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Importance of Heat-Labile Enterotoxin in Colonization of the Adult Mouse Small Intestine by Human Enterotoxigenic *Escherichia coli* Strains

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Enterotoxigenic *Escherichia coli* (ETEC) infections are a significant cause of diarrheal disease and infant mortality in developing countries. Studies of ETEC pathogenesis relevant to vaccine development have been greatly hampered by the lack of a suitable small-animal model of infection with human ETEC strains. Here, we demonstrate that adult immunocompetent outbred mice can be effectively colonized with the prototypical human ETEC H10407 strain (colonization factor antigen I; heat-labile and heat-stable enterotoxin positive) and that production of heat-labile holotoxin provides a significant advantage in colonization of the small intestine in this model.

Enterotoxigenic *Escherichia coli* (ETEC) infections are a significant cause of diarrheal disease worldwide. Infections caused by this heterogeneous group of pathogens remain major causes of diarrheal morbidity and infant mortality in developing countries (11, 33, 61), are perennially associated with disease in travelers (4, 7, 43) and in soldiers deployed to developing countries (8, 32), and have recently been associated with large outbreaks in developed countries, including the United States (1, 13).

In the presently accepted paradigm for ETEC pathogenesis, fimbrial (or fibrillar) colonization factors (CFs) (10, 22, 27) mediate colonization of the small intestine, where organisms elaborate heat-labile enterotoxin (LT) and/or heat-stable enterotoxin (ST) (21, 68). Vaccine development has largely focused on the CFs; however, development of a broadly protective ETEC vaccine has been hampered due to the considerable heterogeneity of the known CFs (6, 52, 58, 62).

While a number of other surface antigens of ETEC have been described (20, 25, 51), their utility as potential vaccine candidates has not been effectively explored, largely due to the lack of a suitable animal model for testing. Animal models that have been used in previous studies of ETEC include infant (3, 16–18, 28, 47) and adult (9) mice, rats (34, 35), and rabbits (19, 46, 51). All of these models have inherent difficulties in utilization or require anesthesia and/or significant surgical manipulation. Some of the models have not been thoroughly evaluated.

We sought to develop a murine model of small-intestinal colonization with human ETEC isolates using adult immunocompetent mice. Here, we report our initial use of this model using the prototypical human ETEC H10407 strain. Furthermore, similar to recent studies of porcine ETEC infection of gnotobiotic piglets (2), we demonstrate that elaboration of the heat-labile toxin provides a distinct advantage to the organism in establishing early colonization of the small-intestinal mucosa.

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MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are shown in Table 1. The prototype H10407 human ETEC strain was originally isolated from a child with cholera-like diarrheal illness (21), and the present culture used in our laboratory was forwarded from the Walter Reed Army Institute of Research, where it was shown to be fully virulent in human volunteer challenge studies.

Bacterial preparation. Bacterial strains used in these experiments were stored at −80°C until needed. Each strain was grown in 2 ml LB overnight at 37°C. H10407-S was grown in the presence of 25 μg/ml streptomycin. Prior to inoculation, the strains were diluted 1:100 in 100 ml LB (with or without antibiotics) and grown to an optical density at 600 nm of 0.14. Subsequently, a 40-ml sample of each culture was centrifuged to pellet the bacteria, and each pellet was resuspended in 1.6 ml of sterile phosphate-buffered saline (PBS) (pH 7.4), yielding a bacterial concentration of approximately 1×10^9 CFU/ml. Dilutions of this suspension in PBS were plated onto Luria agar and incubated overnight at 37°C to determine the actual inoculum for each strain. Immediately prior to the inoculation of mice, this original suspension was diluted in PBS, and aliquots of 400 μl were dispensed into sterile microcentrifuge tubes on ice.

Inoculation of mice in vivo. All experimental procedures were reviewed and approved by the University of Tennessee Health Science Center Institutional Animal Care and Use Committee. Experimental procedures were performed in compliance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* (46a).

