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The Oral, Live Attenuated Enterotoxigenic *Escherichia coli* Vaccine ACE527 Reduces the Incidence and Severity of Diarrhea in a Human Challenge Model of Diarrheal Disease

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An oral, live attenuated, three-strain recombinant bacterial vaccine, ACE527, was demonstrated to generate strong immune responses to colonization factor and toxin antigens of enterotoxigenic *Escherichia coli* (ETEC) in human volunteers. The vaccine was safe and well tolerated at doses of up to 10^{11} CFU, administered in each of two doses given 21 days apart. These observations have now been extended in a phase 2b study with a total of 70 subjects. Fifty-six of these subjects were challenged 28 days after the second dose of vaccine with the highly virulent ETEC strain H10407 to obtain preliminary indicators of efficacy against disease and to support further development of the vaccine for both travelers and infants in countries where ETEC is endemic. The vaccine had a significant impact on intestinal colonization by the challenge strain, as measured by quantitative fecal culture 2 days after challenge, demonstrating the induction of a functional immune response to the CFA/I antigen. The incidence and severity of diarrhea were also reduced in vaccinees as measured by a number of secondary and *ad hoc* endpoints, although the 27% reduction seen in the primary endpoint, moderate to severe diarrhea, was not statistically significant. Together, these observations support the hypothesis that the ACE527 vaccine has a dual mode of action, targeting both colonization factors and the heat-labile enterotoxin (LT), and suggest that it should be further developed for more advanced trials to evaluate its impact on the burden of ETEC disease in field settings.

In regions of the world with poor sanitation and limited or no access to clean water, diarrheal diseases are the second-highest cause of mortality in children under the age of 5 years after pneumonia. The number of deaths worldwide in children under 5 years due to diarrhea is estimated to be between 1.3 million (95% confidence interval [CI], 0.8 to 2.0 million) (1) and 1.9 million (95% CI, 1.6 to 2.2 million) (2). Additional health burdens of multiple diarrheal episodes early in life are the impairment of physical, intellectual, and economic development (7, 24, 25). Enterotoxigenic *Escherichia coli* (ETEC) strains are estimated to cause 10% to 20% of cases of diarrheal disease in developing countries, potentially accounting for 280 to 400 million cases of diarrheal disease and 300,000 to 500,000 deaths in children under the age of 5 years every year, with an additional 100 million and 400 million cases in the 5- to 14-year and ≥15-year age groups, respectively (34).

ETEC is also a major cause of diarrhea in travelers to developing countries, including both civilian and military personnel, and is currently estimated to cause 10 million cases annually (28, 32, 33). With the projected number of travelers estimated to increase from more than 30 million in 2013 to up to 55 million by 2023, the number of traveler's diarrhea cases will only increase, representing a significant medical need for preventative measures.

A new, oral vaccine known as ACE527 is being developed to prevent moderate to severe ETEC disease. The vaccine comprises three live attenuated strains of ETEC from which enterotoxin genes and antibiotic resistance determinants have been deleted and in which knockout mutations have been made in the chromosomal genes *aroC*, *ompC*, and *ompF*. ACE527 expresses colonization factor antigen I (CFA/I), CS1, CS2, CS3, CS5, CS6, and heat-labile enterotoxin subunit B (LT-B) and was demonstrated to be well tolerated at doses of up to 10^{11} CFU in a phase 1 trial in healthy adult volunteers (17). The vaccine was also shown to generate immune responses to colonization factors expressed on each of the three strains in the majority of subjects. A positive immune response to the heat-labile enterotoxin (LT) was induced in 100% of vaccinees and was of a magnitude comparable to that induced by exposure to wild-type, virulent ETEC. This paper describes a follow-on phase 2b trial with 70 healthy adult volunteers in which the safety and immunogenicity profiles of ACE527 were expanded, and the efficacy of the vaccine against experimental challenge with the highly virulent H10407 strain of ETEC was investigated in 56 subjects.

MATERIALS AND METHODS

Primary and secondary objectives. The primary objective was to demonstrate that ACE527 vaccination produces a statistically significant reduction in moderate to severe ETEC illness induced by a wild-type, heat-stable enterotoxin (ST)/LT-positive ETEC strain expressing CFA/I antigen. The main secondary objectives were as follows: to further assess the safety, tolerability, and immunogenicity of the vaccine in an expanded number of subjects; to investigate systemic and mucosal immune responses as potential markers for protection and compare them with the corresponding responses induced by wild-type ETEC challenge; and to assess the impact of vaccination on colonization by the challenge strain.

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Production and administration of vaccine. The ACE527 vaccine was prepared by the research pharmacist, Johns Hopkins Bloomberg School of Public Health (JHBSPH), and administered as previously described (17) as a suspension in 200 ml of CeraVacx Buffer (Cera Products, Columbia, MD); the placebo consisted of buffer alone. The dose of vaccine administered was approximately 3×10^{10} CFU of each of the three strains, or approximately 10^{11} CFU total live cells. Subjects were not permitted to ingest solids or liquids for at least 90 min before and after vaccination and were observed closely for 60 min following vaccination to ensure no regurgitation. Both doses were administered on an outpatient basis 21 days apart.

Study design. ACE527 protocol 102 was a randomized, placebo-controlled, double-blind evaluation of the protective efficacy of oral vaccination with ACE527 against challenge with virulent ETEC strain H10407 (ST/LT; CFA/I).

Recruitment of subjects. Subjects were healthy male and nonpregnant female volunteers age 18 to 50 with a body mass index (BMI) of 19.0 to 34.0 kg/m^2, as for the previous phase 1 study (17). All volunteers were fully informed about the content of the trial and provided signed informed consent following demonstration of understanding via a written test. Major exclusion criteria were significant medical conditions, including chronic, immunosuppressive, malignant, or gastrointestinal diseases or abnormalities. Subjects who were food handlers or who had household or professional contact with potentially immunocompromised individuals were also excluded.

After a comprehensive screening evaluation, 70 subjects were enrolled and randomized to receive either the placebo or ACE527 at an approximate dose of 10^{11} CFU (~1:1 ratio). Subjects received two doses of ACE527 or placebo 3 weeks apart, were actively monitored for 27 days after the second dose, and were admitted to the inpatient unit to confirm their continued eligibility for challenge. A total of 29 ACE527 recipients and 27 placebo recipients were eligible for challenge at 4 weeks after their second immunization.

Challenge of subjects with ETEC strain H10407 (O78:H11; CFA/I; LT; ST). As previously described, the protocol included an optimized challenge procedure using ETEC strain H10407 at a target dose of 2×10^7 CFU (16). Subjects were admitted to the inpatient unit of the Center for Immunization Research (CIR) 27 days after the second vaccination. On the following morning, after approximately 9 h of fasting, subjects ingested 120 ml of USP sodium bicarbonate solution (13.35 g/liter) followed by another 30-ml bicarbonate solution containing 2×10^7 CFU of H10407. Subjects were not allowed food or drink (except sips of water) for 90 min following challenge. This protocol and the rationale for the chosen dose and inclusion of the overnight fast are described in detail elsewhere (16). Following challenge, subjects were monitored and cared for in the CIR inpatient facility until discharge between 6 and 10 days later after receipt of a 3-day course of antibiotics and production of two stool samples which were negative for H10407 by microbiological culture. All subjects were followed up with a telephone call to check for any serious emergent medical conditions approximately 4 months after their first vaccination. The clinical protocol was performed under BB-IND13,982 and was approved by the Institutional Review Board of JHBSPH, the Western Institutional Review Board (Olympia, WA), and the Johns Hopkins Institutional Biosafety Committee. The use of the challenge strain was approved under BB-IND12,234.

