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Construction of a Potential Live Oral Bivalent Vaccine for Typhoid Fever and Cholera-*Escherichia coli*-Related Diarrheas

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We used the *Salmonella typhi* galactose epimerase (*galE*) mutant strain Ty21a, shown to be a safe, effective, living, attenuated oral typhoid vaccine, as a recipient for a recombinant plasmid containing the gene for production of the nontoxic B subunit of the heat-labile enterotoxin of *Escherichia coli*. The *S. typhi* derivative, strain SE12, produced heat-labile enterotoxin subunit B that was structurally and immunologically indistinguishable from heat-labile enterotoxin subunit B produced by strains of *E. coli* harboring the same plasmid. Tests in mice and guinea pigs showed that strain SE12 was safe when given orally and was capable of inducing a significant antitoxic antibody response when injected parenterally. Moreover, it retained the galactose sensitivity of the parent strain, preserving its utility as a typhoid vaccine. This strain may prove to be a useful live oral bivalent vaccine strain for typhoid fever and cholera-*E. coli*-related diarrheas.

The extensive diarrhea of cholera results from the elaboration of a potent exo-enterotoxin which causes the activation of adenylate cyclase and a subsequent increase in intracellular levels of cyclic AMP. This enterotoxin (choleragen) is an 84,000-dalton polymeric protein composed of two major, noncovalently associated, immunologically distinct subunits designated A and B (11). Of these, the 56,000-dalton B subunit is responsible for the binding of the toxin to the host cell receptor (12). The B subunit is nontoxic and appears to be the immunodominant moiety of the holotoxin (17, 28, 35).

A variety of cholera vaccines have been developed since the identification of the causative organism by Koch (recently reviewed by Levine et al. [28]), the most widely used being parenterally administered killed whole-cell vaccines. Unfortunately, these vaccines give rise to only limited protection of short duration (10). A number of toxoid vaccines have been proposed, but have never been proven to be safe or effective. Formalin toxoid, although capable of eliciting antitoxin antibodies, reverted to toxicity (33), whereas glutaraldehyde toxoid was stable but poorly antigenic (9). Subunit toxoid vaccines (B subunit toxoid) have also been proposed, and Svennerholm et al. (38) have found increasing titers of local intestinal secretory immunoglobulin A antitoxin after oral or intramuscular immunization with cholera toxin subunit B (CT-B). In the most recent efforts to develop an effective immunoprophylactic for cholera, researchers have used mutagenesis (1, 13, 18) or recombinant DNA technology (19, 20, 30) to construct strains of *Vibrio cholerae* genetically incapable of producing the toxic A fragment of the enterotoxin. Some of these strains, when studied in human volunteers, have provided a degree of protection against challenge with *V. cholerae*, with an overall reduction in the occurrence of diarrhea (27).

A number of enteric pathogens, principally enterotoxigenic *Escherica coli*, have been shown to produce a plasmid-mediated heat-labile enterotoxin (LT) which is immunologically related to choleragen (3, 4). The role of antitoxic immunity in protection against diarrhea caused by these organisms has never been clearly established. Levine et al. (29) have shown that in volunteers, previous disease caused by LT-producing *E. coli* does not protect against challenge with a heterologous LT-producing strain; and Svennerholm and Åhrén (37) have shown that passive protection in rabbits is improved when antibacterial antibodies are included with antitoxic antibodies. Other studies have shown that immunization with LT or the B subunit of LT (LT-B) can stimulate production of mucosal secretory antitoxic immunoglobulin A (23) and that these antitoxic antibodies are protective (22–24). Moreover, Klipstein et al. (25) have shown that immunization with LT-B raises an antitoxin response that provides protection against LT or CT, whereas immunization with CT-B induces much weaker cross-protection. These results indicate that LT-B may be a more effective immunoprophylactic against cholera and enterotoxigenic *E. coli* than CT-B.