Techniques for inoculation of mice were adapted from experimental methods previously used to distinguish the colonizing abilities of *E. coli* (28, 69). Briefly, female ICR (Institute for Cancer Research) mice (5 to 8 weeks old) purchased from Charles River Laboratories (Charles River designation, CD-1), Wilmington, MA, were used in all experiments. Upon arrival, the mice were allowed to acclimate for 72 h prior to any experimental manipulation. The mice were housed in autoclaved microisolator cages with autoclaved bedding. Drinking water was autoclaved, and food (Harlan Teklad, Madison, WI) was irradiated. All mice received streptomycin (5 g/liter) in their drinking water 24 to 48 h prior to ETEC inoculation to eradicate normal resident bacterial flora in the intestinal tract (69). The streptomycin-treated water also contained fructose (6.7%) (Karo Syrup; ACH Food Companies, Inc., Memphis, TN) to encourage water consumption prior to ETEC inoculation. Food was withdrawn for 12 hours prior to inoculation, and fructose-treated water was replaced with unsweetened, sterile water 4 hours prior to ETEC inoculation. Cimetidine (50 mg/kg; Abbott Laboratories, Chicago, IL) was administered intraperitoneally to all mice 1 to 3 h prior to inoculation with ETEC to reduce the effect of stomach acidity on the bacterial organisms (56). Each mouse of a group of 5 to 10 mice per dosage group was

TABLE 1. bacterial strains

Strain	Relevant genotype or description[a]	Reference
jf394	WT ETEC strain H10407: serotype 078:H11; LT^+ ST^+ CFA/I^+	22
jf085	H10407-S;• spontaneous Sm^r mutant	20
jf571	Δ*eltA lac*ZYA^+ ST^+ CFA/I^+	15
jf946	*lac*ZYA LT^+ ST^+ CFA/I^+	15
jf571+	Merodiploid strain; *eltA*-FLAG LT^+ ST^+	15
jf1124	Isogenic Δ*gspM* mutant CFA/I^+	15
AAEC191A	*E. coli* afimbriate control strain	5

[a] CFA/I, coloniation factor antigen I.

orally inoculated with a suspension of ETEC or control organisms in a final volume of 400 μl via a 20-gauge gavage needle (Bio-Medical Needles; Popper & Sons, Inc., New Hyde Park, NY). The mice were allowed to survive for 24 or 72 h following inoculation, depending on the experiment protocol. Twelve hours prior to euthanasia, food was withdrawn. The mice were euthanized with isoflurane (Isoflurane USP; Halocarbon Laboratories, River Edge, NJ) anesthesia to effect, followed by cervical dislocation.

Competition experiments. In competition experiments, each mouse in a group of five received a mixture of approximately equal numbers of each strain in a final volume of 400 μl. Immediately following inoculation, the mice were allowed access to food. The mice were euthanized 24 h following inoculation.

Isolation of bacteria from the small-intestinal mucosa. Bacteria were harvested from the small intestine using a modification of a technique previously described for neonatal mice (28). Briefly, following isolation of the small intestine, two segments of ileum (3 cm each), beginning within 0.5 cm of the ileocecal junction and extending proximally 6 cm, were removed and opened longitudinally. In one experiment, two segments of duodenum (3 cm each) were selected from the region beginning within 0.5 cm distal to the pyloroduodenal junction, and similarly sized segments of jejunum were selected from the midportion of the small-intestinal tract. The two segments from each representative location were placed in 2 ml of sterile saponin solution (5% in PBS). The sample was vortexed for 5 seconds, incubated for 10 min at room temperature, and vortexed again for 5 seconds. One hundred microliters of the resulting suspension, as well as 10^{-1} and 10^{-2} dilutions in PBS, was plated in duplicate onto Luria agar plates and incubated at 37°C overnight. In competition experiments, bacteria were plated onto media containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (40 μg/ml) and IPTG (isopropyl-β-D-thiogalactopyranoside) (1 mM).

To determine fluid accumulation in the small intestine, ligatures of 5-0 silk were placed at the junctions of the pylorus and duodenum and the ileum and cecum prior to removal of the entire small intestine to prevent fluid loss. The entire small intestine of each mouse was weighed prior to collection of the ileal segments.

