Safety and Immunogenicity of Two Different Lots of the Oral, Killed Enterotoxigenic Escherichia coli-Cholera Toxin B Subunit Vaccine in Israeli Young Adults

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Enterotoxigenic Escherichia coli (ETEC) is one of the leading causes of diarrhea among Israeli soldiers serving in field units. Two double-blind placebo-controlled, randomized trials were performed among 155 healthy volunteers to evaluate the safety and immunogenicity of different lots of the oral, killed ETEC vaccine consisting of two doses of whole cells plus recombinantly produced cholera toxin B subunit (rCTB). The two doses of vaccine lot E005 and the first dose of vaccine lot E003 were well tolerated by the volunteers. However, 5 (17%) vaccinees reported an episode of vomiting a few hours after the second dose of lot E003; none of the placebo recipients reported similar symptoms. Both lots of vaccine stimulated a rate of significant antibody-secreting cell (ASC) response to CTB and to colonization factor antigen I (CFA/I) after one or two doses, ranging from 85 to 100% and from 81 to 100%, respectively. The rate of ASC response to CS2, CS4, and CS5 was slightly lower than the rate of ASC response induced to CTB, CFA/I, and CSI. The second vaccine dose enhanced the response to CTB but did not increase the frequencies or magnitude of ASC responses to the other antigens. The two lots of the ETEC vaccine induced similar rates of serum antibody responses to CTB and CFA/I which were less frequent than the ASC responses to the same antigens. Based on these safety and immunogenicity data, an efficacy study of the ETEC vaccine is under way in the Israel Defense Force.

Globally, enterotoxigenic Escherichia coli (ETEC) is responsible for approximately 500 million diarrheal episodes resulting in more than 700,000 deaths annually (8). Most of these cases of morbidity and mortality occur in developing countries, among children below 5 years of age (8). In addition, ETEC is strongly associated with traveler’s diarrhea, characterized by watery diarrhea occurring in people traveling to regions with poorer sanitary conditions, and is isolated in 20 to 50% of these episodes (3, 16).

Traveler’s diarrhea caused by ETEC is an important medical problem for all military personnel on duty in the developing world or under field conditions where sanitation is inadequate. American troops experienced high rates of diarrheal diseases during various military operations in the Middle East (7, 18). Diarrhea rates of over 50% were experienced in some of the units under surveillance, making diarrhea one of the most important medical problems encountered during Operation Desert Shield/Storm. Bacteriological studies showed that a bacterial pathogen was isolated in 50% of troops with diarrhea. ETEC strains were isolated in 21%, Shigella species were isolated in 19%, and dual infections were found in 9%. Salmonellae and Campylobacter jejuni were isolated from less than 2% of troops (7).

ETEC is one of the most important non-Shigella enteropathogens involved in the etiology of diarrheal diseases occurring among Israeli soldiers serving in field units. In the summers of 1993 to 1996, during 5 to 6 months of follow-up of cohorts comprising 1,000 to 1,753 soldiers per year, the incidence of ETEC-associated diarrhea was in the range of 43 to 80 per 1,000 soldiers under follow-up, and the detection rate of ETEC in stool samples from diarrhea cases was in the range of 15 to 32% (D. Cohen, unpublished data). Adequate conditions of sanitation, food handling, and hygiene should be sufficient to prevent diarrhea. However, in military populations serving in field units it is frequently difficult, if not impossible, to provide such conditions. In such circumstances, effective vaccination may be the only reliable means to reduce the attack rate of ETEC-associated diarrhea.

An oral ETEC vaccine, consisting of formalin-killed E. coli expressing colonization factor antigen 1 (CFA/I) and the different coli surface (CS1, CS2, and CS3) antigen components of CFA/II and CFA/IV (CS4, CS5, and CS6), has been developed (14, 15). The enterotoxin component was provided as recombinantly produced cholera toxin B subunit (rCTB). The vaccine strains represented common ETEC O groups that express the different fimbriae in high concentrations. Formalin inactivation killed the bacteria without causing significant loss in antigenicity of the CFA (14). SBL Vaccine AB (Stockholm, Sweden) has manufactured different lots of the ETEC vaccine. Lot E001, tested in Swedish, Bangladeshi, and American adult volunteers, exhibited minimal reactivity and stimulated relevant mucosal immune responses (2, 9, 17). Lot E003, in which two of the five original ETEC component strains were replaced, showed safety and immunogenicity data comparable to those exhibited by the E001 lot, in studies carried out among adult volunteers in the United States, Sweden, and Egypt (A. Trofa, unpublished data) (9, 12). Before the initiation of an efficacy trial of the oral, killed
ETEC/crCTB vaccine among Israeli soldiers naturally exposed to ETEC infections, we performed two double-blind placebo-controlled, randomized trials to establish the safety and immunogenicity of lots E003 and E005 of this ETEC vaccine among the candidate populations for the efficacy trial. The results of these studies are presented in this paper.