It is against this background that we constructed a potential live oral vaccine for cholera- and *E. coli*-related diarrhea. As a vehicle, we used the galactose epimeraseless (*galE*) mutant of *Salmonella typhi* (15), which has been shown to be a safe, highly effective, live, oral vaccine for typhoid fever (16, 42). This strain, *S. typhi* Ty21a, has been used by Formal et al. (14) to construct a potential bivalent dysentery-typhoid vaccine, whose safety and antigenicity have recently been demonstrated in volunteers (40). This communication describes the construction and characterization of an *S. typhi* Ty21a derivative containing a plasmid that codes for *E. coli* LT-B.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used were *E. coli* K-12 derivatives MM294 *hsdR thi hsdM*$^+$ *endA*$^+$ (ATCC 33625) and JM83 *ara* Δ*lac-pro strA thi* ϕ80*dlacZ* ΔM15 (kindly provided by M. Corb, Tulane University School of Medicine, New Orleans, La.); *S. typhi* Ty21a, a galactose epimeraseless (*galE*) mutant of *S. typhi* (kindly provided by S. Formal, Walter Reed Army Institute of Research, Washington, D.C.); and *S. typhi* SE12, an *S. typhi* Ty21a derivative transformed with a 3.5-kilobase (kb) plasmid which contains the gene for production of *E. coli* LT-B (this study). Plasmid pDF87 is a pBR322 derivative containing the gene for production of LT-B (6, 7). Plasmid pUC8, the M13-derived cloning vector (41), was obtained from Bethesda Research Laboratories, Inc., Gaithersburg, Md.

Isolation of plasmid DNA. Plasmid DNA was isolated by the procedure of Bolivar and Backman (2).

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Restriction endonuclease digestion. Restriction endonucleases were obtained from Bethesda Research Laboratories, Inc. Reactions were carried out as prescribed by the manufacturer.

Electrophoresis. Agarose gel electrophoresis was performed on 1% vertical slab gels in 0.04 M Tris–0.2 M sodium acetate–0.002 M EDTA, pH 7.8 (2). Phage λ DNA fragments generated by *Hin*dIII digestion were used as molecular weight standards. Low-melting-point agarose gel electrophoresis was performed in the same Tris-acetate buffer in 1.2% low-melting-point agarose (Bethesda Research Laboratories, Inc.) overlaid with 0.9% agarose in a vertical slab gel. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the technique of Laemmli (26).

Ligation. Ligation reactions were carried out in 66 mM Tris-hydrochloride (pH 7.6)–6.6 mM $MgCl_2$–10 mM dithiothreitol–66 μM ATP. T4 DNA ligase (Bethesda Research Laboratories, Inc.) was added to a concentration of 10 U/μg of DNA, and the mixture was incubated at 4°C for 18 h.

Transformation. Transformation was carried out as described by Bolivar and Backman (2). *E. coli* JM83 transformants were selected on YT plates (8 g of tryptone [Difco Laboratories, Detroit, Mich.], 5 g of NaCl, 5 g of yeast extract, 1.5 g of agar per liter of water) containing 100 μg of ampicillin per ml and supplemented with 200 μg of 5-bromo-4-chloro-3-indoyl-β-D-galactoside per ml. *S. typhi* transformants were selected on YT plates containing 100 μg of streptomycin and 100 μg of ampicillin per ml.

Bioassay. Assays in mouse Y1 adrenal cells were performed essentially as described by Sack and Sack (36). Rat ligated ileal loops were treated as previously described (21). Briefly, a graded toxin dose was instilled into a single 10-cm ligated loop of distal ileum for 18 h. The volume of the loop (in microliters) was compared with the length (in centimeters), and a ratio was determined. Each data point was determined by testing three rats.

Galactose-induced cell lysis. The sensitivity of *S. typhi* Ty21a and SE12 to lysis by galactose was determined by inoculating each strain into ML medium (10 g of tryptone [Difco], 5 g of NaCl, 5 g of yeast extract per liter of water) with and without 0.1% galactose. Cell lysis was followed by monitoring the optical density at 650 nm during a 10-h growth period at 37°C.

Persistence in mouse tissues. Studies to determine the persistence of *S. typhi* Ty21a and the derivative strain SE12 in mouse tissues were conducted essentially as described by Formal et al. (14). Each strain was grown in brain heart infusion broth (Difco) containing 0.1% galactose, washed in sterile normal saline, and suspended in saline to ca. 5×10^7 CFU/ml. Groups of female outbred CD-1 mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were injected intraperitoneally (i.p.) with 0.5 ml of either strain,

TABLE 1. Comparison of adrenal cell activities of LT, LT-B (pDF87), and LT-B (pJC217)

Preparation	Biological activity[a] (ng)
LT	0.038
LT-B (pDF87)	39
LT-B (pJC217)	ND[b]

[a] Amount of trypsin-activated material required to produce significant cell rounding (50%).