To confirm that recovered bacteria were the inoculum strain, bacterial colonies grown on culture plates were routinely tested by PCR using the primers jf030204.1 (5′-CCCCAGTCTATTACAGAA-3′) and jf030204.2 (5′-CTAGTTT TCCATACTGAT-3′), which flank the *eltAB* operon encoding the LT holotoxin of H10407.

Routine histopathology. Mouse intestinal samples harvested following infection were immediately washed briefly in PBS and then fixed in 10% formalin in PBS overnight at 4°C and imbedded in paraffin prior to sections being cut for staining with hematoxylin and eosin (HE).

Immunofluorescence microscopy. Slides prepared from paraffin sections of colonized intestine were treated with xylene to remove the wax and then washed with PBS and blocked overnight at 4°C with a solution of 1% bovine serum albumin in PBS (Pierce Blocker). Specific detection of H10407 (serotype O78:H11) was carried out using rabbit polyclonal antibodies directed against the O78 oligosaccharide (Penn State *E. coli* Reference Center) at a dilution of 1:1,000, followed by highly cross-absorbed goat anti-rabbit immunoglobulin G (heavy and light chains) (Molecular Probes; 1:2,000). After being washed with PBS, the slides were stained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) at a final concentration of 300 nM in PBS for approximately 30 min in the dark. After being washed with PBS and deionized water, the slides were examined by fluorescence microscopy at ×100 (Zeiss, Axiophot). Z-stack images of adherent organisms were acquired using Zeiss Axiovision software with 0.1-μm sections on a Zeiss Axioplan 2 instrument. The images were subsequently reassembled using NIH Image J version 1.34.

Scanning electron microscopy. After removal of the small intestine from an uninfected control mouse, a suspension containing ~1×10^5 CFU/ml of ETEC H10407 in PBS was instilled into the intestinal lumen and incubated at 37°C for 15 min. Intestinal-loop fragments colonized with ETEC were incised to expose the luminal surface, washed with PBS, and fixed with a 2.5% solution of glutaraldehyde in 0.05 M cacodylate for 1 h and then washed with 0.18 M cacodylate buffer. Following fixation, samples were processed with 1% osmium in 0.1% Na cacodylate buffer for 1 h, followed by additional washes in 0.18 M cacodylate buffer.

Statistical analysis. The geometric mean number of CFU per group with the corresponding infecting bacterial strain was calculated. Comparisons between groups were performed using a two-tailed Student's *t* test.

RESULTS

Colonization of adult immunocompetent mice with a prototypical human ETEC strain. In our initial attempt to establish an adult immunocompetent mouse model of intestinal colonization with human ETEC strains, we selected the outbred genetically heterogeneous CD-1 strain of mice that had been used previously to investigate intestinal colonization with human intestinal *Escherichia coli* isolates (41). In a pilot experiment, mice were challenged with approximately 1×10^8 CFU of either the prototypical human ETEC strain H10407 or AAEC191-A, an afimbriate *E. coli* K-12 laboratory isolate. The small-intestinal mucosa of mice inoculated with H10407 yielded significantly more bacteria than those inoculated with AAEC191-A (2.23×10^5 versus 9.79×10^2 CFU/ml, respectively; $P = 0.029$) (Fig. 1A). Intestinal segments from control mice given PBS alone following streptomycin treatment were typically sterile or yielded fewer than 100 CFU/ml. To further examine the ability of ETEC to colonize the mouse small intestine, we employed a streptomycin-resistant version of the wild-type (WT) isolate (H10407-S) (20) in a dose escalation study. While we could detect colonization in only a single mouse (1/4) infected with 10^2 CFU of H10407-S after 72 h, mice inoculated with doses ranging from 10^3 to 10^7 CFU were colonized at a density of organisms proportional to the original inoculum (Fig. 1B).

To more precisely define the location of colonization within the murine intestine, we isolated segments from the duodenum, jejunum, and ileum in five mice following colonization with H10407. As shown in Fig. 1C, in each of these mice, the ileum appeared to be more heavily colonized than either the duodenum or jejunum.

