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# Randomized, Controlled Human Challenge Study of the Safety, Immunogenicity, and Protective Efficacy of a Single Dose of Peru-15, a Live Attenuated Oral Cholera Vaccine

Mitchell B. Cohen,[1]* Ralph A. Giannella,[2] Judy Bean,[3] David N. Taylor,[4] Susan Parker,[5] Amy Hoeper,[5] Stephen Wowk,[1] Jennifer Hawkins,[1] Sims K. Kochi,[6] Gilbert Schiff,[5] and Kevin P. Killeen[6]

*Division of Pediatric Gastroenterology, Hepatology and Nutrition,[1] Center for Epidemiology and Biostatistics,[3] and Gamble Program for Clinical Studies,[5] Children's Hospital Medical Center, and Digestive Diseases, University of Cincinnati and VA Medical Center,[2] Cincinnati, Ohio; Walter Reed Army Institute of Research, Washington, D.C.[4]; and AVANT Immunotherapeutics, Inc., Needham, Massachusetts[6]*

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**Peru-15 is a live attenuated oral vaccine derived from a *Vibrio cholerae* O1 El Tor Inaba strain by a series of deletions and modifications, including deletion of the entire CT genetic element. Peru-15 is also a stable, motility-defective strain and is unable to recombine with homologous DNA. We wished to determine whether a single oral dose of Peru-15 was safe and immunogenic and whether it would provide significant protection against moderate and severe diarrhea in a randomized, double-blind, placebo-controlled human volunteer cholera challenge model. A total of 59 volunteers were randomly allocated to groups to receive either $2 \times 10^8$ CFU of reconstituted, lyophilized Peru-15 vaccine diluted in CeraVacx buffer or placebo (CeraVacx buffer alone). Approximately 3 months after vaccination, 36 of these volunteers were challenged with approximately $10^5$ CFU of virulent *V. cholerae* O1 El Tor Inaba strain N16961, prepared from a standardized frozen inoculum. Among vaccinees, 98% showed at least a fourfold increase in vibriocidal antibody titers. After challenge, 5 (42%) of the 12 placebo recipients and none (0%) of the 24 vaccinees had moderate or severe diarrhea (≥3,000 g of diarrheal stool) ($P = 0.002$; protective efficacy, 100%; lower one-sided 95% confidence limit, 75%). A total of 7 (58%) of the 12 placebo recipients and 1 (4%) of the 24 vaccinees had any diarrhea ($P < 0.001$; protective efficacy, 93%; lower one-sided 95% confidence limit, 62%). The total number of diarrheal stools, weight of diarrheal stools, incidence of fever, and peak stool *V. cholerae* excretion among vaccinees were all significantly lower than in placebo recipients. Peru-15 is a well-tolerated and immunogenic oral cholera vaccine that affords protective efficacy against life-threatening cholera diarrhea in a human volunteer challenge model. This vaccine may therefore be a safe and effective tool to prevent cholera in travelers and is a strong candidate for further evaluation to prevent cholera in an area where cholera is endemic.**

Cholera continues to be a major public health problem in nearly all developing countries (15). The disease is endemic in some areas, while in other areas, epidemics can result from social strife and crowding conditions, such as in refugee camps (2). The death rate is highly dependent on the availability of treatment facilities. Like other enteric bacterial infections, cholera exhibits a spectrum of clinical illness. Mild cholera is indistinguishable from other etiologies of diarrheal disease. What makes cholera a significant public health problem is its ability in some patients to cause severe, potentially fatal dehydration; this condition is known as cholera gravis. Cholera gravis is characterized by the copious purging of electrolyte-rich rice water stools, which can result in fluid loss equal to or greater than the patient's blood volume. If appropriate rehydration therapy is administered, cholera gravis is a readily treatable disease, and even under field conditions, fatality rates can be kept below 1%. In developing countries, cholera fatalities occur because patients with cholera gravis do not have timely access to treatment centers or because explosive epidemics of cholera overwhelm the ability of the treatment centers to cope with the number of severely dehydrated patients that present within a short period of time.

