a cholera or ETEC vaccine; the complete inclusion and exclusion criteria are published in http://clinicaltrials.gov/show/NCT00654108. The subjects were admitted to the research isolation ward 1 day prior to vaccination for acclimatization purposes. The next day, the subjects fasted for 90 min before and after the ingestion of the vaccine or placebo.

Following ingestion of the blinded product, the subjects remained inpatient for the following 9 days to be closely monitored for illness. The consistency of stool was graded as normal, loose (readily taking the shape of the container), or watery. Diarrhea was defined as the passage of two or more loose or watery stools or <400 g of loose stools in a 24-h period. Every stool was graded, and if loose or watery, it was weighed. Moderate diarrhea was defined as 4 to 5 diarrheal stools or 400 to 800 g of loose stools in a 24-h period, while severe diarrhea was defined as ≥6 diarrheal stools or >800 g of loose stools in a 24-h period. Any subject who developed diarrhea or vomiting was offered oral rehydration salt (ORS) solution or intravenous fluids for rehydration. Blood cultures were performed on any subject experiencing fever of ≥38°C. Starting on day 7, ciprofloxacin was administered at 500 mg twice daily for 5 days to eradicate shedding of vaccine organisms. The subjects were discharged on day 9 only upon confirmation of two consecutive sequential negative stool cultures for vaccine organisms, separated by 12 h.

Following discharge, the subjects recorded daily oral temperatures and the occurrence of solicited adverse events (reactogenicity) over the subsequent 6 days, including diarrhea, nausea, vomiting, abdominal pain, malaise, myalgia, anorexia, and headache. A stool specimen or rectal swab was collected during the inpatient stay and as an outpatient on days 10, 14, 21, and 28. Clinical safety laboratories evaluated the samples on days 3, 7, 14, and 28 and included tests for hematology (white blood cells with differential, hemoglobin, hematocrit, and platelet count) and chemistry parameters (sodium, potassium, creatinine, and alanine transaminase). Telephone interviews for longer-term safety follow-up were conducted at 2, 4, and 6 months. The study was reviewed and approved by the Cincinnati Children’s Hospital Medical Center institutional review board.

Vaccine shedding. Stool specimens were plated directly onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar plates (Difco Laboratories, Detroit, MI) and/or inoculated into alkaline peptone water (APW) enrichment broth (Fisher Scientific, Pittsburgh, PA) for overnight incubation before plating on TCBS agar. Up to two stools each day were quantitatively cultured to determine the number of vaccine organisms per g of stool. A rectal swab was obtained if no stool was passed. Suspicious colonies were agglutinated with specific Inaba anti-O1 antisemum (Difco, Sparks, MD).

Immunology. The serum specimens for antibody assessments were obtained before vaccination and at days 7, 10, 14, and 28 after vaccination. Serum Inaba vibriocidal antibody was measured by performing a vibriocidal antibody assay to measure complement-mediated antibody inhibition of V. cholerae growth.