MATERIALS AND METHODS

Vaccine and placebo composition. The ETEC/crCTB vaccine (lots E003 and E005) was produced by SBL Vaccin AB. One vaccine dose, suspended in 6.0 ml of phosphate-buffered saline (PBS), contained 5.5 × 10^{10} formalin-inactivated bacteria. Each vaccine dose included the following inactivated ETEC strains: SBL 101 (O78, CFA1, LT/ST²), SBL 106 (O6, C51, LT/ST²), SBL 107 (OR, C52, LT/ST²), SBL 104 (O25, CS4, CS6, LT/ST²), and SBL 105 (O167, CS5, CS6, LT/ST²). The concentration of CFA1 was slightly lower and the concentrations of CS2 and CS5+CS6 were slightly higher in lot E005 than in lot E003. Each dose of lots E003 and E005 was given in 150 ml of water with a raspberry-flavored bicarbonate-citric acid buffer containing 4 g of sodium bicarbonate per dose (Recip AB, Stockholm, Sweden). The placebo preparation, containing a suspension of heat-killed E. coli K-12 with an optical density (OD) equivalent to that of the ETEC vaccine, was administered in the same buffered solution as the vaccine.

Study population and procedures. The protocols, both placebo controlled, randomized, and double blind, were carried out between May 22 and July 10, 1995, and between April 1 and June 18, 1997, at the Israel Defense Force (IDF), Medical Corps, School of Military Medicine. The volunteers were healthy men and women and were recruited among the School of Military Medicine cadets or the Medical Corps Headquarters staff. Sixty-five volunteers (59 males and 6 females) were recruited in the first study, in which lot E003 of the ETEC vaccine was evaluated; 90 volunteers (87 males and 3 females) were enrolled in the second study, in which the E005 lot was examined. The ages of 53 (82%) of the volunteers in the first study ranged between 18 and 21 years, while the ages of the remaining 12 (18%) ranged between 23 and 43 years. The ages of the 90 volunteers in the second study ranged between 18 and 21 years with the exception of 3 volunteers, of whom 2 were 24 and 1 was 41 years old.

The volunteers underwent a general physical examination, and those fulfilling the enrollment criteria were randomly divided into vaccine and placebo groups. All 65 volunteers in the first study and all 90 volunteers in the second study received a first dose of vaccine or placebo, and 60 and 79 of them also received a second dose of lots E003 and E005, respectively, on day 14 (range, 13 to 15). Sixteen subjects either dropped out of the study or were not given the second dose on the basis of the exclusion criteria for dose 2. The specific reasons for nine volunteers not receiving the second dose were abdominal cramps in the 48 h preceding immunization (three volunteers), follicular tonsillitis and fever on the day following vaccination (two volunteers), and an entry error due to a dosing error for 3 days preceding the second-dose vaccination day (one volunteer), unavailability for follow-up due to a temporary transfer to another army base (three volunteers), and exclusion upon the safety monitoring committee’s recommendation (one volunteer).

Protection of human subjects. The protocols of the two studies were reviewed and approved by the IDF Medical Corps Committee for Research on Human Subjects, the IDF Surgeon General, the Human Subjects Research Review Board of the Defense Research Board, the Army Surgeon General, the U.S. Food and Drug Administration. Informed, witnessed, written consent was obtained after all aspects of the study protocols had been explained in depth to the volunteers.

Allocation of volunteers. After enrollment, subjects were randomized to receive vaccine or placebo in a double-blind fashion. In the first study, group randomization was used so that each group was assigned two letters, and each volunteer was opened at random to one of the four resulting letter groups. The association between a letter group and a vaccine/placebo group was determined by a computer and was kept locked from both volunteers and investigators for the duration of the study. The second study used individual randomization. A blocked randomization scheme was constructed off-site. Subjects were assigned a unique participant identification number (101 to 190) at the time of the first dose and received the corresponding study vaccine/placebo lot. The investigation team remained blinded until all safety and immunogenicity data were generated, computerized, cleaned, and locked.