Endpoints and definitions. The primary efficacy endpoint was the incidence of moderate to severe diarrhea in the two treatment groups, and the protective efficacy of the vaccine was defined as the proportional reduction of the risk of meeting this definition in vaccine versus placebo recipients. A number of secondary parameters were defined to measure the impact on diarrheal stool output and associated clinical symptoms. The primary safety endpoints were evaluations of adverse events (AEs) in the 7 days following each dose via focused medical interviews using written memory aid tools, laboratory tests, and physical examinations. As a secondary safety endpoint, the duration of shedding of each of the three vaccine strains was evaluated.

Clinical diarrhea definitions. During the outpatient period following vaccination, diarrhea was defined as three or more unformed stools (not keeping their shape in the toilet pan) within 24 h as reported by the subject; other unformed bowel movements were recorded as "loose stools." During the inpatient period, each stool passed was collected, weighed, and graded as follows: grade 1, firm formed; grade 2, soft formed; grade 3, viscous opaque liquid or semiliquid which assumed the shape of the container; grade 4, watery opaque liquid; and grade 5, clear watery or mucoid liquid. Stools defined as grade 3, 4, or 5 were considered to be loose and to potentially contribute to an episode of diarrhea. The composite definition of diarrhea defined in the protocol and used to monitor the primary endpoint was as follows: severe diarrhea, at least six grade 3 to 5 stools or more than 800 g of grade 3 to 5 stools in 24 h; moderate diarrhea, four or five grade 3 to 5 stools or 401 to 800 g of grade 3 to 5 stools in 24 h; and mild diarrhea, one grade 3 to 5 stool of more than 300 g or at least two grade 3 to 5 stools totaling at least 200 g during any 24-hour period. Episodes of grade 3 to 5 stools that did not meet any of the above definitions were classified as loose stools. In calculating the total number and weight of diarrheal stools following challenge only stools which contributed to an episode of diarrhea according to these definitions were included.

In *post hoc* evaluation of the impact of ACE527 on the severity of diarrhea, we used a modified scale, based only on the maximum output of grade 3 to 5 stools passed within 24 h: no diarrhea, no loose stools; mild diarrhea, 1 to 400 g loose stools; moderate diarrhea, 401 to 800 g loose stools; severe diarrhea, 801 to 1,600 g loose stools; and very severe diarrhea, more than 1,600 g loose stools.

Continuous measure of diarrhea severity. The evaluation of diarrheal output on an individual-subject basis, with arbitrary output cutoffs to distinguish mild, moderate, and severe outcomes, reduces the power of a study to observe differences in treatment groups, particularly when the researchers are constrained by logistical and ethical considerations to use relatively few subjects. Accordingly, we adopted a continuous approach in a further *post hoc* analysis. Using individual records of the time and volume of every stool passed, it is possible to calculate the accumulated volume per treatment group from a certain point in time. This can be either fixed for all subjects (e.g., the time of challenge) or relative to each subject (e.g., the time of a subject's first diarrheal stool). In this study, we used the latter approach. The total accumulated volume of loose stool divided by the number of subjects in the group was then plotted against time from the index point (for an example, see Fig. 5).

Clinical monitoring and treatment. Medical interviews and physical examinations were performed daily by the principal investigator, and additional medical assessments and vital sign measurements were performed by the study team at least three times daily. Active solicitation regarding the following symptoms took place during the medical interview: fever, vomiting, nausea, abdominal pain, abdominal cramping, myalgias, malaise, bloating, headache, lightheadedness, chills, constipation, and anorexia. Fever was defined as an oral temperature of at least 100.4°F. Fever severity was categorized as mild (≥100.4°F and ≤101.1°F), moderate (>101.1°F and ≤102.0°F), or severe (>102°F). Vomiting was classified as mild (one episode within a 24-hour period), moderate (two episodes within a 24-hour period), or severe (more than two episodes within a 24-hour period). Other constitutional symptoms were graded as follows: mild (discomfort noted but no disruption of normal daily activities; relieved with or without symptomatic treatment), moderate (discomfort sufficient to reduce or affect normal daily activity; only partially relieved with symptomatic treatment), or severe (discomfort sufficient to reduce or affect normal daily activity considerably; not relieved with symptomatic treatment).

Once a subject passed a diarrheal stool, oral rehydration was initiated using oral rehydration solution (Ceralyte; Cera Products, Inc., Columbia, MD) and other fluids, with the aim of replacing the subject's output. If a

subject had severe vomiting, passed a large stool (≥300 g) at diarrhea onset, or for other reasons was unable to consume adequate amounts of oral replacement fluids to maintain hydration, intravenous fluids (lactated Ringer's solution) were given. Prior to discharge, a 3-day course of antibiotic therapy was initiated to clear the infection. Unless it was contraindicated, subjects were administered ciprofloxacin (500 mg twice daily) as first-line therapy. Trimethoprim-sulfamethoxazole (160 mg/800 mg twice daily) and amoxicillin (500 mg three times daily) were additional options if ciprofloxacin could not be used. Early antibiotic treatment was provided to subjects who had severe diarrhea, moderate diarrhea for 2 days, or mild or moderate diarrhea with two or more of the following symptoms: fever (≥100.4°F), vomiting, or severe constitutional symptoms, including abdominal pain or cramping, headache, myalgias, or nausea. In addition, the principal investigator could initiate early treatment if it was warranted for other reasons. Subjects who did not meet criteria for early antibiotic therapy were treated with antibiotics approximately 120 h after challenge. To be eligible for discharge, subjects needed to be clinically well and to have at least two negative stool cultures for ETEC H10407.

Stool microbiology. Stool samples were collected on days 3, 7, 21, 24, 28, 31, and 48 after the first vaccination for evaluation of vaccine shedding. ACE527 colonies were identified by spreading samples on MacConkey agar plates (typical lactose-positive pink appearance) and replica picking onto M9 minimal agar plates with and without supplementation of aromatic compounds (21) where the *aroC* auxotrophic mutation prevents growth on M9 alone. Three days after each vaccination, pools of aromatic-dependent colonies were made and screened in a multiplex PCR to identify the presence or absence of the three individual vaccine strains (17).