The currently licensed parenteral vaccine provides approximately 50% protection for a short duration and commonly elicits systemic and local adverse reactions. A more-effective, better-tolerated vaccine that could be administered orally is therefore desirable. A number of oral, killed whole-cell vaccines, with or without the B subunit of cholera toxin (CT) have been evaluated and have a combined efficacy of 51% at 1 year (6). Natural infection with *Vibrio cholerae* confers long-standing immunity and both antitoxin and antivibriocidal responses. Nonetheless, the precise mechanism by which protection against cholera is achieved is not completely understood. Possibly because it closely resembles natural infection, a single-dose, live oral recombinant vaccine has the potential for generating both rapid onset of immunity and durable protection without subsequent booster doses. Therefore, two live recombinant oral vaccines (7, 11–13, 16) have been developed. The availability of a safe, immunogenic vaccine that would provide a high level of long-term protection to those at high risk for illness would be highly desirable.

* Corresponding author. Mailing address: Division of Pediatric Gastroenterology, Hepatology and Nutrition (MLC 2010), Children's Hospital Medical Center, 3333 Burnet Ave., Cincinnati, OH 45229. Phone: (513) 636-4953. Fax: (513) 636-5581. E-mail: mitchell.cohen@chmcc.org.

The candidate cholera vaccine, Peru-15, was created from a *V. cholerae* O1 El Tor Inaba strain isolated in Peru in 1991 (7, 17). Peru-15 has been attenuated by a series of genetic deletions and modifications, including deletion of the entire CT core genetic element (which contains the genes for CT and other virulence determinants) along with the *attRS1* insertion-like sequences. This latter deletion renders the organism unable to reacquire the toxin core element by site-specific recombination. As an additional precaution, the gene for the nontoxic B subunit of CT has been fused with a heat shock promoter and inserted into the *recA* gene. The resulting strain is a *recA* mutant and therefore cannot integrate exogenous DNA. Finally, Peru-15 is a stable, motility-defective strain.

To predict the usefulness of Peru-15 as a public health tool in the control of cholera, we first wished to determine the degree of protection provided against moderate and severe infection with El Tor *V. cholerae*. Because the basis for immunity to cholera is not completely understood, vaccines must be tested in efficacy studies using either a challenge model or a field trial. The present study design permits us to estimate the protective efficacy of Peru-15 in a controlled environment, i.e., a human challenge study, before proceeding to field trials. The challenge model has also been accepted for evaluating efficacy of a cholera vaccine in travelers (16). In the challenge model, volunteers who had total diarrheal stool outputs of 3,000 g or more are defined as having moderate cholera and those who had total diarrheal stool outputs of 5,000 g or more are defined as having severe cholera (14, 16). Volunteers with moderate or severe cholera in the experimental challenge model have lost prodigious, clinically important, volumes of body water and electrolytes, which is analogous to cases of cholera gravis in the field. The important difference between the volunteer model and natural cholera in the field is the prompt and continuing fluid and electrolyte replacement given to the volunteers which precludes the development of dehydration. Thus, we undertook to test Peru-15 in a human cholera challenge model to obtain an estimate of the safety, immunogenicity, and protective efficacy of this vaccine.

## MATERIALS AND METHODS

**Study design, inclusion criteria, and informed consent.** The study and consent forms were approved by the Institutional Review Board of the Children's Hospital Medical Center (CHMC). Fifty-nine volunteers were recruited and screened with the understanding that they would be asked to return for challenge with wild-type *V. cholerae* O1 El Tor Inaba approximately 3 months later. All volunteers received prestudy counseling and gave informed, written consent. Separate consent was obtained prior to vaccination and prior to challenge. To ensure comprehension of the study and to document that informed consent had been elicited, the volunteers had to pass a written examination before inoculation with the challenge strain. Volunteers were thoroughly screened to document their mental and physical health before challenge. Although the human challenge model has been used now a number of times, it is a complex study that requires a number of safeguards to ensure the protection of participants.