This assay employs a wild-type strain of V. cholerae (N16961) to calculate the titer of functional anti-V. cholerae antibodies in the serum samples of immunized or placebo human clinical trial subjects. The vibriocidal antibody assay compares the amount of V. cholerae growth achieved in a 96-well plate when mixed with guinea pig complement (catalog no. C300-0050, lot no. 19365, 50-ml frozen bottle, protein concentration, 83.0 mg/ml; Rockland, Limerick, PA) of a standard activity and serial dilutions of the heat-inactivated human serum samples, all assayed in duplicate. A well-characterized negative control, reference standard, and positive control were also included with each plate to ensure the validity of the assay. A working cell suspension (WCS) was prepared by diluting the target cell suspension with saline. A positive cell suspension (PCS) was prepared by adding 4.3 ml/plate of WCS to 1.2 ml/plate of guinea pig complement. This was swirled or inverted but not vortexed. A negative cell suspension (NCS) was similarly prepared. Serial dilutions were performed in saline. The plates were statically incubated and covered for 1 h ≥ 10 min at 37°C ± 2°C. Following a 1-h incubation, 150 μl of brain heart infusion (BHI) broth was added to all wells, and the plates were covered and incubated statically for 2 h ≥ 10 min at 37°C ± 2°C. Absorbance was then read at 600 nm. The dilution just prior to the point at which 50% of the largest amount of growth on the plate was inversely inhibited became the titer of the serum sample. The reagents used included thiosulfate-citrate-bile salts-sucrose (TCBS) agar, 0.85% saline, Luria-Bertani (LB) medium, and brain heart infusion (BHI) broth (NorthEast Laboratories, Winslow, ME), which were used as previously described (15, 17). Spectrophotometric readings at 600 nm, with a 1-cm path length, were made on a Molecular Devices precision microplate reader model EMax spectrophotometer (serial no. E10868; Sunnyvale, CA), with SoftMax PRO 4.8 for Windows software. All assays were repeated, and the samples for all subjects in a cohort were run at the same time.

Anti-CT and -LT IgG and IgA antibodies were measured by enzyme-linked immunosorbent assay (ELISA) (18). The critical reagents used in these assays were as follows: cholera toxin B subunit (catalog no. 103B, lot no. 10327A, 1.0 mg; List Biological Laboratories, Inc., Campbell, CA), which was resuspended in 0.2 ml deionized (DI) water for a final concentration of 5.0 mg/ml, and stored at 2 to 8°C; goat anti-human IgA (catalog no. 109-035-011, lot no. 78949, peroxidase-conjugated AffiniPure goat anti-human IgA, α-chain specific; Jackson Immunoresearch Laboratories, Inc., West Grove, PA); goat anti-human IgG (catalog no. 109-035-011, lot no. 78949, peroxidase-conjugated AffiniPure goat anti-human IgG, whole molecule; Jackson Immunoresearch Laboratories, Inc.); and 0.5 mg heat-labile toxin from E. coli (catalog no. EB8656, lot no. 028K4046; Sigma-Aldrich, Inc.), which was resuspended in 5 ml of DI water for a final concentration of 0.1 mg/ml and stored at 2 to 8°C. For these assays, CTB or LTB was directly coated on the surface of the wells in a 96-well polystyrene-96-well polystyrene-
TABLE 1 Subject demographics by dose

| Characteristic | Placebo (n = 15) | 10^7 CFU (n = 12) | 10^8 CFU (n = 11) | 10^9 CFU (n = 12) | 10^10 CFU (n = 62) | All |
|----------------|-----------------|-----------------|-------------------|------------------|-------------------|-----|
| Gender (no. [%]) |                 |                 |                   |                  |                   |     |
| Male           | 9 (60)          | 7 (58.3)        | 5 (41.7)          | 6 (54.5)         | 9 (75)            | 36 (58.1) |
| Female         | 6 (40)          | 5 (41.7)        | 7 (58.3)          | 5 (45.5)         | 3 (25)            | 26 (41.9) |
| Ethnicity (no. [%]) |         |                 |                   |                  |                   |     |
| Non-Hispanic/Latino | 15 (100) | 12 (100)        | 12 (100)          | 11 (100)         | 12 (100)          | 62 (100) |
| Hispanic or Latino | 0             | 0               | 0                 | 0                | 0                 | 0     |
| Race (no. [%]) |                 |                 |                   |                  |                   |     |
| Black/African-American | 13 (86.7) | 11 (91.7)       | 11 (91.7)         | 9 (81.8)         | 9 (75)            | 53 (85.5) |
| White          | 2 (13.3)        | 1 (8.3)         | 0                 | 2 (18.2)         | 3 (25)            | 8 (12.9) |
| Asian          | 0               | 0               | 0                 | 0                | 0                 | 0     |
| Multiracial    | 0               | 0               | 1 (8.3)           | 0                | 0                 | 1 (1.6) |
| Age (yr)       |                 |                 |                   |                  |                   |     |
| Mean (SD)      | 27.7 (9.5)      | 32.2 (7.4)      | 25.8 (7.3)        | 26.5 (8.3)       | 29.8 (8.0)        | 28.4 (8.3) |
| Median         | 27.0            | 33.5            | 23.0              | 25.0             | 29.0              | 28.0 |
| Min, max*     | 18, 45          | 19, 43          | 18, 40            | 18, 42           | 18, 45            | 18, 45 |

* Min, minimum; max, maximum.