[b] ND, None detected when tested at 25,000 ng per well.

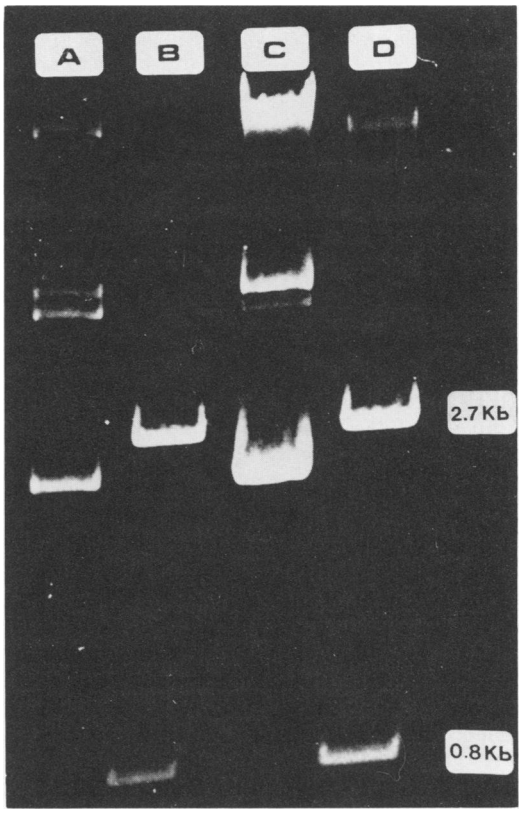

FIG. 1. Agarose gel electrophoresis of partially purified lysates of A, *E. coli* JM83(pJC217); B, *E. coli* JM83(pJC217) cleaved with *Hin*dIII; C, *S. typhi* SE12; and D, *S. typhi* SE12 cleaved with *Hin*dIII. Plasmid DNA was prepared by the procedure of Bolivar and Backman (2) and examined by electrophoresis in 0.7% agarose slab gels (0.04 M Tris, 0.2 M sodium acetate, 0.002 M EDTA [pH 7.8]).

and the spleens of the animals in each group were removed aseptically on days 1, 3, 5, 8, 10, 13, or 15 post-inoculation. Spleens were homogenized and serially diluted in saline, and plated on Trypticase soy agar (BBL Microbiology Systems,

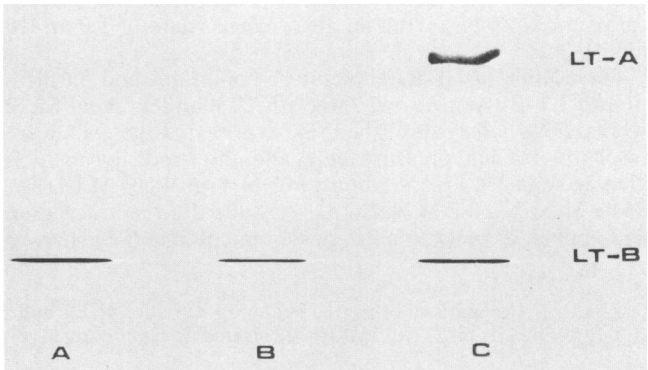

FIG. 2. Analytical discontinuous electrophoresis in SDS-PAGE of agarose affinity-purified preparations of LT-B from A, *S. typhi* SE12; B, *E. coli* JM83(pJC217); and C, LT. Preparations derived from *S. typhi* SE12 and *E. coli* JM83(pJC217) contained a band corresponding to monomeric LT-B. Neither contained a band corresponding to LT-A (lane C, upper band). SDS-PAGE was performed in 10% slab gels by the technique of Laemmli (26). Each well contained 100 μg of protein and was heated to 100°C for 3 min in 0.1% SDS before loading.