Stool samples were taken on up to three occasions each day between challenge and discharge to monitor excretion of the H10407 challenge strain. On the second and fourth days after challenge, quantitative culture was performed to determine the level of H10407, which was expressed as CFU/g stool. Where stool samples were not available, a rectal swab was used to make a qualitative assessment of colonization. Quantitative results were obtained for 23/29 and 19/29 ACE527 recipients at days 2 and 4 after challenge, respectively, and for 24/27 and 17/27 placebo recipients at days 2 and 4 after challenge, respectively.

Immunology. Systemic and mucosal antibody responses to LT-B, CFA/I, CS3, and CS6 following vaccination were evaluated to monitor the immunogenicity overall and those of the three individual strains. Serum IgG and IgA responses were measured on days 0, 21, 31, and 49 and were analyzed by the level of responses (geometric mean titers) as well as the frequency with which subjects seroconverted, as measured by a 2.5-fold or more increase over the preimmunization levels, following the same assay methodology as used in the previous study (17) as defined in the clinical protocol.

Mucosal immune responses to vaccination were measured primarily using the antibody in lymphocyte supernatant (ALS) assay (3), which measures the IgA secreted by peripheral blood mononuclear cells (PBMC) circulating to the mucosal inductive sites, peaking at 7 to 10 days following oral immunization. PBMC were isolated on the day of and at 7 days following each immunization. ALS responses were considered positive if, at any time point, they reached 4-fold or more over baseline at day 0. This protocol was modified for logistic reasons from that employed in the earlier phase 1 trial (17), where ALS responses were measured at both 7 and 10 days following each vaccination.

Systemic and mucosal antibody responses to O78 lipopolysaccharide (LPS) following challenge with H10407 were evaluated as a surrogate marker for inhibition of colonization (and hence immunogenicity) by vaccination. Serum IgG and IgA responses were measured on days 7 and 28 after challenge and were analyzed by the frequency with which subjects seroconverted, as measured by a 2.5-fold or more increase over the prechallenge levels.

Data capture and analysis. All AEs and clinical test results were recorded on source documents and transferred to an electronic case report form (CRF). All clinical data were 100% source document verified before analysis.

Statistical methods and sample size. The primary endpoint of the trial was the incidence of moderate to severe diarrhea in the two treatment groups, and the protective efficacy of the vaccine was defined as follows: protection = [1 − (rate in vaccine recipients/rate in placebo recipients)] × 100%. The null hypothesis stated that the incidences of moderate to severe diarrhea in the vaccine and placebo groups were the same, versus the alternative hypothesis that the incidence of moderate to severe diarrhea was lower in the vaccine group (H0, vaccine efficacy ≤ 0%, versus H1, vaccine efficacy > 0%). Accordingly, this hypothesis and other data comparisons were evaluated using one-sided tests and *P* values.

The sample size was based on estimated incidences of 70% in placebo recipients and 30% in vaccinees (protective efficacy of 57%), requiring challenge of 28 subjects in each group to show a significant difference between the two groups ($\alpha = 0.05$; $\beta = 80\%$). The estimated attack rate of 70% in placebo recipients was based on a prior challenge model refinement study performed at the same study site in an equivalent population of subjects (16). Accordingly, 36 and 34 subjects each were vaccinated with ACE527 and the placebo, respectively, in order to allow for dropouts before challenge.

Dichotomous results were analyzed using Fisher's exact test and quantitative parameters using Wilcoxon's rank sum test or the Mann-Whitney U test, as the variables were not normally distributed. The numbers of subjects who had no loose stools were analyzed by simple odds regression, and the proportions that experienced no, mild, moderate, severe, or very severe diarrhea output were analyzed by a proportional odds regression model.

TABLE 1 Subject demographics (safety analysis set, $n = 70$)

Demographic variable	Vaccine group ($n = 36$)		Placebo group ($n = 34$)		All ($n = 70$)	
	No.	%	No.	%	No.	%
Sex						
Male	28	77.8	26	76.5	54	77.1
Female	8	22.2	8	23.5	16	22.9
Race						
Black/African American	34	94.4	29	85.3	63	90.0
White	2	5.6	3	8.8	5	7.1
Other	0	0.0	2	5.8	2	2.8
Age, yr (mean ± SD)	35.4 ± 8.78		35.7 ± 9.16		35.6 ± 8.90	

RESULTS

The demographics of the study population are shown in Table 1. Overall, 77% of subjects were male and 90% were African-American. The mean age was 35.6 years; there were no significant differences between the two study groups. The disposition of subjects through the study is shown in Fig. 1.

Safety. There were no serious AEs following vaccination, but one occurred in a placebo recipient during long-term follow-up postchallenge. This event was not considered to be related to participation in the study. There were no early terminations or withdrawals due to vaccine safety or tolerability. The vaccine was safe and, for the most part, well tolerated, extending the observations from the phase 1 study (17). The frequency of subjects experiencing AEs possibly related to vaccination was 47% in both the vaccine and placebo groups (Table 2), and the majority of AEs were of

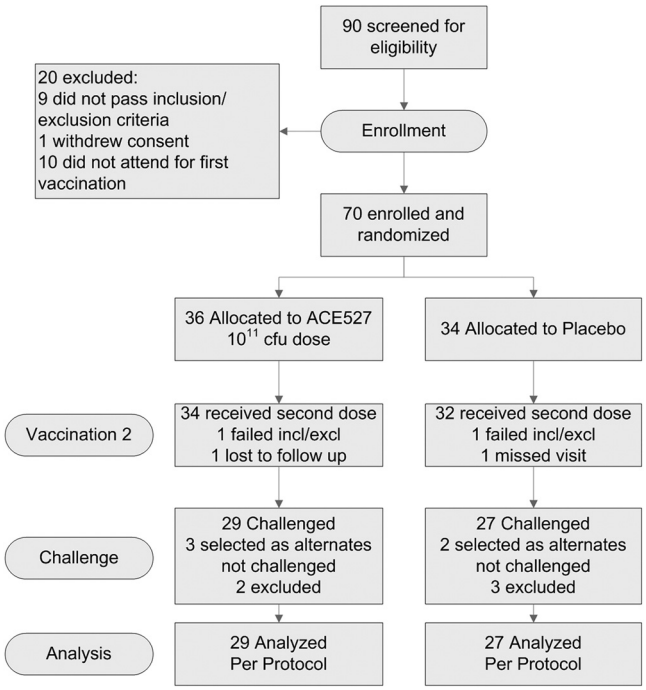

FIG 1 Subject allocation and retention through study.

mild intensity. In the present study, there were trends toward an elevated frequency of gastrointestinal AEs recorded in the vaccine recipients, the majority of which were of mild intensity, as seen in the phase 1 study. These occurred predominantly on the day of vaccination and resolved spontaneously within 24 h (data not shown).