Active surveillance for adverse experiences. In both studies, the subjects filled in a symptom diary which was measured their temperature every day for 4 days following the first and second dosings. They were required to report any unusual symptoms to the study physician and were interviewed and physically examined whenever necessary. Side effects assessed were loss of appetite, general malaise, headache, nausea, vomiting, abdominal cramps, abdominal gurgling, bloody stool, mucus stool, temperature (precise measurement with a thermometer given to each subject), and number of stools per day and their quality. Diarrhea was defined as three or more soft or liquid stools in any 24 h during the follow-up period. The definition of vomiting was defined as peroral excretion of stomach contents one or more times in the follow-up period. All symptoms were graded in the following scale: absent; very mild, mild, or moderate (with no impairment on normal activity); moderate-severe (with impairment on normal daily activities but not incapacitating); or severe (incapacitating and requiring medical care). When a complete report on presence or absence of symptoms or complaints in one or more days following up was missing from the symptom diary, the volunteers, data from the physician’s interview, when available, were added for that specific day.

Blood collection and processing. At days 0, 14, and 28 (range, 28 to 29) following vaccination with lot E003 and on days 0 and 28 (range, 27 to 30) following vaccination with lot E005, blood samples were obtained from volunteers to determine the humoral antibody response using enzyme-linked immunosorbent assay (ELISA). Sera were separated and kept frozen at −20°C until tested. Immunoglobulins and the two ETEC fimbrial antigens (CFA/I and CS1) were tested in pairs against two ETEC fimbrial antigens (CFA/I and CS1). At days 0, 7, and 21 following vaccination with lot E003 and at days 7 and 21 following vaccination with lot E005, additional blood samples were taken from a subset of volunteers to determine the level of antibody-secreting cell (ASC) responses to ETEC antigens (CFA/I, CFA/I, CS2, CS3, and CS5) after the first and second doses of the ETEC investigational vaccine.

Measurement of IgA ASCs to ETEC antigens. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples by Ficoll-Hypaque gradient centrifugation and finally suspended at a concentration of ~2 × 10^6 cells/ml in RPMI 1640 to 5% fetal calf serum. Specific immunoglobulin A (IgA) ASCs were measured using the ELISPOT technique (6, 17) with fresh MNC preparations. Briefly, individual wells of 96-well nitrocellulose-bottomed plates (Millipore Corporation, Bedford, Mass.) were incubated overnight at 4°C with 0.1 ml of PBS containing purified CFA/I (20 μg/ml), CS1 (20 μg/ml), CS4 (20 μg/ml), CS5 (20 μg/ml), or GM1 ganglioside (5 μg/ml; Sigma, St. Louis, Mo.). After washing PBS, the GM1-coated wells were incubated with rCTB (2.5 μg/ml) in PBS for 2 h. Following washing with PBS, the wells were blocked for 1 h at 37°C with RPMI-5% 1640 fetal calf serum. Thereafter, 50 μl of MNC suspension (10^6 cells) was added to each well, and the plates were incubated for 3 h at 37°C in 7.4% CO₂. Plates were then washed with PBS and incubated for 5 min with 100 μl of 10% H₂O₂ in PBS. After additional washing with PBS-0.05% Tween (PBS-T), the plates were incubated overnight at 4°C with goat anti-human IgA conjugated to horseradish peroxidase (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) and finally developed with H₂O₂ and 3-amin-9-ethylcarbazole chromogen substrate (Sigma). Spots, corresponding to zones of antibodies secreted by individual cells, were enumerated in triplicate wells under low magnification (×40), and data were adjusted to the number of spot-forming cells per 10^6 MNCs.

ELISPOT detection of serum antibodies to ETEC antigens. Sera were added to plates coated with ELISA as previously described (5), with some modifications. Briefly, 96-well polystyrene microtiter plates (model 3590; Costar, Cambridge, Mass.) were incubated for 1 h at 37°C coated with coating buffer (0.05 M carbonate buffer/0.02 M CaCl₂/0.02 M MgCl₂, pH 9.6; 100 μl/well; 1:250 dilution). For the analysis of anti-CTB antibodies, plates were incubated for 2 h at 37°C with PBS containing 1% gelatin (1.5 μg/ml; Sigma), washed twice with PBS-T, and incubated for additional 2 h at 37°C with coating buffer containing CFA/I and CS1 (1:250 dilution). After the coating buffer was blocked for 1 h at 37°C with PBS supplemented with casein and bovine serum albumin (each at 5 gl). The plates were then washed twice in washing buffer. Sera were added to the wells in eight twofold dilutions in blocking buffer, starting at 1:50, and the plates were incubated overnight at room temperature. After four further washings, goat anti-human IgA or IgG conjugated to alkaline phosphatase (Kirkegaard & Perry Laboratories, diluted 1:500 in blocking buffer, was added to the plates. The plates were incubated overnight at room temperature and washed four times, and ELISA was completed by the addition of enzyme-substrate solution containing p-nitrophenylphosphate (1 mg/ml; Sigma) in diethanolamine buffer (pH 9.8). The reactions were terminated with 3 M NaOH, and OD was read at 405 nm with an automatic ELISA Bio-Kinetics EL340 reader (Bio-Tek Instruments, Winoski, Vt.). Control sera were included in every microwell to allow the OD to be adjusted. The calculated cutoff values for a positive ASC response were ≥2 spots/10^5 cells in the first study and ≥1 spot/10^5 cells in the second study.