To be included in the study, volunteers were required to be healthy, between age 18 to 40 years, and have a normal medical history and normal physical examination. Volunteers were excluded if they had clinically significant abnormalities on urinalysis, complete blood count, serum hepatic transaminases, glucose, creatinine, blood urea nitrogen, electrolytes, or electrocardiogram. Additional exclusion criteria included travel to an area where cholera was endemic in the previous 5 years, history of cholera or enterotoxigenic *Escherichia coli* challenge, history of recent antibiotic use, an abnormal stool pattern, or regular use of laxatives. Further exclusion criteria included the failure to pass a psychological screening; allergy to tetracycline or ciprofloxacin; pregnancy or breast feeding; positive serology for human immunodeficiency virus, hepatitis $B_s$ antigen, or hepatitis C antibody; stool culture positive for an enteric pathogen; or failure to pass the written examination. In addition, since this was a study requiring participants to remain in the hospital for the duration of the study, individuals unlikely to be able to comply were excluded.

**Method of randomization.** Subjects with blood group O and non-O blood group were randomly assigned to two cohorts using SAS PROC PLAN. Volunteers were stratified by blood group (O versus non-O), because persons of blood group O are predisposed to develop more-severe forms of cholera diarrhea (3, 5), and we wished to preferentially include them in the challenge group. The sponsor, National Institute of Allergy and Infectious Diseases (NIAID), generated the randomization code. This study was a blind one. Investigators did not know of the vaccine status of all volunteers until the data were locked, and the code was broken after the challenge had been completed.

**Vaccination.** Volunteers were randomly assigned to groups in a double-blind manner to receive, with buffer, a single oral dose of either Peru-15 or placebo (CeraVacx buffer alone) in a 2:1 ratio. For the day of vaccination and the following 3 days as outpatients, the volunteers kept a symptom diary to record all stools and to determine the occurrence of adverse reactions, such as diarrhea, nausea, vomiting, abdominal cramps, malaise, anorexia, headache, and fever. A study nurse who was unaware of the group assignment reviewed the diary.

**Vaccine and placebo formulations.** The vaccine strain was manufactured and packaged by the Walter Reed Army Institute for Research, Forest Glen Facility (Building 501), under current good manufacturing practices. The product consisted of lyophilized ampules of Peru-15 containing $5 \times 10^8$ CFU. Prior to vaccination, these ampules were stored at an NIAID-approved repository and shipped on dry ice to CHMC where they were maintained at $-20C^\circ$ until use in the trial. On the day of vaccination at CHMC, lyophilized Peru-15 vaccine was reconstituted in 5 ml of sterile distilled water. After reconstitution, the vaccine was stored for no more than 60 min at room temperature. Immediately prior to administration, an aliquot of 2 ml of reconstituted vaccine, containing $\approx 2 \times 10^8$ CFU, was diluted in 200 ml of CeraVacx buffer (Cera Products, Columbia, Md.). This buffer, which contains rice syrup, sodium bicarbonate, and trisodium citrate, was prepared according to the manufacturer's instructions (dilute one packet into 200 ml of drinking water) (13).

The placebo formulation consisted of 200 ml of CeraVacx buffer alone, and its appearance was similar to that the vaccine suspension cocktail. Volunteers were asked to take no food or water for 1 h pre- and postinoculation.

**Challenge.** In January 2001, approximately 3 months after immunization (mean, 84 days; range, 59 to 99 days), 36 volunteers (24 vaccinees and 12 placebo recipients) were admitted to the General Clinical Research Center at CHMC in two groups of 18. Volunteers were challenged with approximately $10^5$ CFU of virulent *V. cholerae* O1 El Tor Inaba strain N16961, prepared from a standardized frozen inoculum (14). During the day before ingestion of the challenge inoculum, the volunteers were on the ward, acclimating to the ward, while medical screening was completed. Baseline serum samples were collected for the measurement of antibody response.

The El Tor Inaba *V. cholerae* O1 challenge strain N16961 was thawed and diluted to $\approx 10^5$ organisms per ml (14). The inoculum size was quantitated by the replica spread plate technique before and after challenge. Two grams of $NaHCO_3$ was dissolved in 150 ml of distilled water. Volunteers drank 120 ml of the water containing $NaHCO_3$; 1 min later, they ingested $\approx 10^5$ CFU of the challenge strain suspended in the remaining 30 ml of water containing $NaHCO_3$. Volunteers had not ingested any food or drink for 90 min before and after challenge.