TABLE 2 Numbers of subjects with treatment-related AEs before challenge by group

MedDRA System Organ Class preferred term	Vaccine group ($n = 36$)		Placebo group ($n = 34$)	
	No.	%	No.	%
Any treatment-emergent AE related to vaccine[a]	17	47.2	16	47.1
Gastrointestinal symptoms				
Gastrointestinal sounds abnormal	8	22.2	8	23.5
Abdominal pain	10	27.8	5	14.7
Nausea	11	30.6	4	11.8
Defecation urgency	8	22.2	5	14.7
Loose stools (<3 per 24 h)	8	22.2	4	11.8
Diarrhea (>2 per 24 h)	6	16.7	1	2.9
Vomiting[b]	7	19.4	0	0.0
General symptoms				
Anorexia	10	27.8	3	8.8
Malaise	7	19.4	5	14.7
Chills	3	8.3	2	5.9
Headache	6	16.7	2	5.9
Dizziness	5	13.9	1	2.9
Myalgia	2	5.6	1	2.9

[a] AEs occurring in more than one subject.
[b] $P < 0.05$.

TABLE 3 Numbers of subjects shedding ACE527 and individual strains

Parameter	Day	No.[a]	%
Shedding of individual strains 3 days after either dose			
ACAM2022	3	25	69.4
	24	17	50.0
ACAM2025	3	11	30.6
	24	7	20.6
ACAM2027	3	14	38.9
	24	19	55.9
Shedding of ACE527 (any strain) on specific days	3	28	77.8
	7	12	33.3
	21	4	11.1
	24	24	70.6
	28	6	17.6
	31	5	14.7
	Any day (3–21) (first dose)	31	86.1
	Any day (24–31) (second dose)	24	70.6
	Any day (3–31) (either dose)	35	97.2

[a] $n = 36$ (dose 1) and $n = 34$ (dose 2).

Six ACE527 vaccinees reported diarrhea (4 mild, 1 moderate, and 1 severe), compared to a single moderate episode in the placebo group. In addition, eight vaccinees experienced loose stools not meeting the definition of diarrhea, compared to four placebo recipients. Although the incidence of diarrhea after vaccination was not significantly higher in ACE527 recipients ($P = 0.107$), if the incidence of any loose stools is considered, then the rate was higher in ACE527 recipients (38.9% versus 14.7%; $P = 0.032$). The only AE which was statistically significantly more frequent in ACE527 vaccinees was vomiting, which occurred in seven vaccinees and zero placebo recipients ($P = 0.012$), with four subjects vomiting after both doses of vaccine, two only after the first dose, and one only after the second dose. These events all happened on the day of vaccination following discharge from the clinic (at least 1 h after dosing); two episodes were mild, seven were moderate, and two were rated as severe. Subjects who experienced such AEs following vaccination were not excluded from the challenge phase in the trial. Shedding of vaccine in the stools of the subjects who vomited was detected in 7/11 (63.6%) of instances, which was not significantly different from the overall rate of recovery (78.6%; $P = 0.28$). There was no apparent difference in the immune response to vaccination or in the frequency or severity of symptoms following challenge with H10407 in this subset of subjects (data not shown).

Shedding of ACE527 was monitored by stool culture on days 3, 7, 21, 24, 28, 31, and 48. The results are shown in Table 3. Peak shedding of ACE527 occurs 2 to 3 days following dosing (17); in this study, 78% of subjects shed ACE527 on day 3 and 71% shed on day 24 (3 days after the second dose of the vaccine). The median times to negative cultures were 5 to 6 days after the first dose and 4 to 5 days after the second dose, with 11% of subjects shedding at day 21 immediately before receipt of the second dose. Overall, 97% of vaccinees shed ACE527 on at least one occasion, with 86% shedding after the first dose and 71% after the second. Fifty-six percent of subjects shed after both the first

TABLE 4 Immune responses to vaccine antigens

Antigen	No. (%) of subjects responding[a]							
	ACE527 recipient response to vaccine by day 41, 20 days after second vaccination ($n = 36$)				Placebo recipient response to H10407 by day 70, 21 days after challenge ($n = 27$)			
	Serum IgG	Serum IgA	ALS	Any	Serum IgG	Serum IgA	ALS	Any
LT-B	24 (67)	20 (56)	33 (92)	34 (94)	14 (52)	3 (11)	17 (63)	21 (78)
CFA/I	13 (36)	17 (47)	20 (56)	29 (81)	8 (30)	10 (37)	18 (67)	23 (85)
CS3	0 (0)	4 (11)	20 (56)	22 (61)				
CS6	6 (17)	5 (14)	6 (17)	13 (36)				

[a] Positive responses are defined as a 2.5-fold increase over baseline titer for serum IgG and IgA and as a 4-fold increase over baseline titer for ALS.

and second doses, 31% only after the first dose, and 11% only after the second dose. On the third day following each dose, 94% of subjects shed at least one strain, 61% shed at least two, and 36% shed all three on either day as detected by PCR.

All challenged subjects were stool culture negative for ACE527 by day 48; no subjects required antibiotics to stop them from shedding. Vaccine colonization was self-limiting, with a median time to negative stool culture postimmunization of less than 1 week after each dose.

Immunogenicity. The numbers of subjects mounting positive serum IgG or IgA or mucosal ALS responses to key antigens following vaccination with ACE527, or following challenge with H10407 for those subjects who received placebo, are shown in Table 4. The frequency of responders to LT-B, CFA/I, and CS3 following vaccination with ACE527 were similar to those observed in the earlier phase 1 trial (17), although the responses to CS6, in particular the ALS response, were somewhat lower in the present study. This may be at least in part because the ALS assays in this trial were performed at only one time point (day 7) after each vaccination. Most encouraging was the comparison of the responses to LT-B and CFA/I induced by the vaccine to those induced in placebo recipients following the challenge with H10407. The overall systemic and mucosal response rates to CFA/I were equivalent in vaccinated and challenged subjects, with higher seroconversion rates in the vaccinees. The antitoxin response rates were higher in all three assays in ACE527 recipients than in naive H10407-challenged subjects. As seen previously in the phase 1 study (17), the serum IgG response to LT-B following ACE527 vaccination was substantial. Figure 2 shows the kinetics of the response in both vaccine and placebo recipients. It is clear that the response induced by the first dose of ACE527 was higher than that induced in placebo recipients following challenge with H10407 and that the response increased further following the second vaccination. There was no discernible correlation between the shedding of ACE527 or individual strains and the generation of immune responses to the colonization factors or LT-B.

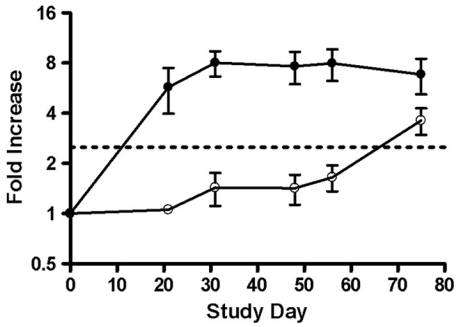

FIG 2 Quantitative serum IgG responses to LT-B following vaccination and challenge. Titers are expressed as fold increase relative to prevaccination levels, on a $\log_2$ scale. Solid circles, ACE527 vaccinees; open circles, placebo recipients. Bars show means and standard errors. Vaccination with ACE527 or placebo was on days 0 and 21, and challenge with H10407 was on day 49. The horizontal dashed line at a 2.5-fold increase is the threshold for a positive response.