Statistical analyses. All subjects who received a dose of study agent were included in the analysis of postdosing reactogenicity. Differences in proportions of reactions and differences in ASC response rates between the two treatment groups were compared by Fisher’s exact test. Differences were considered significant at a p value of <0.05. No adjustments for multiple comparisons were made, and therefore the probability
vomited after receiving the second dose of the vaccine; none of the vaccinees complained of nausea, and five of these were detected between vaccinees and placebo recipients after differences in reported gastrointestinal or other symptoms compared to two placebo recipients (Table 1). Ten of the vaccinees (eight of whom also reported nausea and/or vomiting) complained of malaise following the second vaccination, eight of whom also reported nausea and/or vomiting. They reported interference with their regular activities, but this was of limited duration and ceased following vomiting.

The majority of the volunteers who were given the two doses of vaccine or placebo either had no complaint or had minor symptoms which did not impair normal daily activities. Six (13%) of the vaccinees and 2 (4%) of the placebo recipients reported symptoms which temporarily impaired their normal activities after the first dose. However, none of these volunteers sought medical assistance due to these complaints. Follow-up after the second dose revealed similar complaints in one vaccinee and two placebo recipients.

### Immunogenicity of the ETEC vaccine (lots E003 and E005).

#### (i) ASC response. The ASC response before vaccination at (day 0) was examined in 10 volunteers receiving lot E003 of the ETEC vaccine and in 10 placebo recipients. Minimal numbers of spots were detected against CS1 (5 spots/10⁶ MNC) and CS4 (3 spots/10⁶ MNC) in the blood samples of 1 of the 10 vaccinees and against CS2 (4 spots/10⁶ MNC) in blood samples of 1 of the 10 placebo recipients. All blood samples from both vaccinees and placebo recipients were negative when tested against CTB and CFA/I.

Table 3 displays the rate and magnitude of the ASC responses to CTB and fimbrial antigens following vaccination with the two vaccine lots. The rate and magnitude of the ASC response induced by CTB and CFA/I were similar following vaccination with the two different lots. Both lots of vaccine induced significant anti-CTB and anti-CFA/I responses after the first dose, ranging from 85 to 90% and from 81 to 100%, respectively, with similar geometric mean (GM) numbers of spots (Table 3). A higher ASC response against CS1 was observed following vaccination with lot E005 than after administration of lot E003, after both the first and second immunizations. The difference was statistically significant after the second dose, when the rate of ASC response and the GM of spots per 10⁶ mononuclear cells were 82% and 3.0 for lot E005, compared to 32% responders and GM of 0.7 spots per 10⁶ cells for lot E003 (P = 0.01) (Table 3). The ASC response to CS2, CS4, and CS5 was examined only in the first study following vaccination with the E003 lot. The rates of significant ASC response to CS2, CS4, and CS5, respectively, were 73, 53, and 45% after the first dose of vaccine and 32, 32, and 31% after the second dose.

Two vaccinees in the first study and two in the second study, who were nonresponders for CTB after the first dose of lots E003 and E005, respectively, showed a significant ASC response after the second dose of the ETEC vaccine. The enhanced response to CTB after the second dose of both vaccine lots is shown in Table 4.

### RESULTS

#### Safety of the ETEC vaccine (i) Lot E003. No significant differences in reported gastrointestinal or other symptoms were detected between vaccinees and placebo recipients after the first dose of vaccine or placebo (Table 1). However, nine (30%) of the vaccinees complained of nausea, and five of these vomited after receiving the second dose of the vaccine; none of the placebo recipients reported similar symptoms (P = 0.002 for nausea and P = 0.052 for vomiting) (Table 1). Ten of the vaccinees (eight of whom also reported nausea and/or vomiting) complained of malaise following the second vaccination, compared to two placebo recipients (P = 0.012). Four of the five vaccinees who complained of vomiting and seven of the nine vaccinees who complained of nausea reported the onset of these symptoms during the day they received the second dose. All of these complaints were of short duration, most of them were defined by the volunteers as very mild or mild, and none of them incapacitated. There was no clustering of the vaccinees reporting malaise, nausea, and vomiting in one of the two different bases. None of the 65 volunteers visited the unit clinic due to any gastrointestinal symptom occurring during the 4 days following ingestion of the first and second doses of vaccine or placebo. None of the volunteers complained of any severe symptom during the follow-up periods (Table 1).