Following the ingestion of vibrios, the volunteers were closely monitored to detect any signs or symptoms of illness. An investigator interviewed the volunteers at least twice daily. Every stool was saved, examined, graded, and if loose, weighed. The consistency of the stool was ranked according to five grades: grade 1, firm; grade 2, soft; grade 3, thick liquid; grade 4, opaque watery; and grade 5, rice water. The total diarrheal stool weight (for stool grades 3 to 5) was determined.

Any volunteer who developed diarrhea after challenge received oral glucose-electrolyte solution (ORS) to prevent dehydration. Oral rehydration was offered in a volume 1.5 times the diarrheal stool volume after each loose stool. Intravenous rehydration with a balanced polyelectrolyte solution was administered to volunteers who could not stay hydrated using ORS. Tetracycline, 500 mg four times a day for 5 days, was given when the volunteers exceeded 5,000 g of total diarrheal stool output or on day 4, whichever occurred first. Volunteers were discharged when they were asymptomatic and had received a course of tetracycline and when their stool cultures were negative for *V. cholerae* for 3 consecutive days.

**Definitions of illness.** For outpatient volunteers, after administration of vaccine or placebo, diarrhea was defined as four or more loose stools in a 24-h

TABLE 1. Severity of symptoms for subjects receiving Peru-15 vaccine or placebo on the day of vaccination (day 0)

| Sign or symptom | No. (%) of subjects receiving vaccine or placebo with symptom severity[a] of: | | | | | | | | *P* value (vaccine vs placebo) |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 1 | | 2 | | 3 | | |
| | Vaccine (*n* = 40) | Placebo (*n* = 19) | Vaccine (*n* = 40) | Placebo (*n* = 19) | Vaccine (*n* = 40) | Placebo (*n* = 19) | Vaccine (*n* = 40) | Placebo (*n* = 19) | |
| Loss of appetite | 36 (90) | 16 (84) | 4 (10) | 2 (11) | 0 (0) | 1 (5) | 0 (0) | 0 (0) | 0.43 |
| Loss of energy | 34 (85) | 17 (89) | 5 (13) | 2 (11) | 1 (3) | 0 (0) | 0 (0) | 0 (0) | 0.99 |
| Abdominal cramps | 33 (83) | 19 (100) | 5 (13) | 0 (0) | 2 (5) | 0 (0) | 0 (0) | 0 (0) | 0.19 |
| Headache | 26 (65) | 19 (100) | 12 (30) | 0 (0) | 2 (5) | 0 (0) | 0 (0) | 0 (0) | 0.004 |
| Vomiting | 40 (100) | 19 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1.00 |
| Nausea | 33 (83) | 17 (89) | 5 (13) | 2 (11) | 2 (5) | 0 (0) | 0 (0) | 0 (0) | 0.99 |

[a] Severity of symptoms graded on a scale of 0 to 3 as follows: 0 for absent or none, 1 for mild, 2 for moderate, and 3 for severe.

period. For subjects under surveillance in the hospital, after challenge, diarrhea was defined as the passage of two or more unformed stools (grades 3 to 5) over a 48-h period that equaled or exceeded 200 g or a single stool of 300 g or greater. Cholera was defined as follows: cholera, a positive stool culture for *V. cholerae* O1 plus meeting the definition of diarrhea; moderate case of cholera, passage of at least 3,000 g of diarrheal stool (grades 3 to 5) during the study; and severe case of cholera, passage of least 5,000 g during the study. Fever was defined as an oral temperature of >38°C.

**Serology.** Blood was collected before and 9 or 10 days after vaccination to provide sera for measurement of vibriocidal antibodies and antibodies to CT. Blood was collected before and on days 9 and 14 after challenge for serologic studies. Coded sera from each subject were tested for vibriocidal antibodies by using *V. cholerae* O1 El Tor Inaba strain 89 as a target strain and for immunoglobulin G (IgG) antitoxin by enzyme-linked immunosorbent assay (ELISA) as previously described (1, 4, 8–14). An increase in serum vibriocidal titer of fourfold or greater over that of the baseline specimen was considered significant. For anti-CT antibody, a 0.20 rise in optical density (OD) units between pre- and postvaccination or challenge specimens was considered significant (14, 16).