While there appeared to be slightly more fluid accumulated in the small intestines of some animals inoculated with higher doses of bacteria, the differences were not statistically significant, and none of the animals appeared ill or developed diarrhea prior to euthanasia and harvest of the small intestines (data not shown). However, together, these results suggested that human ETEC strains effectively colonize the intestines of adult immmunocompetent outbred mice.

Although inoculation of rabbit ileal loops with ETEC H10407 has been shown to result in significant mucosal inflammation (51), we found no evidence of a similar inflammatory response on histopathologic examination of the mouse small intestine following colonization with this strain (Fig. 2A and B). However, scanning electron microscopic examination of the murine small intestine infected ex vivo with H10407 demonstrated multiple aggregates of bacteria adherent to the mucosal surface (Fig. 2C), suggesting that while these organisms

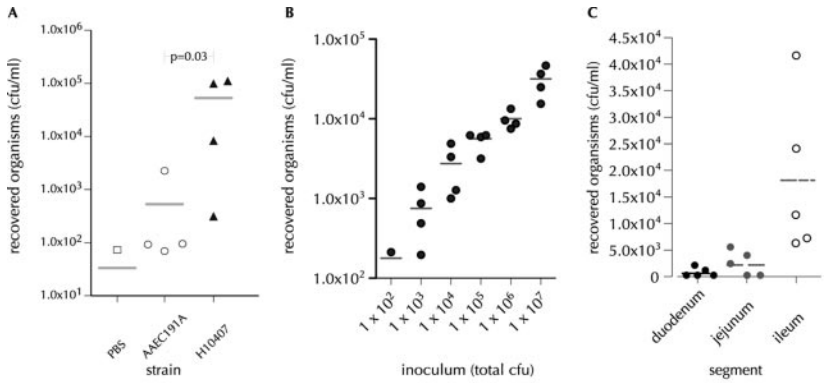

FIG. 1. Intestinal colonization of mice with human ETEC strain H10407. (A) Intestinal colonization of mice with WT ETEC (H10407) or a nonpathogenic afimbriate *E. coli* strain (AAEC191A). Mice were pretreated with oral streptomycin solution to eradicate colonization and cimetidine to reduce gastric acidity and then inoculated with the strains by gavage using ~1×10^8 CFU. After 24 h, bacteria were recovered from segments of small intestine in PBS containing saponin (5%). The recovered organisms were diluted in PBS and plated onto Luria agar. (The data are adjusted for CFU/inoculum.) (B) Dose-ranging study of colonization using a streptomycin-resistant isolate (H10407-S). For these studies, mice were maintained on streptomycin-treated water throughout the experiment, and at the end of 72 h, the mice were sacrificed and recovered organisms were plated onto Luria agar plates containing 25 μg/ml streptomycin. (C) Localization of ETEC within the murine small intestine 24 h after challenge with 6×10^5 CFU of H10407. The horizontal lines within each group reflect geometric means.

can adhere to the small-intestinal surface, they do not elicit a discernible inflammatory response in this model. To further investigate the adherence of H10407 to the murine small intestine in vivo, we examined histologic sections of ileum obtained from mice infected with H10407 (serotype O78-H11) or uninfected controls by immunofluorescence using primary antibodies directed against the O-78 antigen. These studies identified organisms within the lumen and adjacent to enterocytes of intestines from H10407-infected animals (Fig. 2D to F), but not in controls, further supporting specific colonization of the intestine by this human ETEC isolate.