(ii) Lot E005. No statistically significant differences in reported gastrointestinal or other symptoms were detected between vaccinees and placebo recipients after the first and second doses of vaccine or placebo (Table 2). After the first dose, however, a greater percentage of vaccinees (67%) experienced any symptom compared with the placebo recipients (47%), but the difference did not reach statistical significance (P = 0.056). No such difference was found following the second dose. None of the subjects in either group reported fever at any time during the study. None of the vaccinees reported diarrhea after the first or second vaccine dose. Two subjects in the placebo group reported passage of three loose or liquid stools in one of the 4 days of follow-up after the first dose of placebo.

Two of the subjects receiving the first dose of vaccine vomited on the day of vaccination, compared with none in the placebo group. Both of these vaccinees vomited once, 4 and 6 h after dosing. They reported interference with their regular daily activities, but this was of limited duration and ceased following vomiting.

The majority of the volunteers who were given the two doses of vaccine or placebo either had no complaint or had minor symptoms which did not impair normal daily activities. Six (13%) of the vaccinees and 2 (4%) of the placebo recipients reported symptoms which temporarily impaired their normal activities after the first dose. However, none of these volunteers sought medical assistance due to these complaints. Follow-up after the second dose revealed similar complaints in one vaccinee and two placebo recipients.

#### Immunogenicity of the ETEC vaccine (lots E003 and E005).

(i) ASC response. The ASC response before vaccination (at day 0) was examined in 10 volunteers receiving lot E003 of the ETEC vaccine and in 10 placebo recipients. Minimal numbers of spots were detected against CS1 (5 spots/10⁶ MNC) and CS4 (3 spots/10⁶ MNC) in the blood samples of 1 of the 10 vaccinees and against CS2 (4 spots/10⁶ MNC) in blood samples of 1 of the 10 placebo recipients. All blood samples from both vaccinees and placebo recipients were negative when tested against CTB and CFA/I.

Table 3 displays the rate and magnitude of the ASC responses to CTB and fimbrial antigens following vaccination with the two vaccine lots. The rate and magnitude of the ASC response induced by CTB and CFA/I were similar following vaccination with the two different lots. Both lots of vaccine induced significant anti-CTB and anti-CFA/I responses after the first dose, ranging from 85 to 90% and from 81 to 100%, respectively, with similar geometric mean (GM) numbers of spots (Table 3). A higher ASC response against CS1 was observed following vaccination with lot E005 than after administration of lot E003, after both the first and second immunizations. The difference was statistically significant after the second dose, when the rate of ASC response and the GM of spots per 10⁶ mononuclear cells were 82% and 3.0 for lot E005, compared to 32% responders and GM of 0.7 spots per 10⁶ cells for lot E003 (P = 0.01) (Table 3). The ASC response to CS2, CS4, and CS5 was examined only in the first study following vaccination with the E003 lot. The rates of significant ASC response to CS2, CS4, and CS5, respectively, were 73, 53, and 45% after the first dose of vaccine and 32, 32, and 31% after the second dose.

Two vaccinees in the first study and two in the second study, who were nonresponders for CTB after the first dose of lots E003 and E005, respectively, showed a significant ASC response after the second dose of the ETEC vaccine. The enhanced response to CTB after the second dose of both vaccine lots is shown in Table 4.

### TABLE 1. Self-reported symptoms during 4 days after the first and second doses of oral ETEC vaccine (lot E003) or placebo

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Dose 1 Placebo (n = 31)</th>
<th>Dose 1 Vaccine (n = 33)</th>
<th>Dose 2 Placebo (n = 29)</th>
<th>Dose 2 Vaccine (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>16 (55)</td>
<td>21 (64)</td>
<td>15 (52)</td>
<td>16 (53)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2 (7)</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fever</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Abdominal cramps</td>
<td>8 (26)</td>
<td>12 (36)</td>
<td>7 (24)</td>
<td>8 (27)</td>
</tr>
<tr>
<td>Nausea</td>
<td>5 (16)</td>
<td>4 (12)</td>
<td>0 (0)</td>
<td>9 (30)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>5 (17)</td>
</tr>
<tr>
<td>Headache</td>
<td>8 (26)</td>
<td>12 (36)</td>
<td>6 (21)</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Malaise</td>
<td>5 (16)</td>
<td>8 (24)</td>
<td>2 (7)</td>
<td>10 (33)</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>2 (7)</td>
<td>6 (18)</td>
<td>2 (7)</td>
<td>4 (13)</td>
</tr>
</tbody>
</table>

Note: *P* (0.002) and # (0.021) by Fisher exact test; all the other differences were not statistically significant.