Immune response to H10407 following challenge. The number of subjects mounting positive serum IgG or IgA responses to O78 LPS following challenge with H10407 for the two groups of subjects who received ACE527 or placebo are shown in Table 5. There was no apparent impact of vaccination on the high frequency of subjects who seroconverted with IgA antibodies against O78 LPS. The frequency of subjects who mounted an IgG serum response to the same antigen, however, was significantly reduced in those who had been vaccinated with ACE527 before challenge. At day 7 after challenge, 8 of 27 placebo recipients but only 2 of 29 vaccinees mounted an IgG response (76.3% reduction; $P = 0.038$ by two-sided Fisher exact test). By day 28 after challenge there were more responders in either group; however, there was still a 49.9% reduction in the frequency of IgG seroconversion in the ACE527 group.

Efficacy. At this stage in the development of the vaccine, the clinical protocol included a number of prospectively defined primary and secondary endpoints to evaluate the potential impact of vaccination on disease outcome. In addition, a smaller number of *post hoc* analyses were performed on the data to further investigate the potential effects of vaccination on diarrhea severity and possibly help to define endpoints for future studies. These endpoints and results are shown in Table 6 with calculated protective efficacy and *P* values obtained using the appropriate statistical tests.

Primary endpoint. The rates of moderate or severe diarrhea, by the composite protocol definition, were 51.7% in ACE527 vac-

TABLE 5 Serum antibody responses to O78 LPS

Group	No. (%) of positive responses[a]			
	Day 7 postchallenge		Day 28 postchallenge	
	Serum IgG	Serum IgA	Serum IgG	Serum IgA
ACE527 ($n = 29$)	2 (6.9)	23 (79.3)	7 (24.1)	22 (75.9)
Placebo ($n = 27$)	8 (29.6)	24 (88.9)	13 (48.1)	22 (81.5)

[a] Positive responses are defined as a 2.5-fold increase over baseline titer.

TABLE 6 Incidence and severity of symptoms of ETEC disease after challenge

Parameter	Value for group		Protective efficacy (%)	*P* value (1 sided)[a]
	ACE527 ($n = 29$)	Placebo ($n = 27$)		
Primary endpoint				
No. (%) of subjects with moderate or severe diarrhea	15 (51.7)	19 (70.4)	26.6	0.12
Secondary endpoints				
No. (%) of subjects with severe diarrhea	14 (48.3)	16 (59.3)	18.5	
No. (%) of subjects with diarrhea of any severity	16 (55.2)	20 (74.1)	25.5	0.12
Mean total wt (g) of grade 3–5 stools[b] per subject	1163.3	1,724.6	35.3	
Median total wt (g) of grade 3–5 stools[b] per subject	390.0	1,128.0	65.4	0.09[c]
Mean no. of grade 3–5 stools[b] per subject	7.2	9.6	25.0	
Median no. of grade 3–5 stools[b] per subject	5.0	10.0	50.0	0.13[d]
No. (%) of subjects with moderate to severe nausea	11 (37.9)	12 (44.4)	14.6	
No. (%) of subjects with moderate to severe vomiting	8 (27.6)	8 (29.6)	6.8	
No. (%) of subjects with moderate to severe abdominal pain/cramps	12 (41.4)	12 (44.4)	6.7	
No. (%) of subjects with moderate to severe anorexia	8 (27.6)	14 (51.9)	46.8	0.06
No. (%) of subjects who would have reduced their daily activity if traveling	13 (44.4)	19 (70.4)	36.9	0.05
Mean time (h) to onset of diarrhea	60.05	55.95	6.8	0.14[c]
No. (%) of subjects with moderate to severe ETEC illness	14 (48.3)	16 (59.3)	18.5	
CFU of challenge strain per g of stool (geometric mean)	1.1×10^6	2.2×10^7	90.0	0.002
No. (%) of subjects requiring early antibiotic treatment	12 (41.4)	16 (59.3)	30.2	0.14
No. (%) of subjects requiring intravenous fluids	8 (27.6)	10 (37.0)	25.4	
Post hoc analyses				
Severity based only on total vol of loose stool				
No. (%) of subjects producing >800 g of grade 3–5 stools	12 (41.4)	19 (70.4)	41.2	0.03
No. (%) of subjects producing no grade 3–5 stools	9 (31.0)	3 (11.1)		0.04
Diarrheal output produced in 24 h following first diarrheal stool				
Mean total wt (g)	599.8	1,102.4	45.6	
Median total wt (g)	310.0	488.0	36.5	0.06[d]

[a] Determined by Fisher's exact test unless otherwise indicated; shown only where $P < 0.15$. As stool number and output data were not normally distributed, nonparametric tests were applied to assess the significance of differences in the median values.
[b] Only stools which contributed to an episode of diarrhea are included.
[c] Determined by Wilcoxon rank sum test.
[d] Determined by Mann-Whitney test.

cinees and 70.4% in placebo recipients. This represents a 26.5% reduction in incidence (95% CI, −12.8 to 52.1%; $P = 0.124$), as shown in Table 6. Thus, while the trend indicated a reduction in vaccinees, the difference was not statistically significant. The use of the modified severity definitions based only on diarrhea output did not change this result (protective efficacy = 29.6%; 95% CI, −14.9 to 54.4%; $P = 0.131$).

Prospectively defined secondary measures of diarrheal disease severity and infection. All of the 14 secondary endpoints defined in the protocol showed a positive impact of vaccination with ACE527. For 6 of these, the *P* value was 0.15 or greater due to the small size of the effect or the variability between subjects; a further 6 endpoints showed trends toward significance (P values of 0.06 to 0.15). Two of them had significant *P* values of ≤0.05, namely, the number of subjects who considered that their illness would have been severe enough to interfere with their activities if they had been traveling and the shedding of H10407 at day 2 after challenge.

Vaccination was observed to have an effect on the incidence and severity of diarrheal disease, with a reduction of 26% in the proportion of subjects experiencing any diarrhea and a considerable reduction in the output of loose stools passed during the study period. Due to the nonnormal distributions of these data, they were compared using nonparametric tests for the differences between the median values. The median numbers of diarrheal stools during the observation period after challenge were 5 for ACE527 vaccinees and 10 for placebo recipients, a 50% reduction, and the median total weights were 390 g and 1,128 g, respectively, a 65% reduction (Table 6).

A measure for the general level of gastrointestinal illness in subjects in challenge studies, independent of diarrheal output, is the extent to which they report loss of appetite. In this study, the incidences of reduced appetite rated as moderate or severe were 51.9% in placebo recipients and 27.6% in vaccinees. This represents a 47% reduction ($P = 0.056$). As an additional measure, subjects were asked before discharge whether they thought their symptoms would have significantly reduced their activities had they been traveling. This was the case for 70.4% of placebo recipients and 44.4% of vaccinees, a 37% reduction ($P = 0.048$) (Table 6). Other endpoints designed to measure nondiarrheal symptoms of ETEC infection (nausea, vomiting, and abdominal pain or cramps) were not significantly affected in vaccinees.