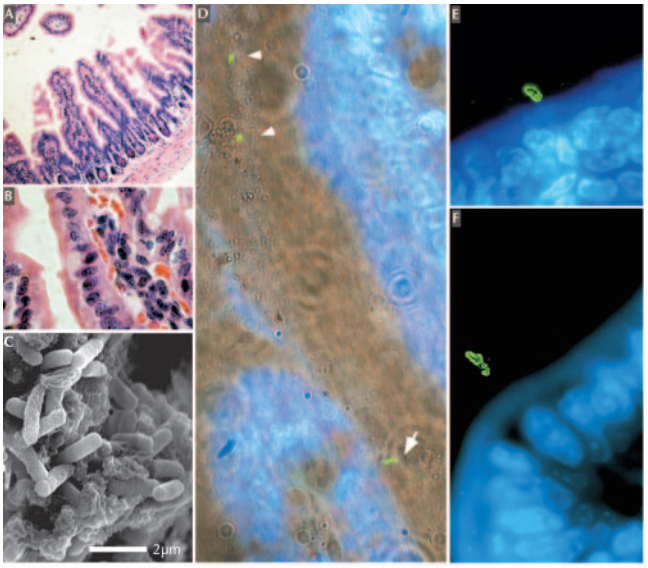

FIG. 2. Microscopy of mouse intestine colonized with human ETEC H10407. Low-power (A) and ×100 (B) images of HE-stained mouse ileum following colonization with 10^8 CFU of ETEC H10407. (C) Scanning electron microscopy image of ETEC H10407 adherent to mouse small-intestinal mucosa. (D) Immunofluorescence image of ETEC H10407 (serotype O78:H11) colonization of mouse ileum. Intraluminal (arrowheads) and enterocyte-associated (arrow) bacteria were detected using rabbit polyclonal anti-O78 primary antibodies and secondary goat anti-rabbit immunoglobulin G (heavy and light chains) labeled with Alexa Fluor 488 (Molecular Probes). Eukaryotic cells were stained with DAPI. The image represents the overlay of two layers obtained using (i) simultaneous differential interference contrast microscopy to obtain the light image coupled with UV activation of DAPI (blue) and (ii) a 485-nm band pass filter to detect fluorescent bacteria (green). (E and F) reassembled Z-stack images of H10407 adherent or in close proximity to enterocytes of mouse ileal mucosa.

Heat-labile toxin facilitates intestinal colonization by ETEC. Interestingly, prior studies have suggested that heat-labile toxin and the closely related cholera toxin promote mucosal colonization by porcine ETEC (2) and *Vibrio cholerae* (53), respectively. Therefore, to investigate the role of LT in colonization in the present animal model of ETEC infection, we challenged mice with either the parent strain or strain jf571, which bears a mutation in the gene encoding the A subunit of LT.

Although the jf571 strain bearing a deletion in the A subunit of the toxin was able to colonize the mouse small intestine, the WT strain colonized more efficiently, with nearly 10-fold more organisms recovered from the mucosa of animals infected with H10407 (1.86×10^5 CFU/ml) than from those infected by the toxin-deficient mutant (2.01×10^4 CFU/ml) ($P = 0.015$). Restoration of toxin production in strain jf571+ resulted in colonization similar to WT levels (2.75×10^5 CFU/ml) (Fig. 3).

To examine further the contribution of LT to colonization, we performed competition experiments between the *eltA* mutant (jf571) and a derivative of the WT, strain jf946, in which the *lac* operon has been interrupted. The *lacZYA* jf946 strain is fully capable of secreting LT holotoxin and activates cyclic AMP (cAMP) production in target monolayers as efficiently as the WT strain, but it appears white when plated onto media containing the β-galactosidase indicator X-Gal, allowing us to easily distinguish jf946 from *lac*$^+$ strains. Furthermore, we found no differences in intestinal colonization by jf946 and the WT strain. When both the jf946 (*lacZYA eltA*$^+$*B*$^+$) and jf571 (*lacZYA*$^+$ *eltAB*$^+$) strains were introduced into mice in equal numbers, the LT-A-producing strain dramatically outperformed the LT-A-deficient strain in colonization of the mu-