#### TABLE 2. Self-reported symptoms during 4 days after the first and second doses of oral ETEC vaccine (lot E005) or placebo

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Dose 1 Placebo (n = 45)</th>
<th>Dose 1 Vaccine (n = 45)</th>
<th>Dose 2 Placebo (n = 41)</th>
<th>Dose 2 Vaccine (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>21 (47)</td>
<td>30 (67)</td>
<td>16 (39)</td>
<td>12 (32)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2 (4)</td>
<td>12 (27)</td>
<td>4 (10)</td>
<td>4 (11)</td>
</tr>
<tr>
<td>Fever</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Abdominal cramps</td>
<td>8 (18)</td>
<td>15 (33)</td>
<td>4 (10)</td>
<td>7 (19)</td>
</tr>
<tr>
<td>Nausea</td>
<td>5 (11)</td>
<td>7 (16)</td>
<td>2 (5)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0 (0)</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Headache</td>
<td>10 (22)</td>
<td>15 (33)</td>
<td>7 (17)</td>
<td>7 (19)</td>
</tr>
<tr>
<td>Malaise</td>
<td>6 (13)</td>
<td>12 (27)</td>
<td>4 (10)</td>
<td>4 (11)</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>5 (11)</td>
<td>10 (22)</td>
<td>3 (7)</td>
<td>2 (5)</td>
</tr>
</tbody>
</table>
lots is also reflected by the higher GM number of spots counted in the vaccinees on day 21 than on day 7 (Table 3). No additional responders to CFA/I or CS1 were detected following the second dose, and no boosting of the ASC response was observed in those volunteers who showed a significant response after the first dose of vaccine (Table 3).

In both studies, none to three of the placebo recipients showed positive ASC responses to CTB and/or fimbrial antigens after either the first or the second placebo dose. Except for one placebo recipient who vigorously responded to CS2 on day 21, only minimal numbers of spots were detected in the placebo recipients. All blood samples included in the immunogenicity evaluation were negative when tested against bovine serum albumin (negative control antigen) and positive against goat anti-human IgG F(ab\(^9\))\(_{2}\) (positive control antigen).

**Serum antibody response.** Serum samples obtained from vaccinees and placebo recipients on days 0, 14, and 28 were tested for IgG and IgA antibodies against CTB, CFA/I, and CS1. In both studies, the rates of significant serum antibody response and particularly those to the fimbrial antigens were lower than the corresponding ASC responses. A significant IgG and IgA anti-CTB response after either the first or second dose of lot E003 was detected in 82 and 50% of the vaccinees. A 71% response for IgG and a 69% response for IgA to CTB were detected among vaccinees in the second study (Table 4). The GM titers (GMT) of IgA and IgG to CTB rose from 51.6 to 144.6 and from 217.0 to 1,893, respectively, after the administration of two doses of lot E005. Two doses of lot E005 induced a rise in GMTs to CFA/I from 78.7 to 160.7 for IgA and from 231.7 to 441.0 for IgG. The serum antibody responses to CS1 were examined only after vaccination with lot E005, and the rates of significant response were 26% for IgG and 31% for IgA (Table 4). After two doses of lot E005, the GMTs for IgA and IgG to CS1 rose from 113.7 to 254.0 and from 372.7 to 676.7, respectively. A very few placebo recipients, two in the first study and two in the second study, showed a rise of 2.5-fold or higher in the specific antibodies to CTB, CFA/I, or CS1 (Table 4).

We examined the correlation between significant IgA ASC and serum IgA responses to CTB and CFA/I after either one or two doses of the ETEC vaccine (both lot E003 and lot E005) among vaccinees or placebo recipients. Of 35 subjects with a significant IgA ASC response to CTB, 22 (63%) also had a significant rise in serum IgA, while 2 of 33 subjects with no ASC response to CTB showed significant serum IgA (P < 0.01). Of 22 subjects with a significant IgA ASC response to CFA/I, 14 (64%) also had a significant rise in serum IgA to the same antigen, while none of 26 subjects with no ASC response to CFA/I showed significant serum IgA response to the same antigen (P < 0.01).