The peak shedding of the challenge strain occurs between 2 and 4 days after dosing in studies where subjects are treated with antibiotics once they meet a certain endpoint. The quantitative level of H10407 was measured at 2 and 4 days after challenge in this study and the results of the 2-day cultures are presented in Fig. 3. Vaccination significantly reduced the level of shedding by more than

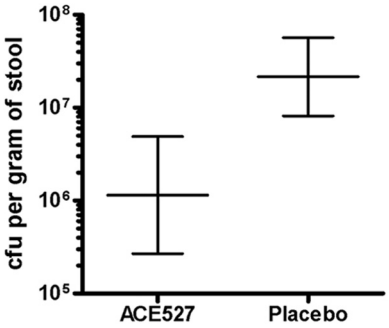

FIG 3 Quantitative level of H10407 shedding at day 2 postchallenge. Bars show geometric means and 95% confidence intervals.

10-fold ($P = 0.002$). Although the levels of H10407 in stools of subjects in 4-day cultures were lower in vaccinees, they were not significantly different (ACE527, 7.5×10^5 CFU/g [95% CI, 7.4×10^4 to 7.7×10^6]; placebo, 2.9×10^6 CFU/g [95% CI, 2.0×10^5 to 4.1×10^7]). There were also significant reductions in the level of H10407 shedding on day 2 after challenge in the subset of subjects who mounted positive anti-CFA/I IgA ALS or anti-LT-B IgA serum or IgA ALS responses postvaccination compared to those who did not respond ($P = 0.009$, $P = 0.026$, and $P = 0.027$, respectively) (data not shown).

***Post hoc* analyses of diarrhea severity.** Vaccination with ACE527 had a positive impact on diarrhea severity when measured by the output of loose stool. The proportion of subjects in each severity category, as defined by the alternative scale based on maximum output in 24 h, is shown in Fig. 4. The most likely outcome of challenge in a vaccinated subject was no diarrheal stools, whereas for a placebo recipient it was very severe diarrhea. The median severities in the vaccine and placebo groups were mild and severe diarrhea, respectively. Fitting a simple proportional odds regression model to the ordered responses demonstrated that the odds of vaccinees falling into a more severe category was roughly halved compared to that for placebo recipients (odds ratio = 0.479 [95% CI = 0.217 to 1.062]; $P = 0.064$) (5). The proportion of subjects in the vaccine group experiencing no diarrheal stools is shown in Table 6 and was significantly higher than that in the placebo group (relative fraction = 2.79; $P = 0.040$ by Fisher's exact test).

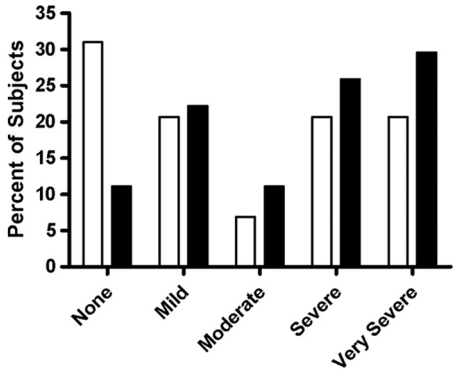

FIG 4 Frequency distribution of severity of diarrhea based on maximum loose stool output in any 24-hour period within 120 h of challenge. None, 0 g; mild, 1 to 400 g; moderate, 401 to 800 g; severe, 801 to 1,600 g; very severe, >1,600 g. Open bars, ACE527 vaccinees; solid bars, placebo recipients.

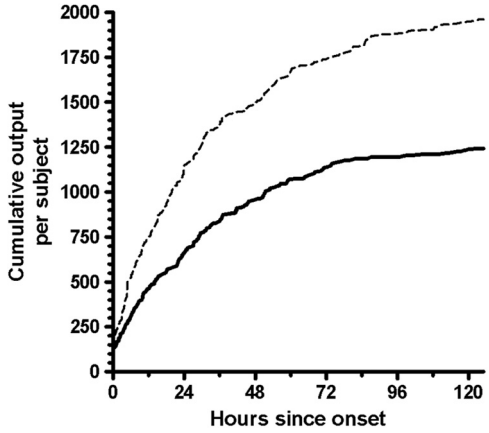

FIG 5 Mean accumulation of loose stools with time from the first diarrheal stool experienced by a subject. Solid line, ACE527 vaccinees; dashed line, = placebo recipients.

The threshold of 800 g of loose stools within 24 h was the protocol-directed definition of severe diarrhea. We therefore asked how many subjects produced 800 g or more in the entire 5-day observation period following challenge. The results are shown in Table 6. Whereas 70.4% of placebo recipients met this output threshold, only 41.4% of ACE527 vaccinees did, a reduction in incidence of 41% ($P = 0.027$).

An alternative analysis of severity based on continuous output of the treatment groups as a whole is shown in Fig. 5. It is apparent that from the onset of diarrhea, the mean unformed stool output was larger in the placebo group, and it remained larger in that group throughout the postchallenge surveillance period; the two curves continue to diverge throughout the full observation period of 5 days after challenge. The output of unformed stool produced in the first 24 h from the start of the episode in the two groups is shown in Table 6. The mean output in the placebo group was 1,102 g, while in ACE527 vaccinees it was 600 g, a reduction of 45.6% (Wilcoxon rank sum test used to analyze difference in medians, $P = 0.056$).

Correlates of protection. While there are no immediately obvious correlations between immune responses to the major antigens (i.e., CFA/I and LT) and the clinical outcome of challenge for individual subjects, the observed reduction in shedding described above in individuals responding to CFA/I or LT-B is of interest. A full analysis of this will be presented elsewhere.

DISCUSSION

This study extends our previous evaluation of the safety, tolerability, and immunogenicity of the three-strain, oral ETEC vaccine, ACE527. In addition, the impact of vaccination on the clinical outcome of experimental challenge with the highly virulent ETEC strain H10407 was evaluated, along with the ability of the vaccine to interfere with colonization by the challenge strain. While the 27% reduction in the incidence of the primary endpoint (moderate to severe diarrhea) was not statistically significant, there were trends toward improved outcomes in the vaccinated subjects in all prospectively defined secondary endpoints, with a significant reduction in the level of colonization by the challenge organism as

measured by fecal shedding. Additionally, significant positive impacts of the vaccine on several *post hoc* secondary endpoints, including signs and symptoms of disease, were observed.