RESULTS

Participants. A total of 62 subjects were enrolled and vaccinated between June 2008 and December 2009, of which 61 subjects completed the day-28 study visit and 57 subjects completed the 6-month phone call. The mean age of the subjects was 28 years (range, 18 to 45 years), and the majority (58%) were male and black/African-American (86%) (Table 1). One subject in cohort 3 (10^9-CFU dosage) withdrew from the study within 2 days of ingesting a blinded product and was excluded from the immunogenicity analysis; this individual completed ciprofloxacin therapy and had two documented negative stool samples prior to discharge.

Vaccine safety. The occurrence of reactogenicity symptoms during the 14 days after vaccination is summarized in Table 2. During the 9 inpatient days, diarrhea was experienced by 4 placebo (27%) recipients and 5 (42%), 2 (17%), 2 (18%), and 6 (50%) subjects receiving the 10^7-, 10^8-, 10^9-, and 10^10-CFU dosages, respectively; however, due to the small sample sizes, there was no statistically significant difference. Although there was no severe-grade diarrhea, there were 3 moderate-grade diarrheal symptoms among the participants receiving 10^7 CFU and 2 moderate-grade diarrhea symptoms among the participants receiving 10^10 CFU. Among all the other reactogenicity symptoms experienced, there were no severe-grade symptoms.

There were a total of 308 nonserious AEs reported during the 6 months of follow-up for each subject, of which 265 were mild, 38 were moderate, and 5 were graded as severe; only 25 were considered to be possibly associated with the vaccine. The 5 severe AEs were categorized as elevated systolic blood pressure or bradycardia that resolved spontaneously and were not considered vaccine related. There was a single serious adverse event for decreased neutrophils, which was deemed to be possibly vaccine related, and that resolved spontaneously.

Vaccine immunogenicity. The serum Inaba vibriocidal antibody GMT and seroconversion rates following vaccination are summarized in Table 3 and Fig. 1. Whereas the mean of the indi-
TABLE 2 Any reactogenicity within 14 days of receiving vaccine or placebo

| Symptom            | Placebo | 10^7 CFU | 10^8 CFU | 10^9 CFU | 10^10 CFU | Placebo | 10^7 CFU | 10^8 CFU | 10^9 CFU | 10^10 CFU |
|--------------------|---------|----------|----------|----------|-----------|---------|----------|----------|----------|-----------|
| Fever              | 0/15    | 1/12     | 0/12     | 0/11     | 0/12      | 0/15    | 0/12     | 0/12     | 0/12     | 0/10      |
| Abdominal pain     | 1/15    | 6/12     | 3/12     | 5/11     | 2/12      | 2/15    | 1/12     | 2/12     | 0/12     | 0/10      |
| Nausea             | 2/15    | 5/12     | 1/12     | 1/11     | 2/12      | 1/15    | 1/12     | 0/12     | 0/10     | 0/12      |
| Vomiting           | 0/15    | 0/12     | 0/11     | 0/12     | 0/15      | 0/15    | 0/12     | 0/12     | 0/10     | 0/12      |
| Anorexia           | 2/15    | 2/12     | 2/12     | 1/11     | 3/12      | 0/15    | 1/12     | 0/12     | 0/10     | 0/12      |
| Malaise            | 0/15    | 2/12     | 1/12     | 0/11     | 0/12      | 1/15    | 1/12     | 0/12     | 0/10     | 0/12      |
| Headache           | 5/15    | 5/12     | 3/12     | 5/11     | 4/12      | 2/15    | 0/12     | 2/12     | 3/10     | 2/12      |
| Myalgia            | 1/15    | 4/12     | 0/12     | 3/11     | 0/12      | 1/15    | 0/12     | 0/12     | 0/10     | 1/12      |

*Diarrhea was defined as ≥2 loose stools within a 24-h period. Moderate grade was defined as 4 to 6 diarrheal stools of 400 to 800 g in a 24-h period. Severe diarrhea was defined as ≥6 diarrheal stools of >800 g in a 24-h period.