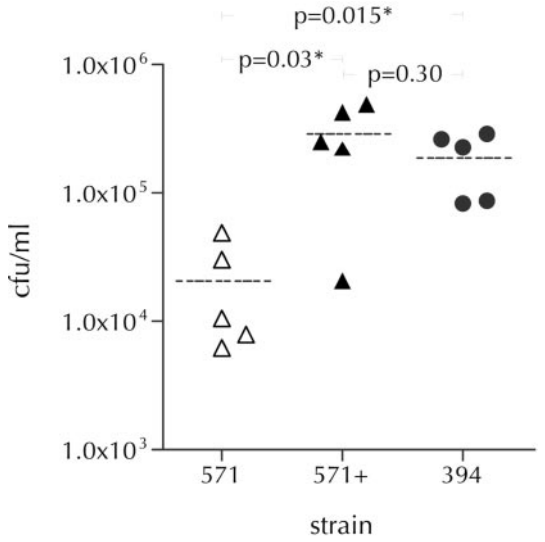

FIG. 3. LT-negative mutants exhibit defective colonization relative to the WT in a murine model. The LT-negative strain jf571 (571), jf571 complemented with a FLAG epitope-tagged version of LT (571+), and WT H10407 (394) were used to colonize groups of five mice each. The numbers of recovered organisms (CFU/ml) are adjusted for the inoculum. The *P* values reflect comparisons of two groups using a two-tailed Student's *t* test: 571 versus 571+ ($P = 0.03$), 571 versus 394 ($P = 0.015$), and 571+ versus 394 ($P = 0.30$).

cosa, with the former yielding 1.2×10^3 CFU/ml and the latter only 3×10^1 CFU/ml ($P = 0.02$) (Fig. 4A and B).

Similar competition experiments were performed with jf946 and jf1124 (15), a mutant bearing a deletion in *gspM* required for secretion of the heat-labile toxin (64). This mutant produces LT holotoxin but does not secrete it from the periplasmic space and is incapable of activating cAMP in target epithelial cell monolayers (15). As shown in Fig. 3C, the Δ*gspM* mutant colonized the mucosa far less efficiently than the competing strain, which secretes LT holotoxin. These findings suggest that both production and effective secretion of LT holotoxin are required to facilitate colonization of the mucosa.

DISCUSSION

The absence of a viable animal model for infection by human strains of enterotoxigenic *E. coli* presents a serious disadvantage in development of candidate ETEC vaccines by hindering the testing of candidate immunogens that may prevent disease and by posing significant limitations on examination of the roles of potential virulence factors. The intent of these studies was to develop a murine model that would minimize animal stress and that could be used to (i) investigate the pathogenesis of ETEC infections and (ii) explore the protective efficacies of candidate vaccine constructs.

Here, we demonstrate that adult immunocompetent mice that have been pretreated with both cimetidine to reduce gastric acidity and streptomycin to eradicate normal flora can be effectively colonized with a prototypical ETEC strain in a dose-dependent fashion. Furthermore, similar to earlier studies of animal models of porcine ETEC (2) and *Vibrio cholerae* infections (53), the data presented here suggest that production of the ADP-ribosylating heat-labile enterotoxin is an important determinant of mucosal colonization.

In recent studies of porcine ETEC strains in gnotobiotic piglets (2), LT enhanced colonization of the small intestine. Among piglets inoculated with LT-negative mutants, there was significantly reduced colonization of the small intestine relative to the WT porcine ETEC strain. Likewise, earlier studies demonstrated that when rabbits are challenged orally with *Vibrio cholerae*, cholera toxin (CT) contributes to the ability of these organisms to colonize the mucosa (53, 65). Interestingly, *V. cholerae* mutants lacking both the CT A subunit and B subunit (A^-B^-) or deficient in only the A subunit, (A^-B^+) were less efficient in mucosal colonization than the parent, while cholera