Experimental challenge of human volunteer subjects is a widely used model to evaluate the efficacy of vaccines or other interventions to protect against enteric diseases such as ETEC, *Shigella*, and *Campylobacter* infection before moving on to large and expensive pivotal phase 3 field trials. In the case of ETEC, the frequency and severity of disease observed in such challenge studies are higher than those seen in naturally acquired illness associated with travel, making them "useful for limited hypothesis testing" (22) rather than reflecting the expected efficacy of a vaccine in the real world. The most commonly used challenge model for ETEC employs the prototypical CFA/I-positive LT/ST strain H10407 (6, 8, 9, 10, 11, 14, 15, 20, 31). In a recent review of the use of challenge models to evaluate ETEC pathogenesis and vaccine efficacy (26), it was clearly shown that the H10407 strain produces significantly more severe symptoms than any other strain evaluated. Recently, the protocol for this model was refined, including an overnight fast before the morning of challenge, to allow reproducible attack rates of more than 70% to be obtained in a total of 35 human subjects following an oral dose of only 2×10^7 CFU (16). Even at this lower dose, however, the severity of disease induced following challenge is comparable to that seen at the higher dose levels and is greater than with any other challenge strain; diarrheal purges of several liters are common, requiring active intervention with antibiotics and oral and intravenous rehydration support to prevent hypotension or hypovolemia.

Sample sizes in challenge studies are limited by both ethical and logistical considerations. Therefore, challenge trials are not generally powered to see statistically significant differences between treatment groups unless those differences are large. Another feature of challenge studies which may underestimate the efficacy of a vaccine is the use of antibiotics as soon as a subject meets a certain clinical definition, effectively shortening the observation period in which a difference in clinical outcome might be observed. While it might be more informative to avoid treatment to see if vaccination shortens the duration of the episodes, this is difficult to do from an ethical perspective.

The definitions of diarrhea severity in the clinical protocol were in line with similar studies performed under investigational new-drug applications in the United States, based on the U.S. Food and Drug Administration and U.S. National Institutes for Health table of AEs (12), and were a composite of frequency and volume of loose stools passed in any 24-hour period. During inpatient studies, the frequency component of these definitions is felt to be less meaningful, and so we have additionally evaluated the efficacy of the vaccine against the severity of diarrhea based only on the peak volume passed during any 24-hour period. We suggest this to be a more objective assessment of the clinical impact of the diarrheal episode on individual subjects and more representative of the potential for the vaccine to prevent serious, dehydrating diarrhea in travelers and particularly in children in settings of endemicity.

The vaccine was safe and generally well tolerated, as observed in the previous phase 1 study (17), with the exception of episodes of vomiting seen in seven vaccinated subjects on the day of vaccination. These episodes were unexpected, given that no such AEs were seen previously. In the phase 1 study, which was the first administration of the multivalent combination to human volunteers, the first dose of vaccine was given on the second day of a 4-day inpatient period as a safety precaution. As a consequence of this, the subjects' diet was carefully controlled; they were given a light breakfast and fasted for approximately 90 min before dosing. In contrast, the subjects in this phase 2b study came into the clinic on the morning of dosing, and many of them had not eaten since the night before (data not shown). It is possible that ingestion of the high dose of vaccine on an empty stomach might have contributed to this reactogenicity. Similar episodes of vomiting postimmunization have been reported for an experimental killed, whole-cell ETEC vaccine (4, 27, 29). This hypothesis is further supported by the increased virulence of the wild-type challenge with H10407 following overnight fasting (16). This will need to be addressed during further development of the vaccine by adjusting the buffer formulation and dose level.

Efficacy. The reduction in the primary endpoint of the study was 26.5%, which was not statistically significant. A number of alternative measures of disease severity did, however, reach statistical significance, and others showed strong trends (Table 6). Figure 4 shows the incidence of diarrhea by severity based on output alone. The impact of vaccination is spread across the whole range of severity, with each category other than "no diarrhea" having a higher incidence in placebo recipients and with almost three times as many subjects in the ACE527 group being free of diarrheal stools altogether ($P = 0.046$). In terms of impact on individual subjects, the most significant effect of vaccination was seen on the output of diarrheal stool produced in the first 24 h of an episode, as shown in Table 6. This suggests a useful impact of vaccination for travelers, who would typically self-medicate within 24 h of the beginning of a diarrheal episode, which would be expected to prevent worsening of symptoms and accelerate resolution. Therefore, a milder disease course in this first 24 h may mean that the vaccine would reduce the number of travelers whose symptoms interfered with normal activities. This observation is supported by the significantly lower number of vaccinated subjects who considered that their illness would have been sufficiently severe to prevent their normal activities if they were traveling (Table 6).

In contrast to some other challenge studies (22), however, the effect on stool output continued throughout the 5-day observation period after challenge. This effect is readily seen from the graphical representation in Fig. 5. The median number and output of diarrheal stools during this period were reduced by 50% and 65%, respectively (Table 6).

When subjects are dichotomized according to the incidence of diarrheal output of greater than 800 g in the 5 days after challenge, there is a 41% reduction in incidence in vaccinees ($P = 0.027$). This effect is greater than that on the peak output in 24 h and results from the earlier truncation of output in vaccinees than in placebo recipients. This is clearly seen in Table 6; the median output in the first 24 h of the episode was 310 g in ACE527 vaccinees and increased to 390 g (26% more) by the end of the surveillance period, whereas in placebo recipients the corresponding increase was from 488 g to 1,128 g (131% more). Thus, more placebo recipients continued to produce significant diarrhea after 24 h from the beginning of the episode, despite the fact that many were treated with antibiotics. H10407 has been demonstrated to produce a longer duration of diarrhea than other ETEC strains in challenge studies (26). It appears from our observations that vaccination with ACE527 truncated this extra duration, perhaps by modulating colonization of the small intestine.

Shedding of H10407 challenge strain. For the first time, this study showed that vaccination with an oral, whole-cell ETEC vaccine had a significant impact on the quantitative level of colonization of subjects as measured by fecal shedding. Geometric mean concentrations of H10407 shed at 2 days after challenge were reduced by 20-fold; this level of reduction in vaccinees is comparable to that seen in other studies.

In a study in which subjects were fed CFA-specific antibodies or a placebo in milk before challenge with H10407 (14), the mean level of the challenge organism was 6.2×10^7 CFU/g in milk globulin-treated subjects versus 4.5×10^8 in placebo recipients, a 7.3-fold reduction. Treated subjects experienced 90.5% (95% CI, 33.9% to 98.6%) protection against diarrhea. In a study aimed at refining the H10407 challenge model, subjects fed 2×10^7 CFU shed geometric mean maximum concentrations of 8×10^7 CFU/g, whereas subjects rechallenged with the same dose shed only 3×10^5 CFU/g (16), a 270-fold reduction, and were protected against diarrhea.

In a previous ETEC vaccination challenge study using a spontaneous toxin-deleted ETEC strain (E1392-75/2A) as a vaccine and a different ETEC challenge strain (E24377A) (19, 30), 75% protection against diarrhea was seen, and a reduction in colonization by the challenge strain was observed in vaccinees as measured in small-bowl aspirates. In that study, there was no difference seen in the level of stool shedding, although it is not clear from the literature at what times stool samples were assayed for shedding after challenge. The authors hypothesized that vaccination with the live vaccine strain had induced protective immune responses in the small intestines of immunized subjects and that this interfered with colonization of this niche and the subsequent induction of diarrheal disease. In the present study, a significant reduction was seen in the level of shedding of H10407 in stool on day 2 after challenge in vaccinees, whereas by day 4 their level of shedding, although still lower, approximated that in placebo recipients. We hypothesize that this corresponds to an inhibition of small intestinal colonization early after dosing in vaccinees, followed by a later colonization of the large intestine equally in both groups, as previously described by Levine and colleagues (19, 20). As colonization of the large intestine by ETEC does not cause diarrhea, a vaccine does not need to inhibit this to exert a protective effect against disease.