**Bacteriology.** After challenge, up to two stools per day per subject were plated directly onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Difco Laboratories, Detroit, Mich.), as well as inoculated into alkaline peptone water enrichment broth (Fisher Scientific, Pittsburgh, Pa.), for overnight incubation before plating onto TCBS agar (10, 14, 16). Up to two stools each day were also cultured quantitatively to determine the number of vaccine organisms per gram of stool. A rectal swab was obtained if no stool was passed. Suspicious colonies were agglutinated with specific *V. cholerae* O1 Inaba antiserum (Difco Laboratories).

**Statistical analysis.** The frequency of adverse reactions among vaccinees and placebo recipients was compared by use of the Fisher's exact test (FET). The maximum daily recorded severity for each of these reactions was compared between groups by use of the Wilcoxon test. The total number of days of diarrhea and peak temperatures of vaccinees and placebo recipients were compared by using the Wilcoxon test.

The vibriocidal and anti-CT seroconversion rates were compared between vaccinees and placebo recipients by FET. Pre- and postimmunization vibriocidal antibody titers were compared between groups by using the Wilcoxon test. Vibriocidal antibody titers against the Inaba serotype on days 0 and 9 were performed in Cincinnati at CHMC and verified independently by AVANT Immunotherapeutics. These titers were compared by linear regression analysis on log-transformed data.

The point estimate of protective efficacy was calculated as the difference in attack rates among placebo recipients and vaccinees divided by the attack rate among placebo recipients. To construct the 95% one-sided confidence interval for protective efficacy, the variance formula used was the one for relative risk. The study was designed to have 77% power to detect an 80% protective efficacy against moderate and severe diarrhea by using a one-tailed analysis evaluated at a *P* of 0.05.

## RESULTS

**Demographic characteristics of the study volunteers.** Fifty-nine subjects participated in the vaccine phase. Of these subjects, 37% (22 of 59) were men, 29% (17 of 59) were African American, 69% (41 of 59) were Caucasian, and 1% (1 of 59) was Asian. Forty volunteers received vaccine, and 19 received placebo. Sixty-four percent (38 of 59) of all subjects were of blood group O. The demographic characteristics of the 36 subjects (24 vaccinees and 12 placebo recipients) who participated in the challenge phase were not significantly different from those who participated in the vaccine phase or those who did not participate in the challenge phase. Among the challenged subjects, 12 (50%) vaccinees and 8 (67%) placebo recipients were of blood group O.

**Reactions to vaccine.** Volunteers kept a symptom diary beginning on the day of vaccination (day 0) and for three additional days (days 1 to 3). In general, the vaccine was well tolerated. There were no significant differences in symptoms reported by subjects who had ingested Peru-15 and those who had ingested buffer alone except for headache. This symptom was more frequently reported by vaccinees on days 0 and 3 ($P = 0.002$ and 0.05, respectively) and approached statistical significance on days 1 ($P = 0.08$) and 2 ($P = 0.15$). Another symptom that approached but did not reach statistical significance was abdominal cramps on days 0, 2, and 3.

In addition to the presence of symptoms, volunteers were asked to rate the severity of their symptoms. When analyzed by this method (symptom severity graded as follows: 0 for no symptom, 1 for mild symptom, 2 for moderate symptom, and 3 for severe symptom), headache severity was greater in vaccinees on day 0 ($P = 0.004$) (Table 1) and day 3 ($P = 0.04$ [not shown]). Most (12 of 14 or 86%) of the volunteers who experienced headache on day 0 reported it to be mild; 2 of 14 (14%) reported moderate headache (Table 1). The only other symptom that was significantly different between the two groups by this method of analysis was abdominal cramps on day 3. A total of 31 of 40 (78%) vaccine recipients and 18 of 19 (95%) placebo recipients reported no abdominal cramps, 9 of 40 (23%) vaccinees reported mild cramps, and 1 of 19 (5%) placebo recipients reported moderate cramps ($P = 0.02$) on day 3. No volunteer experienced fever after ingestion of Peru-15 or buffer (0 of 40 in the vaccine group; 0 of 19 in the placebo group).