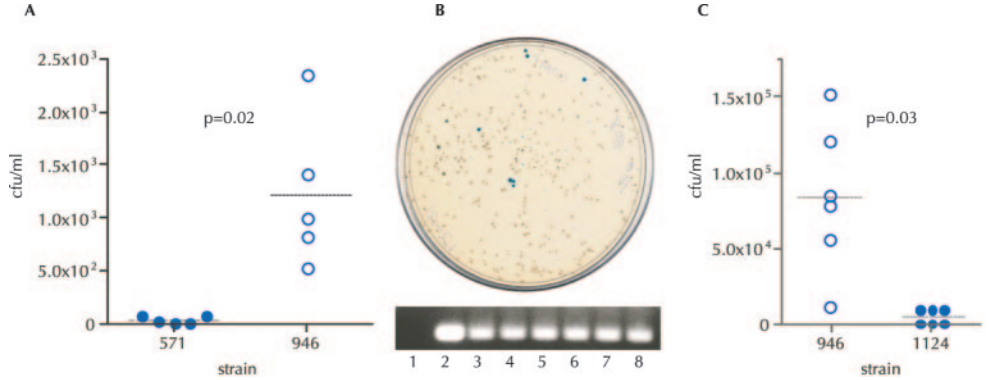

FIG. 4. Secretion of LT holotoxin promotes colonization. (A) Summary of competition experiments in which five individual mice were simultaneously inoculated with $\sim 1 \times 10^4$ CFU of both the LT^+ and LT^- strains. The closed blue circles represent the numbers of recovered blue CFU (jf571 *eltA eltB*$^+$ *lacZYA*$^+$), and the open circles represent the numbers of white CFU (jf946 *eltA*$^+$ *eltB*$^+$ *lacZYA*). (B) A representative X-Gal plate containing bacteria recovered from one mouse. Shown below the plate are representative colony PCR results from the experiment in panel A performed to confirm the identities of ETEC strains. Primers jf030204.1 (5′-CCCCAGTCTATTACAGAA-3′) and jf030204.2 (5′-CTAGTTT TCCATACTGAT-3′) were used to amplify the terminal 308 bp of the *eltB* gene from six randomly chosen blue and white colonies recovered from three different animals. Lane 1, negative control (water); lane 2, positive control (H10407 genomic DNA); lane 3, white colony plate A (mouse 1); lane 4, blue colony plate A; lane 5, white colony plate B (mouse 3); lane 6, blue colony plate B; lane 7, white colony plate C (mouse 5); lane 8 blue colony plate C. (C) Competition between jf946 and a derivative of H10407 (jf1124) bearing a deletion in *gspM* required for secretion of the heat-labile toxin. Six mice were challenged with $\sim 10^7$ CFU of both strains.

holotoxin was able to restore colonization by the nontoxigenic (A^-B^-) strain. Earlier studies also demonstrated that repeated mucosal colonization of rabbits with *V. cholerae* El Tor strains yields hypertoxigenic mutants by selecting for duplication (40) of the chromosomal region (70) encoding CT, further suggesting that in vivo enterotoxin production provides a selective advantage in populating the small intestine.

While these studies suggest that production of these very similar toxins (12) by their respective pathogens greatly enhances the ability of ETEC and *Vibrio cholerae* to populate the mucosal surface, the mechanism responsible for this phenomenon has not been adequately explored. As demonstrated in the A^-B^+ *Vibrio cholerae* mutants (53), delivery of the A subunit, the enzymatically active toxin moiety, appears to be required.

The cellular events that occur following toxin uptake by target host cells have been elucidated in some detail. The enzymatically active A_1 portion of the A subunit must be translocated across the cellular membrane to permit the allosteric interaction of A_1 with ADP-ribosylating factors to affect the ADP ribosylation of Gsα, an intracellular guanine nucleotide protein (66). The inhibition of Gsα GTPase activity leads to the constitutive activation of adenylate cyclase. Consequently, increased levels of intracellular cAMP activate the cystic fibrosis transmembrane regulator (57) Cl^- channel, followed by the ultimate secretion of electrolytes and water that leads to the voluminous watery diarrhea characteristic of clinical ETEC and cholera infections (55). Most of the effects of cAMP in the cell are orchestrated through protein kinase A (PKA) and phosphorylation of transcription factors. Increases in intracellular cAMP concentrations enhance interaction of the cyclic nucleotide with the regulatory subunit of PKA, promoting translocation of the PKA catalytic subunit to the nucleus, where it phosphorylates transcription factors, such as CREB (cAMP response element binding protein), that bind to cAMP response elements in the promoters of a wide variety of cAMP-responsive genes (36, 39).