**DISCUSSION**

This study presents data on the immunogenicity and reactogenicity of two lots of the oral ETEC vaccine evaluated among young and healthy volunteers located in a region where ETEC infection is endemic. The two studies described above

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**TABLE 3.** ASC response to CTB and fimbrial antigens of two lots of ETEC vaccine

<table>
<thead>
<tr>
<th>Antigen</th>
<th>ETEC lot E003</th>
<th>ETEC lot E005</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After dose 1</td>
<td>After dose 2</td>
</tr>
<tr>
<td></td>
<td>Frequency (%)</td>
<td>GM no. of spots</td>
</tr>
<tr>
<td>CTB</td>
<td>19/21 (90)*</td>
<td>11.4</td>
</tr>
<tr>
<td>CFA/I</td>
<td>17/21 (81)*</td>
<td>13.3</td>
</tr>
<tr>
<td>CS1</td>
<td>11/15 (73)*</td>
<td>5.7</td>
</tr>
<tr>
<td>CS2</td>
<td>11/15 (73)*</td>
<td>2.0</td>
</tr>
<tr>
<td>CS4</td>
<td>8/15 (53)*</td>
<td>2.3</td>
</tr>
<tr>
<td>CS5</td>
<td>5/11 (45)*</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* significantly higher than the rate among placebo recipients (P < 0.01); † significantly higher than the rate among recipients of the second dose of lot E003 (P = 0.01); ND, Not done.

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**TABLE 4.** Frequency of volunteers with 2.5-fold or higher IgG and IgA response to CTB and fimbrial antigens following administration of either of the two doses of the ETEC vaccine or E. coli K-12 placebo

<table>
<thead>
<tr>
<th>Antigen</th>
<th>ETEC lot E003</th>
<th>E. coli K-12</th>
<th>ETEC lot E005</th>
<th>E. coli K-12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (%)</td>
<td>Frequency (%)</td>
<td>Frequency (%)</td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>CTB</td>
<td>23/28* (82)</td>
<td>14/28* (50)</td>
<td>1/26 (4)</td>
<td>2/23 (9)</td>
</tr>
<tr>
<td>CFA/I</td>
<td>7/18 (39)</td>
<td>8/18* (44)</td>
<td>1/12 (8)</td>
<td>1/12 (8)</td>
</tr>
<tr>
<td>CS1</td>
<td>ND</td>
<td>ND</td>
<td>9/35* (26)</td>
<td>11/35* (31)</td>
</tr>
</tbody>
</table>

* Significant higher (P < 0.01) and †(P < 0.05), significantly higher than the corresponding rate among placebo recipients.

b Determined only on day 28, therefore, the comparisons for E005 are just between days 0 and 28.

ND, Not done.
were carried out using the same methodology and in similar
groups of volunteers.

The results of the two studies demonstrate that lots E003 and
E005 of the ETEC vaccine were well tolerated by Israeli
adult volunteers. None of the volunteers who received the two
lots complained of diarrhea during the follow-up periods after
dosing. An excess of nausea, vomiting, and malaise was re-
ported by the vaccinees who received a second dose of lot E003
in 1995 but not after the first dose. These symptoms appeared
very few hours after ingestion of the vaccine, were of short
duration, and did not interfere with the daily activities of the
volunteers or otherwise incapacitate them. The excess of these
specific symptoms after the second dose of vaccine lot E003
remains an unusual finding. It did not recur following vaccina-
tion of a similar population with either of the two vaccine doses
of lot E005, nor had it been reported in any of the other studies
of the killed ETEC/rCTB vaccine carried out among volun-
tees living in countries of low and high endemicity for ETEC
infections (9, 12–14).

The findings of controlled phase 2 studies of the various
ETEC/rCTB vaccine lots carried out in Sweden, the United
States, and Egypt also indicated a good safety profile for this
vaccine in adults and children (9, 12, 13) (Trofa, unpublished).
The mild postdosing gastrointestinal symptoms such as diar-
rhea and vomiting occurring with similar frequencies among
both treatment groups were attributed to receipt of the antacid
vehicle at a relatively high concentration (9, 12, 13). Sanchez et
al. have shown in a phase 2 study of the oral, whole cell/rCTB
vaccine in North American volunteers that the bicarbonate
buffer alone, at the concentration needed to protect the rCTB
against the stomach acidity, may cause mild gastrointestinal
symptoms (11). The possibility that the mild symptoms in the
placebo group could be due to some minimal reactogenicity of E.
coli K-12 was ruled out by the extensive data demonstrating
the safety of this control (4).

The extent of the immune stimulation of the intestinal mu-
cosa by the ETEC vaccine was assessed by measuring circulat-
ing B-cell responses to fimbrial antigens and CTB. It has pre-
viously been shown that the ASC correlates with immune
response measured in intestinal lavage fluid (2). The rate and
magnitude of ASC responses induced by CTB and CFA/I were
similar following vaccination with the two different lots. Both
lots of vaccine mounted a rate of significant anti-CTB and
anti-CFA/I response after one or two doses, ranging from 85 to
100% and from 81 to 100%, respectively. CS1 induced a higher
ASC response following vaccination with lot E005 than with lot
E003, which may be explained by a somewhat higher concen-
tration of CS1 in lot 005 than in lot 003. This difference
reached statistical significance after the second dose. The rate
of ASC response to CS2, CS4, and CS5 was examined only
after vaccination with lot E003 and was lower than the ASC
response induced by CTB and CFA/I.