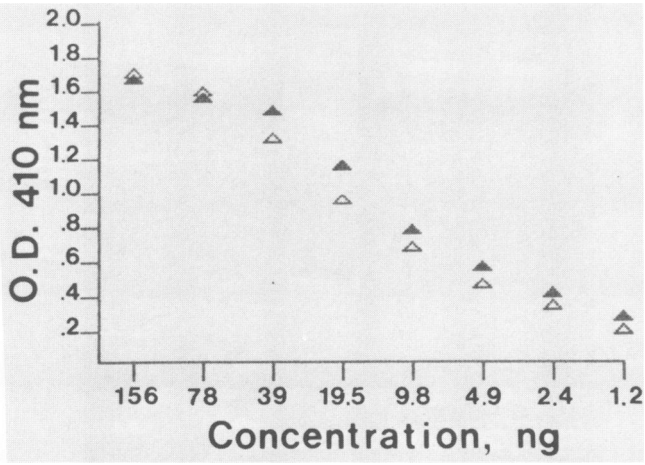

FIG. 3. ELISA comparison of agarose affinity-purified preparations of LT-B from *E. coli* JM83(pJC217) (△) and *S. typhi* SE12 (▲) with monospecific antiserum to LT-B. Samples were assayed in twofold serial dilutions in PBS-Tween-20. O.D., Optical density.

Cockeysville, Md.) and on Trypticase soy agar containing 100 μg of ampicillin per ml.

Oral test in guinea pigs. The derivative strain SE12 was grown in brain heart infusion broth containing 0.1% galactose, washed in sterile normal saline, and suspended in saline to 3×10^9 CFU/ml. Five female Hartley albino guinea pigs (Charles River Breeding Laboratories), each weighing 350 to 450 g, were inoculated orally with 1 ml each and observed for signs of diarrhea for 14 days.

Vaccine efficacy. Female outbred CD-1 mice were used and given a primary i.p. injection with 2.5×10^7 CFU of strain SE12 in 0.5 ml of saline. After 2 weeks, mice to be boosted were injected i.p. with either 2.5×10^7 CFU of strain SE12 or 100 μg of purified LT-B. Serum samples were obtained periodically up to 4 weeks after primary immunization and assayed for anti LT-B antibodies by enzyme-linked immunosorbent assay (ELISA) (see below).

ELISA. The ELISAs for LT-B and for antibodies to LT-B were performed as previously described (6). Samples to be assayed were serially diluted in phosphate-buffered saline (pH 7.4)–0.05% Tween 20. Titer was defined as the reciprocal of the dilution giving an absorbance value of 1.0 at 410 nm.

Purification of LT-B. The culture conditions and purification of LT-B from *E. coli* JM83(pJC217) and *S. typhi* SE12 were as previously described (5), except that the cells were lysed by French pressure cell, and the crude lysate was dialyzed against TEAN buffer (0.05 M Tris, 0.001 M EDTA, 0.003 M NaN_3, 0.2 M NaCl [pH 7.5]) (3) after centrifugation and applied directly to a 2.5-by-80-cm column of Sepharose 4B (Sigma Chemical Co., St. Louis, Mo.) equilibrated with TEAN buffer.

Antiserum. Antiserum to LT-B was prepared by immunizing a goat with 1 mg of guanidine-purified LT-B (8) suspended in 2 ml of Freund complete adjuvant.

Guidelines used for recombinant DNA experiments. The experiments reported here were performed under conditions as specified in the Guidelines for Recombinant DNA Technology published by the National Institutes of Health, Bethesda, Md.

TABLE 2. Comparison of ligated ileal loop activities of LT and LT-B purified from *E. coli* JM83(pJC217) and *S. typhi* derivative SE12

Preparation	Amt tested (μg)	FA ratio[a] (μl/cm)
LT	1	65
LT	10	421
LT	100	818
LT-B JM83(pJC217)	100	0
LT-B SE12	100	0

[a] FA, Fluid accumulation. FA ratios > 50 are considered to be positive.

RESULTS

Construction of *E. coli* JM83(pJC217). We have previously described the construction of a plasmid (pDF87) containing the gene for production of LT-B from a human isolate of *E. coli* H10407 (6, 7). LT-B was isolated from an *E. coli* K-12 transformant containing this plasmid and was found to be structurally identical to the native B subunit when examined by SDS-PAGE, immunologically identical by ELISA, and to contain no demonstrable A subunit in either assay. Despite deletion of the A subunit, LT-B purified from such transformants did induce morphological alterations in cultured mouse Y1 adrenal cells at high concentrations (Table 1) (J. D. Clements, D. C. Flint, R. F. Engert, and F. A. Klipstein, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, B49, p. 31), an indication of residual toxicity.