Given the highly significant reduction in early shedding of H10407 observed in the vaccine group, it is important to investigate whether this has any functional impact on the incidence or severity of disease. All subjects from whom we were able to collect quantitative shedding data at 2 days after challenge (23 of 29 vaccinees and 24 of 27 placebo recipients) were grouped according to whether their shedding was above or below the median level (5×10^6 CFU/g). The time to reach the primary endpoint of moderate to severe diarrhea (composite definition based on volume or number of loose stools in 24 h) was investigated for these groups using a Kaplan-Meier survival analysis, as shown in Fig. 6. Subjects who did not meet the endpoint during the 120 h following challenge were censored. The hazard ratio for meeting the primary endpoint in the high-shedding group was 2.98 (95% CI, 1.51 to 6.74%), and the survival curves were statistically different ($P = 0.002$ by the log rank test). Thus, it has been clearly shown that vaccination with ACE527 significantly reduces the shedding of H10407 after challenge and that in subjects with lower shedding

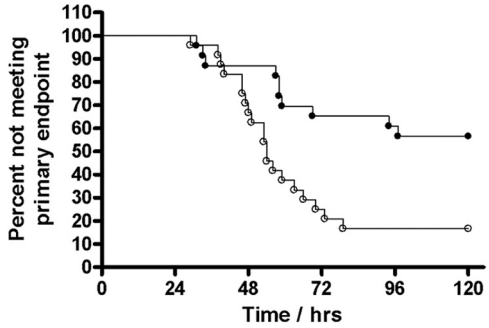

FIG 6 Kaplan-Meier survival analysis of the time to reach the primary endpoint (moderate or severe diarrhea) according to the quantitative level of shedding of H10407 in stool at 2 days after challenge. All subjects for whom quantitative shedding data were available were sorted by the level of shedding. Those below the median level (6.4×10^6 CFU/g) were considered "low shedders," represented by filled circles, and those above or at the median were considered "high shedders," represented by open circles.

there is a highly significant reduction in the odds of meeting the primary endpoint of the study.

The subgroup of subjects who mounted positive mucosal responses (IgA ALS) to both CFA/I and LT-B ($n = 11$, all of whom were ACE527 recipients) showed a significant reduction in the level of shedding of H10407 in stool at day 2 after challenge compare to subjects who responded to neither antigen ($n = 22$) ($P = 0.009$) (data not shown). This may indicate the involvement of these specific responses in the reduction of colonization by H10407, or else they may simply be markers of having received the ACE527 vaccine, as there was a significant reduction in shedding in the vaccine group as a whole.

Immune response to H10407 challenge strain. Vaccination with ACE527 reduced the rate of IgG seroconversion to the O78 LPS antigen expressed by the H10407 challenge strain, most obviously in the early response at day 7 after challenge. This correlates with the impact of vaccination on the colonization of subjects by the challenge strain and provides additional evidence that the interaction of H10407 with the mucosal surface and immune system was impaired in those subjects who were vaccinated with ACE527.

Comparison with previous challenge studies. Previously, as part of the development of the live attenuated vaccine approach, a similarly attenuated CS1- and CS3-expressing strain of ETEC (PTL003, a derivative of E1392-75/2A lacking any LT toxin antigen) was evaluated in phase 1 and phase 2 studies (21, 23), which failed to show efficacy against challenge with virulent strain E24377A (LT, ST, CS1, CS3) in the latter. That challenge trial was performed with a different immunization schedule (2 doses 10 days apart), a lower vaccine dose (2×10^9 CFU), and a higher challenge dose (3×10^9 CFU), and the vaccine contained no LT antigen. The results of this trial may therefore not be relevant to the potential efficacy of ACE527, which contains three different ETEC strains and induces strong immune responses against LT. In the study reported here, we addressed these shortcomings by increasing the vaccine dose, leaving a longer interval between doses, including LT, and using a low-dose refined challenge protocol. As a result, we demonstrated a clear reduction in many of the parameters of ETEC diarrhea severity as well as a trend toward a reduction in the incidence of moderate to severe diarrhea, the primary endpoint of the study, and a statistically significant reduction in

the shedding of the challenge organism. It should be noted that this is the first time that such protective efficacy has been seen in an ETEC challenge study with a multistrain vaccine, where only one of three vaccine strains matches the CFA type of the challenge organism.

This contrasts with another recent challenge study, performed using challenge strain E24377A, with a vaccine comprising LT delivered in a skin patch (22). Although there were reductions in the number and volume of stools following challenge in subjects vaccinated with the patch, this effect was concentrated in the 24 h following challenge, after which the groups became more similar, and there was no impact on the overall incidence of moderate to severe diarrhea. As expected for a vaccine lacking colonization factors, there was no impact on fecal shedding of the E24377A challenge strain. Despite this modest impact in an inpatient challenge setting, however, the LT patch vaccine went on to show impressive efficacy against traveler's diarrhea in a phase 2 trial (13) and against diarrhea caused by LT ETEC strains in an expanded phase 3 trial (18). This suggests that ACE527 may show similar or higher efficacy in a field setting, where subjects are exposed to significantly lower levels of ETEC, are usually not fasted, and do not protect the ETEC organism by buffering the stomach pH. In such settings subjects are exposed to a variety of ETEC strains expressing multiple CFA and toxin types, most of which may be of lower virulence than H10407.

Final conclusion. This phase 2b study was designed to provide early evidence for the efficacy of ACE527 using a preliminary formulation and a high dose considered to be appropriate for controlled clinical trials. While the study population may be pertinent to the future development of the vaccine for use in travelers from countries where ETEC is not endemic to less-developed regions, it is not expected to be particularly relevant to the evaluation of either the safety or efficacy in pediatric populations in developing countries. These parameters can be studied only in the appropriate target populations. ACE527 induced clinically significant attenuation of diarrheal illness and reduced ETEC intestinal colonization in a stringent ETEC H10407 human challenge model. The reduction in the shedding of H10407 following vaccination with ACE527 and the strong induction of anti-LT immune responses support the dual mechanism of action that the vaccine was designed to exhibit. This dual mechanism suggests that the vaccine may prove to be effective against the majority of wild-type ETEC strains encountered in cases of natural exposure, although this remains to be proven in future field trials. The vaccine was safely administered orally at a total dose of 10^{11} CFU, though further refinements of dosage and formulation are warranted, in conjunction with expanded trials to demonstrate safety, tolerability, and efficacy in at-risk populations. In preparation for future field testing, a follow-on study in healthy adults was recently initiated to evaluate the safety, tolerability, and immunogenicity of a new lyophilized formulation of ACE527, administered at a lower dose, with or without a mucosal adjuvant and according to a dosing schedule more appropriate for pediatric immunization schedules.

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