Table 2 shows the total numbers and percentages of vaccinees and placebo recipients having liquid stools. Two of 40 (5%) vaccinees met the definition of diarrhea on day 3; none had more than five liquid stools, and no one required medical attention because of diarrhea. Although more liquid stools were reported overall by vaccinees, on no day did this reach statistical significance.

TABLE 2. Number of vaccinees and placebo recipients having liquid stools

| Day and group | No. (%) of subjects with the following no. of liquid stools: | | | *P* value (vaccine vs placebo) |
|---|---|---|---|---|
| | 0 | 1–3 | ≥4 | |
| Day 0 | | | | |
| Vaccine ($n$ = 40) | 39 (98) | 1 (3) | 0 (0) | |
| Placebo ($n$ = 19) | 19 (100) | 0 (0) | 0 (0) | 0.68 |
| Day 1 | | | | |
| Vaccine ($n$ = 40) | 39 (98) | 1 (3) | 0 (0) | |
| Placebo ($n$ = 19) | 18 (95) | 1 (5) | 0 (0) | 0.54 |
| Day 2 | | | | |
| Vaccine ($n$ = 40) | 32 (80) | 6 (15) | 2 (5) | |
| Placebo ($n$ = 19) | 18 (95) | 1 (5) | 0 (0) | 0.37 |
| Day 3 | | | | |
| Vaccine ($n$ = 40) | 35 (88) | 5 (13) | 0 (0) | |
| Placebo ($n$ = 19) | 18 (95) | 1 (5) | 0 (0) | 0.65 |

TABLE 3. Seroconversion for vibriocidal assay and CT ELISA[a]

| Group | *n* | No. (%) of subjects who seroconverted | |
|---|---|---|---|
| | | Vibriocidal assay | CT ELISA |
| All vaccinees | 40 | 39 (97) | 11 (28) |
| All placebo recipients | 19 | 0 (0) | 0 (0) |
| Subjects who participated in the challenge study | | | |
| Vaccinees | 24 | 23 (96) | 7 (29) |
| Placebo recipients | 12 | 0 (0) | 0 (0) |
| Subjects who did not participate in the challenge study | | | |
| Vaccinees | 16 | 16 (100) | 4 (25) |
| Placebo recipients | 7 | 0 (0) | 0 (0) |

[a] Seroconversion defined as a fourfold increase in titer pre- versus postimmunization for the vibriocidal assay and as 0.2 OD unit increase for CT ELISA.

**Immune response to vaccine.** Vaccinees showed vigorous Inaba serum vibriocidal responses. Among all vaccinees, 97% (39 of 40) showed at least an increase in vibriocidal antibody titers of at least fourfold. In general, there was a strong correlation between the vibriocidal titers measured in Cincinnati, Ohio, and Needham, Mass. (regression coefficient = 0.95).

A smaller number (11 of 40) or 28% of subjects showed at least a 0.20 rise in OD for anti-CT IgG (Table 3). No placebo recipients seroconverted. As shown in Table 4, the geometric mean inverse antibody titers for vibriocidal antibodies rose in all vaccinees from 30 to 4,159 (a 139-fold increase in antibody titer).

**Volunteers who did not participate in the challenge phase.** Volunteers were selected to participate in the challenge phase without knowledge by the investigators of their immune status. By chance, it was possible that a nonrepresentative group of volunteers could have been selected to participate in the challenge phase. Comparison of immune status and subsequent analysis indicated that the challenged volunteers were representative of the vaccinated group. As shown in Tables 3 and 4, the immunologic profiles of those volunteers who did participate in the challenge and those who did not participate in the challenge were similar. A total of 16 vaccine recipients and 7 placebo recipients did not participate in the challenge phase. Two volunteers did not meet the inclusion criteria for challenge (one had a clinically significant abnormality on hematology screening, and one had a clinically significant abnormality on chemistry screening). Of the remaining volunteers, 11 declined participation in the challenge phase when they were recontacted, 5 developed schedule conflicts preventing them from being confined for 10 days during the challenge phase, and 5 volunteers were lost to follow-up or did not return phone calls.