The rate and magnitude of the ASC response to CTB and
fimbrial antigens induced by the two vaccine lots examined in
our study were similar to those elicited by the ETEC vaccine
among adults and children in both countries where ETEC
infection is endemic and countries where it is not (9, 12, 13, 17)
as well as to the ASC response mounted in young Israeli
recruits after ETEC-associated diarrhea (N. Orr, unpublished
data).

As demonstrated in both studies by the ASC results on day
21, a second dose of vaccine enhanced the ASC response to
CTB but not to the other antigens examined. The same ob-
ervation was reported by the other phase 2 studies carried out in
a region where ETEC infection is endemic among adults and
children aged 6 to 12 (12, 13). Savarino et al. showed, however,
that in preschool children, the magnitude of anti-CFA/I and
anti-CS2 IgA ASC responses tended to be similar or greater
after the second than after the first dose (13). The same au-
authors assume that these discrepancies in the ASC response to
the fimbrial antigens following the second dose of the ETEC
vaccine may reflect the different degrees of priming from re-
peated exposures to natural ETEC infection in the various age
groups living in regions of endemicity (13). If this assumption
is true, then in an adult population living in such a region, a
single dose of the ETEC vaccine might be sufficient to induce
adequate mucosal stimulation. It has also been reported that
ASC responses are lower in subjects with high preexistent ASC
levels at the time of immunization or boosting (10, 13). It
remains unclear whether the second dose of vaccine does or
does not contribute to the immunological memory against fimb-
rial ETEC antigens. A decision as to the number of vaccine
doses needed will have to rely on the accumulation of addi-
tional data on the correlation between the peripheral B-cell
responses and immune responses in intestinal secretions on
one hand, and, protection in humans against natural or induced
infections caused by ETEC bearing vaccine-expressed antigens
on the other hand.

The two lots of the ETEC vaccine induced similar rates of
serum antibody responses to CTB and CFA/I. The rate of
significant serum antibody response to these antigens and to
CS2 in our study was similar to that observed following immu-
nization of Swedish volunteers but lower than that determined
among Egyptian children and adults (1, 9, 13). It is important
to mention that different cutoff values to define significant rise
in antibody levels between pre- and postvaccination specimens
were used in the present study compared with the other studies
(≥2.5- versus ≥2-fold increase, respectively) (2, 9, 13). The
rates of serum response to CTB and CFA/I represented only
about 64% of the peripheral B-cell responses to the same
antigens, suggesting that the local immune response to the
ETEC antigens is only partially reflected in significant rises in
serum antibodies. The pattern of the serum antibody response
to CTB and CFA/I following the first and second vaccine doses
was similar to the pattern of the ASC response to the same
antigens. If the ASC response is considered the “gold stan-
dard” in these comparisons, it clearly appears that at a sensi-
tivity of 64% there are very few or no false positives among
the serum responders to CTB and CFA/I, as only 2 of 33 subjects
with no ASC response to CTB and none of 26 subjects with no
ASC response to CFA/I showed significant serum IgA re-
sponses to the same antigen (positive predictive values of 94% for
the IgA serum antibody response to CTB and of 100% for
the IgA serum antibody response to CFA/I). The circulating
B-cell responses to specific ETEC fimbrial antigens remain at
this stage the primary measure of the immunogenicity of the
vaccine, since these have been shown to reflect immune re-
sponses in intestinal lavage (1, 2, 14). At the level of specificity
described above, and with further improvement of its sensitiv-
ity, the serum antitoxin and antifimbrial detection might serve
as an alternative to the ASC response detection under circum-
stances in which the measurement of the ASC response is not
feasible due to a limited amount of blood drawn (e.g., among
infants) or to the lack of a suitable infrastructure to perform
such analyses on a large scale.

In summary, the two lots of the ETEC vaccine (E003 and
E005) showed a satisfactory safety profile and similar levels of
immunogenicity among young Israeli adults. The second vac-
cine dose enhanced the response to CTB obtained after the
first dose but did not increase the extent of ASC response to
the other antigens. The good ASC response to CTB and CFA/I
was only partially reflected in significant rises in serum anti-
bodies. The safety and immunogenicity data presented above support further evaluation of the protective efficacy of the ETEC vaccine in a phase 3 study among Israeli soldiers exposed to ETEC natural infection while serving under field conditions.

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