Vaccine shedding. The vaccine was excreted in the feces of 67%, 67%, 82%, and 83% of vaccinees, corresponding to the 10^7-, 10^8-, 10^9-, and 10^10-CFU dosages of vaccine, respectively. The peak of the vaccine response generally occurred on day 10 postvaccination.

The serum IgG cholera antitoxin antibody GMT and rates of seroconversion are summarized in Table 4. Overall, 67%, 50%, 80%, and 83% of vaccinees, corresponding to the 10^7-, 10^8-, 10^9-, and 10^10-CFU dosages of vaccine, demonstrated a ≥4-fold increase (seroconversion) in anti-CT IgG responses (comparing baseline to the individual peak response). There was essentially no response among the placebo recipients. Although the single oral dose of vaccine elicited an anti-CT IgG response as early as 7 days after vaccination, the anti-CT response continued to peak through day 28 after vaccination. There was a similar trend for a dose-response relationship, as measured by serum anti-CT IgA and anti-LT IgG and IgA ELISA. There was a good correlation between the serum anti-CT and anti-LT IgG titers at day 28 postvaccination (R^2 = 0.9091).

**DISCUSSION**

This is the first study of Peru-15 pCTB, a candidate live, attenuated vaccine for cholera and ETEC. The parent strain Peru-15 has been found to be safe and immunogenic in >400 subjects, including North American adults (11, 14–16) and Bangladeshi adults (12) and children (13). We demonstrated that a single oral dose of Peru-15 pCTB was without significant reactogenicity or toxicity at dosages up to 10^10 CFU.

The basis for this strain as a potential vaccine against ETEC is that LT and CT share highly conserved sequence homologies and superimposable X-ray crystal structures (19, 20). CT is both functionally and antigenically similar to LT. The parent strain Peru-15 secretes some CTB, but the magnitude of the serum antitoxin antibodies elicited is low; a seroconversion (4-fold increase over baseline) was observed in only 18 to 28% of North American vaccinees (11, 16). Meanwhile, Peru-15 pCTB was engineered to stably overexpress CTB and elicited an ~30-fold higher serum antitoxin IgG than that with Peru-15 in both mice and rabbits (17). Our study demonstrated 80% and 83% anti-CT and 80% and 67% anti-LT seroconversions among individuals receiving the two highest dosages of vaccine (10^9 CFU and 10^10 CFU), respectively. Therefore, the engineering of Peru-15 pCTB appears to have successfully resulted in greater antitoxin responses. However, we did not formally evaluate Peru-15 against Peru-15 pCTB and cannot quantitate the extent of the increased antitoxin response.