**Clinical and bacteriologic responses to challenge.** A total of 5 (42%) of the 12 placebo recipients and none of the 24 vaccinees developed moderate or severe cholera (≥3,000 g of total diarrheal stool weight) after challenge ($P$ = 0.003; protective efficacy, 100%; lower 95% confidence limit, 75%) (Table 5). Seven (58%) of 12 placebo recipients and 1 (4%) of the 24 vaccinees met the definition of any diarrhea ($P < 0.001$; protective efficacy, 93%; lower 95% confidence limit, 62%) (Table 5). The single vaccinated volunteer who experienced diarrhea was not of blood group type O. Three volunteers required intravenous hydration.

All secondary criteria of protective efficacy were also significant. Among all subjects, the total weight of diarrheal stools, number of diarrheal stools, incidence of fever, and peak stool *V. cholerae* O1 excretion was significantly less in Peru-15 vaccine recipients than placebo recipients (Table 5).

## DISCUSSION

This study demonstrates that a single dose of Peru-15 is a well-tolerated and immunogenic oral cholera vaccine that affords protective efficacy against life-threatening cholera diarrhea in a North American human volunteer challenge model. The human challenge model with a frozen inoculum has been validated to gauge protection against moderate and severe forms of cholera in an immunologically naive population (14, 16). Peru-15 vaccine provided complete protection against moderate and severe diarrhea (lower confidence limit, 75%) and 93% protection against any diarrhea (lower confidence limit, 62%). Furthermore, the seroconversion rate for vibriocidal antibodies, a marker that provides the best available predictor of protective efficacy, was 97%. The vaccine also reduced peak vibrio shedding by 10,000-fold. On the basis of the primary outcome variable (protective efficacy against moderate and severe diarrhea) and positive criteria on all secondary measures of efficacy, we believe that this vaccine could be a safe and effective tool to prevent cholera in travelers.

There was an increased incidence of headache in the group that received Peru-15 compared to that of the group that received buffer alone. The explanation for this symptom is unclear, but it was not associated with other constitutional symptoms, such as fever or malaise. Mild abdominal cramps were also seen more frequently in the volunteers who received Peru-15. No side effect of the vaccine was rated severe, and none was judged medically significant by the volunteers. Although the safety profile is highly favorable, a larger number of

TABLE 4. Pre- and postimmunization antibody titers for the vibriocidal assay

| Group | *n* | Geometric mean inverse antibody titer (range) | | Fold increase |
|---|---|---|---|---|
| | | Preimmunization | Postimmunization | |
| All vaccinees | 40 | 30 (10–1,280) | 4,159 (160–40,960) | 139 |
| All placebo recipients | 19 | 35 (10–2,560) | 35 (10–2,560) | 0 |
| Subjects who participated in the challenge study | | | | |
| Vaccinees | 24 | 25 (10–640) | 3,417 (160–40,960) | 137 |
| Placebo recipients | 12 | 45 (10–2,560) | 45 (10–2,560) | 0 |
| Subjects who did not participate in the challenge study | | | | |
| Vaccinees | 16 | 40 (10–1,280) | 5,583 (640–20,480) | 140 |
| Placebo recipients | 7 | 22 (10–1,280) | 22 (10–1,280) | 0 |

vaccinees will need to be studied to provide a better estimate of the number and severity of side effects associated with Peru-15.

These observations are consistent with the immunogenicity profiles and the lack of significant side effects in previous studies involving 85 subjects who ingested various doses of Peru-15 (7, 12, 13). In a controlled study (7), freshly harvested Peru-15 ($2 \times 10^8$ CFU) was administered to 11 volunteers. No vaccinee developed diarrhea, and 10 of 11 had increases in serum vibriocidal titers of greater than fourfold. One month later, five vaccinees and five control volunteers were challenged with wild-type *V. cholerae* O1. Four of five controls developed diarrhea (mean, 1.9 liters). Two Peru-15 vaccinees developed diarrhea, 1 with <0.3 liter and 1 with approximately 1.0 liter; this latter volunteer had not developed a significant vibriocidal immune response to vaccination.

In another set of studies (12), two groups of six inpatient volunteers received freshly harvested vaccine in doses of either $10^7$ or $10^9$ CFU. Loose stools were the most common side effect, but only one volunteer met the definition of diarrhea. In the same study, 50 outpatient volunteers received freeze-dried vaccine in doses of $10^8$ or $10^9$ CFU or placebo in a three-cell, double-masked, placebo-controlled trial (12). Side effects included diarrhea in two volunteers. Peru-15 stimulated high levels of vibriocidal antibodies in most inpatient volunteers and in all (32 of 32) outpatient volunteers.