The cloned LT-B DNA fragment was recloned from pDF87 into the single *Hin*dIII site of the M13-derived cloning vector pUC8 (41). Plasmid pDF87 was cut with *Hin*dIII, and the 0.8-kb fragment which codes for production of LT-B was separated by electrophoresis through low-melting-point agarose. The agarose gel surrounding the fragment was excised, melted, and extracted with phenol and ether. The LT-B DNA fragment was then recovered by ethanol precipitation. Plasmid pUC8 was cleaved with *Hin*dIII and mixed with the purified LT-B DNA fragment, and the mixture was ligated and transformed into *E. coli* JM83. Transformants were selected for resistance to ampicillin, screened for loss of β-galactosidase activity, and tested by ELISA for the ability to make LT-B. One positive clone, designated JM83(pJC217), was selected for further study. Plasmid DNA was purified from this clone, and when recut with *Hin*dIII, it contained only two fragments: one corresponding to the 2.7-kb cloning vector, pUC8, and one of 0.8 kb coding for production of LT-B (Fig. 1). LT-B DNA was efficiently transcribed by the *lac* promoter of pUC8 and expressed in clones containing this plasmid. LT-B was then purified from this clone by agarose affinity chromatography (5), and 50 to 60 mg of pure LT-B was obtained per liter of culture. LT-B isolated from this strain was found to be structurally and immunologically indistinguishable from LT-B isolated from pDF87 and, more importantly, was completely nontoxic when tested in Y1 adrenal cells (Table 1).

Construction of *S. typhi* Ty21a carrying the LT-B plasmid. Plasmid DNA from JM83(pJC217) was purified by cesium

TABLE 3. Distribution of LT-B produced by *S. typhi* derivative SE12

Medium	Amt of LT-B (μg/ml of culture ± SEM) produced	
	Cell-associated (%)	Extracellular (%)
ML	2.51 ± 0.23 (48)	2.73 ± 0.17 (52)
ML + 0.1% galactose	0.47 ± 0.21 (11)	3.65 ± 0.45 (89)

chloride gradient centrifugation and used to transform a streptomycin-resistant mutant of *S. typhi* Ty21a. Transformants were selected for resistance to ampicillin and streptomycin and assayed by ELISA for LT-B production. All Ap^r Str^r transformants tested produced LT-B. One such isolate, designated SE12, was selected for further study.

The *S. typhi* transformant SE12 was examined for the presence of plasmid pJC217. Plasmid DNA was prepared from the transformant and examined by agarose gel electrophoresis. Strain SE12 (Fig. 1D) contained plasmid pJC217, which, when cut with *Hin*dIII, yielded the same two 2.7- and 0.8-kb fragments seen with pJC217 (Fig. 1B).

Characterization of LT-B production by *S. typhi* derivative strain SE12. A major consideration in the construction of a potential bivalent vaccine was that the LT-B produced by the *S. typhi* be structurally and immunologically identical to the LT-B produced by *E. coli*. After purification by agarose affinity chromatography, LT-B from strain SE12 was examined by SDS-PAGE, ELISA, and Ouchterlony analysis. The SE12 LT-B (Fig. 2A) was indistinguishable from the LT-B produced by *E. coli* JM83(pJC217) (Fig. 2B) in SDS-PAGE. Both preparations contained only the single band representing LT-B. As expected, neither preparation contained a band corresponding to LT-A (Fig. 2C). When assayed by ELISA against antiserum to LT-B, the two preparations were immunologically identical (Fig. 3), an observation confirmed by immunodiffusion analysis (data not shown).

The biological activity of LT-B produced by strain SE12 was tested in the rat ligated ileal loop assay. LT produced a positive response in this assay with as little as 1 μg of material, whereas LT-B purified from *E. coli* JM83(pJC217) or from *S. typhi* SE12 had no effect at doses as high as 100 μg (Table 2).

Distribution of LT-B produced by *S. typhi* SE12. When produced by *E. coli*, LT is found to be principally cell associated: more than 95% was found within the cell (5). This was also found to be true for several other genera of *Enterobacteriaceae* containing Ent plasmids (32). Neill et al. (31) have subsequently shown that *V. cholerae* containing an Ent plasmid produced LT that was secreted into the supernatant, as is CT. It was therefore of interest to determine the relative distribution of LT-B produced by the *S. typhi* derivative, and the effect that incorporation of galactose into the culture medium would have on production of LT-B.