TABLE 3 Serum Inaba vibriocidal antibody responses

| Dose group (CFU) | n  | Baseline | 7       | 10      | 14      | 28      | Mean peak titer | Mean peak titer increase |
|------------------|----|----------|---------|---------|---------|---------|-----------------|--------------------------|
| Placebo          | 15 | 27.6 (16.4–46.5) | 74.0 (33.0–166) | 373.1 (150–928) | 52.8 (23.6–118) | 33.3 (18.8–58.8) | 1,080 | 47 |
| 10^7             | 12 | 28.3 (18.2–43.9) | 508 (205–1,258) | 3,417 (794–14,717) | 2,712 (509–4,465) | 1,437 (271–7,612) | 17,760 | 482 |
| 10^8             | 12 | 22.5 (17.4–29.0) | 547 (93.9–1,87) | 11,238 (3,699–34,140) | 3,972 (656–24,057) | 1,162 (218–6,180) | 34,560 | 1,408 |
| 10^9             | 10 | 23.0 (16.8–31.4) | 3,137 (809–12,160) | 32,305 (10,410–100,250) | 19,886 (5,925–66,748) | 9,724 (2,776–34,064) | 67,200 | 3,355 |
| 10^10            | 12 | 31.8 (21.8–46.9) | 2,314 (509–10,531) | 15,252 (4,629–50,251) | 7,065 (1,571–31,772) | 2,922 (699–12,220) | 35,440 | 999 |

*GMt, geometric mean titer; CI, confidence interval.

*Each individual’s peak response was used to calculate these values.*
There were significant vibriocidal antibody responses observed even with the lowest dosage of vaccine, and there appeared to be a dose-response relationship that plateaued with the two highest dosage levels. We cannot explain why there also seemed to be some low-level vibriocidal antibody responses among the placebo recipients. We do not believe there were errors in the blinded study product administration. Since the vibriocidal antibody assay results can be affected by incubation time, assay diluent, or bacterial or complement concentration (21), interassay variability might account for some of the perceived responses of the placebo recipients. Nonetheless, the vibriocidal antibody responses of the vaccinees were orders of magnitude higher than those of the placebo controls. The sample size of this study was limited, and a better approximation of the vibriocidal antibody responses will need to be verified with subsequent studies. We also cannot comment on whether Peru-15 pCTB demonstrates different vibriocidal antibody responses than those of the parent strain Peru-15, since this comparison was not formally tested.

In theory, the presence of CT or LT can act as a mucosal adjuvant to an oral vaccine (22). Our immunogenicity results show that the highest responses occurred with the $10^9$-CFU dosage. Meanwhile, the $10^{10}$-CFU dosage appeared to demonstrate a trend for lower responses. Although this conclusion is limited by the small sample sizes, we have observed trends for lower immune responses with a high dosage ($100^{9}/g$) of orally administered non-reactogenic mutant LT (23). Similar trends for lower immune responses with high dosages of orally administered LT-based adjuvant have also been reported (24,25). Therefore, it is possible that high dosages of CTB, as expressed with the $10^{10}$-CFU vaccine, impeded the vibriocidal immune response. This is consistent with other paradoxical observations regarding CTB. Whereas the whole toxins have adjuvant properties, the B subunits of CT (CTB) and of LT (LTB) have been shown to induce antigen-specific tolerance when administered mucosally with antigens in experimental models, as well as in humans (26).