In a third immunogenicity study, Sack and coworkers (13) compared three different buffers for use with Peru-15: (i) a standard bicarbonate-ascorbic acid buffer, (ii) Alka-Seltzer, and (iii) a buffer containing sodium bicarbonate and sodium citrate rice syrup solids, CeraVacx. Saline served as the control. Thirty-nine healthy adult volunteers received Peru-15 at a dose of $10^8$ CFU with one of the three buffers or saline in a double-blind study. Side effects were minimal in all groups. All 30 volunteers who took Peru-15 with a buffer other than saline showed a significant increase in vibriocidal antibody titer. The magnitude of the increase was highest in the CeraVacx group and was the basis for the selection of CeraVacx as the buffer in the current study. Approximately half of the volunteers in the groups receiving CeraVacx buffer, standard buffer, and Alka-Seltzer buffer showed a cholera antitoxin response. In the present study, fewer volunteers demonstrated an antitoxin response, but the difference was not statistically significant.

Experimental cholera challenge is a useful model for estimating immune response and protective efficacy among immunologically naive travelers from a developed country including military personnel rapidly deployed to areas where cholera is endemic. However, an important potential use of this vaccine would be to protect individuals in developing countries where cholera is endemic or epidemic. At present, we do not have data on the immunologic response to Peru-15 in a population in an area where cholera is endemic or on the protective efficacy of Peru-15 in a field trial.

A field trial of another oral, live cholera vaccine, CVD103-

TABLE 5. Clinical and bacteriologic responses to challenge with *V. cholerae* O1 El Tor Inaba strain N16961 after volunteers received Peru-15 vaccine or placebo

| Parameter | Value for subjects given: | | *P* value[a] |
|---|---|---|---|
| | Vaccine | Placebo | |
| Total no. of subjects | 24 | 12 | |
| No. of subjects with moderate or severe diarrhea (≥3,000 g of diarrheal stool) (%) | 0 (0) | 5 (42) | 0.002 |
| No. of subjects with any diarrhea (%) | 1 (4) | 7 (58) | 0.0006 |
| Mean diarrheal stool wt (g) for all volunteers (range) | 0.26 (0–2,197) | 1,022 (0–12,593) | <0.0001 |
| Mean diarrheal stool wt (g) for all volunteers with diarrhea (range) | 2,197 | 4,504 (10–12,593) | 0.88 |
| Mean no. of diarrheal stools for all volunteers (range) | 0.5 (0–13) | 15 (0–43) | <0.0001 |
| Mean no. of diarrheal stools for all volunteers with diarrhea (range) | 13 | 18 (1–43) | 0.88 |
| Incidence of fever (%) | 2 (8) | 6 (50) | 0.009 |
| Stool *V. cholerae* O1 excretion (%) | 13 (54) | 10 (83) | 0.09 |
| Mean peak stool *V. cholerae* O1 excretion for all volunteers (CFU/g) (range) | $2.1 \times 10^2$ (0–$1.2 \times 10^8$) | $3.9 \times 10^6$ (0–$7.8 \times 10^8$) | 0.003 |

[a] *P* values comparing the values for subjects given vaccine to those for subjects given placebo.

HgR (11), demonstrated no protective efficacy despite good protection (80% against all diarrhea and 91% against moderate and severe diarrhea) in a human challenge model (16). Although both are live oral cholera vaccines, there are several differences between CVD103-HgR and Peru-15. Peru-15 is derived from an El Tor strain (the predominant biotype in the current pandemic) and is excreted in the stool, i.e., colonizes the intestine, albeit less well than a wild-type motile strain. In contrast, CVD103-HgR is derived from a classical strain and colonizes poorly. These differences may be important factors that could facilitate the development of Peru-15-mediated protection against cholera in persons living in a developing country. Data from this study suggest to us that Peru-15 is a strong candidate for further evaluation as a tool to prevent cholera in an area where cholera is endemic.

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