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 choler 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 choler 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 reactogenicity 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/rCTB 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/rCTB 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 a total of 4.8 × 10^10 formalin-inactivated bacteria. Each vaccine dose included the following inactivated ETEC strains: BBL 101 (O78, CFA/I, LT^-/ST^-), BBL 106 (O6, CS1, LT^-/ST^-), BBL 107 (OR, CS2, CS^-/ST^-), BBL 104 (O25, CS4^-/ST^-), and BBL 105 (O167, CS5^-/ST^-). The concentration of CFA/I was slightly lower and the concentrations of CS2 and CS5^- 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 slightly higher in lot E005 than in lot E003. Each dose of lots E003 and E005 was separated in the same buffered solution as the vaccine.

Study population and procedures. The trials, 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, Health Branch Research Unit, and the 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 range 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 tissons and fever on the day of immunization (three volunteers), antibiotic treatment due to acne in the 5 days preceding the second dose (one volunteer), unavailability for follow-up due to a temporary transfer to another army base (two 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 U.S. Army Surgeon General, and 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 openly allotted to one of the four resulting letter groups. The association between a letter group and a vaccine/placebo group was determined by a third party 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 vaccine agent. 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 card, which was measured their temperature every day and kept on 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 thermom- etor 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 in the vaccine and placebo recipients 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 of the 4 days of follow-up was missing from the symptom cards, data from the volunteer's 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. Results were against two ETEC antigens (CTB, CFA/I). 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 (CTB, CFA/I, CS2, CS4, and CS5) after the first and second doses of the ETEC investigational vaccine.

Measurement of IgA ASCs to ETEC antigens. Peripheral blood mononuclear cells (MNCs) 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), CS2 (20 μg/ml), CS4 (20 μg/ml), CS5 (20 μg/ml), or GM1 ganglioside (5 μg/ml; Sigma, St. Louis, Mo.). After washing in PBS, the GM1-coated wells were incubated with rCTB (2.5 μg/ml) in PBS for 2 h at 37°C. Following the antigen washings they 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 15 min with 100 μl of 0.1% H2O2 in PBS. After addition of 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-amino-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.

A positive ASC response to a specific antigen in both vaccinees and placebo recipients was defined as one with a value greater than the mean ± 2 standard deviations found in the placebo group against the same antigen. The ASC values in the placebo group included in this calculation comprised the pooled ASC values measured among volunteers at different times (days 0, 7, and 21) before or after receiving the placebo. The calculated cutoff values for a positive ASC response were 2 spots/10^6 cells in the first study and ≥1 spot/10^6 cells in the second study.

Serum antibodies to ETEC antigens. Serum samples were collected on days 0, 14, and 28 following vaccination with each vaccine. Sera were included in the analysis of postdosing reactogenicity. Differences in proportions were analyzed with the Fisher's exact test (20). Differences in the antibody responses to ETEC antigens were analyzed with the Student's t test (21) or Wilcoxon's rank sum test (22) when appropriate. A p value of <0.05 was considered statistically significant.
of false statistical significance might be in this case increased beyond the ordinary 0.05 level.

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 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/10^6 MNC) and CS4 (3 spots/10^6 MNC) in the blood samples of 1 of the 10 vaccinees and against CS2 (4 spots/10^6 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 10^6 mononuclear cells were 82% and 3.0 for lot E005, compared to 32% responders and GM of 0.7 spots per 10^6 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

### 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 (n = 31)</th>
<th>Dose 2 (n = 33)</th>
<th>Placebo (n = 41)</th>
<th>Vaccine (n = 45)</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>1a (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>

a (P = 0.002) and # (P = 0.021) by Fisher exact test; all other differences were not statistically significant; b 38.0°C reported on day 2 after dosing.

### 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 (n = 45)</th>
<th>Dose 2 (n = 45)</th>
<th>Placebo (n = 41)</th>
<th>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>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</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 GMT titers (GMT) of IgA and IgG to CFA/I 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 35.6 to 73.6 and from 263.3 to 437.3, respectively, after the administration of two doses of lot E003. 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/rCTB vaccine evaluated among young and healthy volunteers located in a region where ETEC infection is endemic. The two studies described above

<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>CTB</td>
<td>19/21 (90)*</td>
<td>17/21 (81)*</td>
<td>11/13 (85)*</td>
<td>7.0</td>
</tr>
<tr>
<td>CFA/I</td>
<td>17/21 (81)*</td>
<td>13.3</td>
<td>11/13 (100)*</td>
<td>9/11 (82)*</td>
</tr>
<tr>
<td>CS1</td>
<td>11/15 (73)*</td>
<td>5.7</td>
<td>7/22 (32)*</td>
<td>0.7</td>
</tr>
<tr>
<td>CS2</td>
<td>11/15 (73)*</td>
<td>2.0</td>
<td>7/22 (32)*</td>
<td>0.8</td>
</tr>
<tr>
<td>CS4</td>
<td>8/15 (53)*</td>
<td>2.3</td>
<td>7/22 (32)*</td>
<td>0.3</td>
</tr>
<tr>
<td>CS5</td>
<td>5/11 (45)*</td>
<td>2.0</td>
<td>4/13 (31)</td>
<td>0.3</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.

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

**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 5. Antigenic responses and serum antibody titers to CTB and fimbrial antigens of two lots of ETEC vaccine**
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 period after dosing. An excess of nausea, vomiting, and malaise was reported 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 vaccination 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 volunteers 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 diarrhea 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 mucosa by the ETEC vaccine was assessed by measuring circulating B-cell responses to fimbrial antigens and CTB. It has previously 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 concentration 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 observation 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 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 repeated 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 fimbrial ETEC antigens. A decision as to the number of vaccine doses needed will have to rely on the accumulation of additional 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 immunization 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 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 standard” in these comparisons, it clearly appears that at a sensitivity 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 responses 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 responses in intestinal lavage (1, 2, 14). At the level of specificity described above, and with further improvement of its sensitivity, the serum antitoxin and antifimbrial detection might serve as an alternative to the ASC response detection under circumstances 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 vaccine 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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