### TABLE 4 Serum IgG and IgA ELISA anti-CT and anti-LT antibody responses

| Dose group (CFU) by antibody response type | n | Antibody GMT (95% CI) on day of vaccination | No. (%) seroconverted<sup>a</sup> |
|------------------------------------------|---|--------------------------------------------|---------------------------------|
| **Antioxid-CT IgG**                      |   |                                            |                                 |
| Placebo                                  | 15| 159 (61.9–414) 174 (67.1–452) 241 (99.0–585) 191 (75.0–486) 191 (76.8–475) 0 |
| $10^7$                                   | 12| 238 (144–393) 283 (154–520) 400 (151–1,061) 635 (196–2,062) 1,068 (349–3,267) 8 (67) |
| $10^8$                                   | 12| 112 (68.7–183) 141 (70.9–282) 189 (73.2–487) 318 (88.4–1,440) 449 (148–1,360) 6 (50) |
| $10^9$                                   | 10| 214 (109–423) 566 (179–1,788) 746 (219–2,540) 857 (247–2,983) 1,493 (547–4,074) 8 (80) |
| $10^{10}$                                | 12| 178 (80.6–394) 378 (158–901) 673 (296–1,529) 800 (281–2,276) 1,796 (692–4,665) 10 (83) |
| **Antioxid-CT IgA**                      |   |                                            |                                 |
| Placebo                                  | 15| 19.0 (13.0–27.7) 19.0 (13.0–27.7) 21.8 (14.4–33.0) 22.8 (15.6–33.3) 21.8 (15.2–31.2) 0 |
| $10^7$                                   | 12| 21.0 (14.4–30.8) 19.8 (13.4–29.3) 23.6 (14.6–38.0) 29.7 (15.1–58.7) 33.4 (18.2–61.3) 2 (17) |
| $10^8$                                   | 12| 21.0 (11.2–39.3) 23.6 (14.1–39.4) 37.5 (16.0–87.6) 44.5 (18.2–109) 33.4 (15.6–71.5) 4 (33) |
| $10^9$                                   | 10| 26.8 (16.4–43.9) 37.9 (14.8–97.1) 50.0 (16.3–153) 75.8 (23.4–245) 123 (38.2–397) 6 (60) |
| $10^{10}$                                | 12| 28.1 (19.4–40.5) 39.7 (20.5–76.7) 84.1 (34.8–203) 119 (43.2–328) 159 (71.0–355) 8 (67) |
| **Antioxid-LT IgG**                      |   |                                            |                                 |
| Placebo                                  | 15| 696 (252–1,927) 635 (243–1,656) 635 (243–1,656) 665 (258–1,712) 635 (238–1,693) 0 |
| $10^7$                                   | 12| 476 (235–964) 534 (268–1,063) 620 (282–1,363) 898 (296–2,720) 1,199 (418–3,437) 5 (42) |
| $10^8$                                   | 12| 599 (336–1,068) 672 (370–1,223) 713 (364–1,397) 951 (410–2,209) 1,068 (499–2,288) 4 (33) |
| $10^9$                                   | 10| 429 (227–811) 985 (299–3,247) 1,300 (346–4,881) 1,493 (429–5,194) 2,599 (937–7,210) 8 (80) |
| $10^{10}$                                | 12| 336 (175–647) 449 (265–759) 755 (389–1,465) 951 (509–1,780) 1,796 (968–3,332) 8 (67) |
| **Antioxid-LT IgA**                      |   |                                            |                                 |
| Placebo                                  | 15| 72.4 (36.2–145) 66.0 (34.0–128) 75.8 (37.9–152) 69.1 (35.6–134) 72.4 (39.2–134) 1 (7) |
| $10^7$                                   | 12| 39.7 (26.8–58.7) 44.5 (28.3–70.1) 47.2 (26.5–84.1) 66.7 (29.8–150) 70.7 (34.6–145) 3 (25) |
| $10^8$                                   | 12| 66.7 (35.4–126) 70.7 (42.3–118) 89.1 (52.7–151) 84.1 (41.5–170) 74.9 (39.2–142) 3 (25) |
| $10^9$                                   | 10| 53.6 (34.7–82.7) 70.7 (33.5–149) 100 (51.6–194) 115 (55.3–239) 107 (47.0–245) 5 (50) |
| $10^{10}$                                | 12| 66.7 (38.7–115) 112 (64.2–196) 134 (77.3–231) 212 (115–389) 225 (118–428) 7 (58) |

<sup>a</sup> Seroconversion is defined as a 4-fold increase in antibody response compared to baseline.
of up to 10^{10} CFU of Peru-15 pCTB is safe, well tolerated, and immunogenic. The incidence of moderate diarrhea in some subjects suggests to us that this should be further evaluated. Since the completion of this study, Avant Immunotherapeutics, Inc. merged with Cellnex Therapeutics, Inc. (in 2008), and the licensing of the cholera and ETEC vaccine technology was acquired by Vaccine Technologies, Inc. (Hainan, China). The status of the clinical development program for this vaccine is unknown, but the present results indicate that either alone or as part of a vaccine cocktail, the use of this approach should continue to be pursued. The protection afforded against ETEC and/or V. cholerae infection will need to be evaluated in future studies.

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