Approximately 50% of LT-B produced by strain SE12 remained cell associated, with an equal amount being released from the cell (Table 3). When galactose was added to the culture medium, the percentage of extracellular LT-B increased to 89%, probably a reflection of galactose-induced cell lysis (see below).

Galactose-induced cell lysis. The safety of *S. typhi* TY21a as a vaccine is due in part to the avirulence resulting from the *galE* mutation. A prerequisite of that avirulence is sensitivity to galactose: strain Ty21a completely lysed after the addition of galactose to the culture medium (15). To be an effective bivalent vaccine candidate, an *S. typhi* Ty21a derivative producing LT-B would need to retain that sensitivity to galactose. The growth curves for *S. typhi* SE12, in the presence or absence of 0.1% galactose, are shown in Fig. 4. Both strains were lysed by the presence of galactose in the culture medium.

Persistence in mouse tissues of *S. typhi* strains Ty21a and SE12. Two groups of 20 mice each were injected i.p. with viable *S. typhi* strains Ty21a or SE12. Spleens were removed from three animals in each group at intervals up to 15 days post-inoculation and assayed for the presence or absence of the challenge organisms. Bacteria were present in the spleens of both groups on day 1 post-inoculation. By day 3, all organisms had been cleared from the SE12 group and reduced 10-fold in the Ty21a group (day 1, $\bar{X}$ = 134 CFU per spleen; day 3, $\bar{X}$ = 13 CFU per spleen). By day 5, and thereafter, all animals gave negative results.

Oral test in guinea pigs. Five guinea pigs were inoculated orally with 3×10^9 CFU each of strain SE12 and observed for a period of 14 days for signs of diarrhea. All remained healthy, no signs of diarrhea were seen, and all the guinea pigs continued to gain weight for the duration of the experiment.

Vaccine efficacy. Mice immunized with 2.5×10^7 CFU of SE12 developed a significant antibody response to LT-B after primary immunization (Table 4). This response was specifically boosted by subsequent immunization with either 2.5×10^7 CFU of viable SE12 or 100 μg of purified LT-B. The titer developed after two immunizations with SE12 (4,500) is approximately equivalent to that seen with two i.p. injections of 10 μg of LT-B each, given 2 weeks apart (unpublished data).

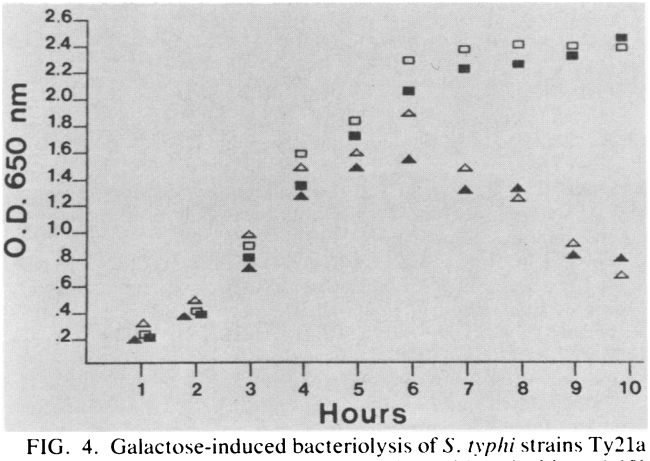

FIG. 4. Galactose-induced bacteriolysis of *S. typhi* strains Ty21a and SE12. Strains were grown in ML medium with and without 0.1% galactose. Symbols: ■, Ty21a; □, SE12; ▲, Ty21a plus galactose; △, SE12 plus galactose. Both strains were lysed by the presence of 0.1% galactose in the culture medium. O.D., Optical density.