One potential explanation for the apparent increase in the ability of LT-producing strains to colonize could be structural alteration of the target host cells. Increases in intracellular cAMP may lead to enhanced production of one or more receptors for bacterial ligands. Interestingly, cellular expression of fibronectin, proposed in the past to be a potential receptor for ligands from ETEC and other *E. coli* strains (26, 37, 59, 67), appears to be significantly enhanced by CREB (14, 45). Cytoskeletal disarrangement has also been noted in target epithelial cells following exposure to either LT or CT, but not after exposure to CT-B (30). Arf1, the ADP-ribosylating factor that associates with cholera toxin A subunit to affect ADP ribosylation of Gsα, also regulates the actin cytoskeleton (54) in the periphery of the cell and in the region of the Golgi apparatus and may be associated with membrane remodeling. Therefore, one mechanism by which these ADP-ribosylating toxins may affect colonization is by modulating the host cell surface to promote adherence.

Another avenue by which LT might facilitate colonization is through alteration of the host response to infection. Increases in cAMP inhibit the activation of a number of cytokines, including tumor necrosis factor alpha and interleukin 8 (IL-8) (29), by interfering with NF-κB-mediated transcription (48, 50), a central element modulating the innate immune response, initiated upon pathogen-associated molecular-pattern recognition (63). Of note, recent studies of clinical ETEC isolates have demonstrated that strains which produce LT are associated with lower IL-8 responses in target cultured epithelial cells than those which produce only ST (31). In addition to cytokines, the production of some antimicrobial peptides expressed in the human intestine, such as human beta-defensin hbd2, is also dependent on NF-κB induction (49).

Theoretically, ETEC, *Vibrio cholerae*, and other diarrheal pathogens are poorly equipped to confront an adaptive host immune response and act by overwhelming innate immunity (42). Production of ADP-ribosylating toxins may be one mechanism by which innate defenses are suppressed long enough for the organisms to proliferate to significant numbers before being disseminated into the environment on a wave of toxin-induced diarrhea.

There are a number of limitations to the current murine model described here. While most of the mice became colonized when given more than 10^2 CFU of the WT strain, none of the mice in these studies developed diarrhea. Significant differences in the responses of humans and mice to colonization with ETEC could be due to a number of factors, including production of cytokines or other factors that may modulate the pathogenic response to colonization. Indeed, mice are missing genes for IL-8, which is a critical chemokine in regulation of neutrophil recruitment to the mucosa, and its CXCR1 receptor (44). Our previous studies in vitro and in the rabbit ileal-loop model suggest that H10407 can induce IL-8 production by target epithelial cells (23), as well as mucosal infiltration of polymorphonuclear leukocytes (51). The absence of an inflammatory response noted in our mouse colonization studies could play a role in limiting the development of diarrhea. Indeed, migration of polymorphonuclear leukocytes to the mucosa could itself lead to diarrhea (24, 38). Exploration of these and other differences between mouse and human factors which influence the host response to colonization could elucidate additional mechanisms involved in the pathogenesis of diseases caused by these organisms, as recently demonstrated in a mouse model of Shigella infection (60).

It is also important to note that there was significant variation in the number of recovered organisms between mice within groups receiving the same dose and strain of bacteria. One explanation for this phenomenon may be that the mice used in our experiments were genetically heterogenous outbred animals, making some variation in susceptibility to ETEC likely. Studies of infant mice have shown that there is considerable phenotypic variation with respect to susceptibility to ETEC or its toxins among different inbred strains of mice (3, 17, 18), indicating that host genetic factors may play a significant role in the acquisition of ETEC infections.

Despite these limitations, the development of an adult immunocompetent murine model for studies involving oral inoculation of human ETEC strains could prove to be useful in the study of small-intestinal adherence and colonization, major events in the pathogenesis of ETEC and principal targets for interventions to prevent diseases caused by these pathogens. Likewise, our studies suggest that the current model has utility in investigation of the pathogeneses of these organisms and that it may provide a platform for testing candidate immuno-

gens to prevent colonization. Furthermore, it should permit strategies such as signature-tagged mutagenesis, which has proven useful in the identification of a variety of novel virulence factors important for mucosal colonization by other pathogens (71).

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