DISCUSSION

Antitoxic immunity is an important component of the protective immune response to cholera and the immunologically related enterotoxic enteropathies typified by enterotoxigenic *E. coli*. Live oral vaccines for cholera which are nontoxigenic or produce CT-B but lack the toxic CT-A fragment could ideally be used to colonize the intestinal epithelium, thereby eliciting a mucosal immune response not only to CT-B, if present, but also to somatic vibrial antigens. The first such strain was a hypotoxinogenic mutant developed by Finkelstein et al. (13) by nitrosoguanidine mutagenesis. Unfortunately, this strain, designated M-13, was found to occasionally revert to toxicity and probably represented a regulatory rather than a structural mutant. After that, another mutagenized candidate designated Texas-Star (18) was proposed, and initial protection studies in infant rabbits suggested some level of protection against virulent wild-type cholera vibrios (1). Subsequent studies in human volunteers showed that Texas-Star provided some protection against challenge with *V. cholerae*, with an overall reduction in the

TABLE 4. Production in mice of antibodies against *E. coli* LT-B after immunization with *S. typhi* strain SE12

Days post immunization[a]	Boost[b]	Titer[c]
1		0
3		0
5		230
28		250
28	SE12	4,500
28	LT-B	40,960

[a] Each mouse was immunized i. p. with 2.5×10^7 CFU of *S. typhi* SE12 in 0.5 ml of sterile saline.

[b] Groups of animals were given boosts of either 2.5×10^7 CFU of *S. typhi* strain SE12 or 100 μg of LT-B in sterile saline on day 15 after primary immunization.

[c] Reciprocal of the dilution giving an absorbance value of 1.0 at 405 nm in ELISA (see text for details). Each data point represents the pooled sera of 3 to 6 mice.

occurrence of diarrhea (27). More recent advances (19, 20, 30) have involved recombinant DNA methodologies to construct recombinant strains with the genes coding for all or a portion of the CT deleted. Unfortunately, some *V. cholerae* strains have been shown to cause limited diarrhea even with all the toxin genes deleted (M. M. Levine, J. B. Kaper, R. E. Black, M. L. Clements, and J. G. Morris, Abstr. 19th Joint Conf. Cholera 1983, p. 56; W. Spira, D. Sack, S. Sanyal, J. Madden, and B. McCardell, Abstr. 19th Joint Conf. Cholera 1983, p. 80–81), thereby indicating that other virulence determinants in *V. cholerae* may elicit fluid movement when these organisms colonize the epithelium of the small bowel. It has been suggested that a shiga-like toxin may play a role in the pathogenesis of these and certain other nonenterotoxigenic organisms (34). Moreover, according to Tokunaga et al. (39), no direct evidence has been presented to date showing development of significant mucosal antitoxic responses after oral administration of any of these $A^- B^+$ mutants of *V. cholerae*.

Unlike *V. cholerae*, which is noninvasive and principally colonizes the proximal small bowel, *S. typhi* is invasive, affects the distal small bowel, and enters the lymphoid tissues of the host. Theoretically, use of a live bacterium (*S. typhi*) to deliver a toxoid antigen directly to the lymphoid tissues should be more effective at inducing antitoxic antibodies than use of an organism (*V. cholerae*) which distributes the same toxoid antigen randomly over the intestinal epithelial surface. Formal et al. (14) have suggested that the stimulation of antibody-forming cells in the lamina propria may contribute to the protection provided by the *galE S. typhi* Ty21a (15, 16, 42) or its derivative containing the form I antigen plasmid of *Shigella sonnei* (14). It therefore seems possible to use a similar approach for the delivery of toxoid antigens directly to the lymphoid tissues to stimulate a protective mucosal antitoxin response effective against cholera and other immunologically related enterotoxic manifestations.

In this study, we used the *S. typhi galE* mutant vaccine strain Ty21a as a recipient for a recombinant plasmid containing the gene for production of the LT-B of *E. coli*. The *S. typhi* derivative strain SE12 was shown to contain the 3.5-kb LT-B plasmid and produced LT-B that was structurally and immunologically indistinguishable from the LT-B produced by strains of *E. coli* harboring the same plasmid and that possessed no demonstrable biological activity. The derivative strain was rapidly cleared after intraperitoneal injection into mice, caused no diarrhea or other manifestations when inoculated orally into guinea pigs, and retained the galactose sensitivity characteristic of the parent strain *S. typhi* Ty21a. More importantly, mice injected intraperitoneally with SE12 developed a significant antibody response which could be specifically boosted with a subsequent injection of either viable organisms or purified LT-B. Since *S. typhi* is a pathogen only of humans and a few primates, the ability of this strain to induce a specific mucosal antitoxic response will be tested in a forthcoming study.

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