Enterotoxigenic *Escherichia coli* (ETEC) is the most common cause of travelers’ diarrhea (TD) in U.S. visitors to Mexico (1). ETEC isolates can produce heat-labile toxin (LT), heat-stable toxin (ST), or both toxins simultaneously (8). The LT of ETEC is highly homologous to the *Vibrio cholerae* toxin (CT) (4) and is composed of five beta-subunits that bind to the intestinal epithelial cell and a single alpha-unit that activates intracellular adenyl cyclase by virtue of its ADP-ribosylating activity. In addition to being an enterotoxin, LT is a potent mucosal adjuvant (5).

In travelers, natural exposure to the ETEC LT is associated with a humoral immune response to LT; however, serum LT antibody titers resulting from naturally acquired ETEC LT do not correlate with long-lasting protection (11, 12). This suggests that other components of the immune system are important in developing protective immunity to ETEC LT.

Little is known about the protective effect that cellular immunity provides against ETEC LT and *V. cholerae* toxin (CT). In this pilot study we evaluated the whole-blood gamma interferon response to CT B in 17 U.S. adults traveling to Mexico. Only one of nine subjects who demonstrated a cellular immune response as determined by whole-blood gamma interferon production to CT B on arrival to Mexico developed diarrhea, whereas five of eight without a cellular response developed diarrhea. Markers of the cellular immune response to ETEC LT could help in identifying individuals immune to ETEC LT, and these markers deserve additional study.

Enterotoxigenic *Escherichia coli* (ETEC), which produces heat-labile toxin (LT), is a common cause of travelers’ diarrhea (TD). The B subunit of ETEC LT is immunologically related to the B subunit of *Vibrio cholerae* toxin (CT). In this pilot study we evaluated the whole-blood gamma interferon response to CT B in 17 U.S. adults traveling to Mexico. Only one of nine subjects who demonstrated a cellular immune response as determined by whole-blood gamma interferon production to CT B on arrival to Mexico developed diarrhea, whereas five of eight without a cellular response developed diarrhea. Markers of the cellular immune response to ETEC LT could help in identifying individuals immune to ETEC LT, and these markers deserve additional study.

We conducted a pilot prospective study to evaluate the cellular immune response to ETEC LT as evidenced by the whole-blood gamma interferon response to the B subunit of CT in U.S. adults traveling to a region where ETEC is endemic. The study was approved by the Committee for the Protection of Human Subjects of the University of Texas Health Science Center at Houston.

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Seventeen white non-Hispanic U.S. adults traveling to Cuernavaca, Mexico, for 3 weeks during July and August 2007 were enrolled in the study. Thirteen were women (76.5%), and the mean age at arrival was 28.6 years (standard deviation [SD], 11.6). TD was defined as the passage of three or more formed stools within a 24-hour period plus one or more abdominal symptom of enteric infection. Six (35.3%) partici-

### TABLE 1. Characteristics of participants in the WBGIR CT B pilot study

| Health status group          | No. (%) of women | No. (%) of men | Age at arrival (yr) | No. (%) with previous travel to Mexico |
|-----------------------------|------------------|----------------|--------------------|---------------------------------------|
| Healthy during travel       | 8 (61.5)         | 3 (75.0)       | 27.9 ± 13.4        | 3 (27.3)                              |
| Developed classic TD        | 5 (38.5)         | 1 (25.0)       | 28.8 ± 8.5         | 3 (50.0)                              |
| Both groups                 | 13 (76.5)        | 4 (23.5)       | 28.6 ± 11.6        | 6 (35.3)                              |

*P* value* 

*NS NS NS NS NS*

*Based on nonparametric comparisons, study subject gender, age, or travel history was not significantly associated with the occurrence of TD.

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pents developed TD. In this pilot study, gender, age, and previous travel history were not significantly associated with the occurrence of TD (Table 1). The mean onset for TD was 12.3 days (SD, 5.6), and there was a mean of 19.5 unformed stools (SD, 4.0) per stay.

We evaluated the whole-blood gamma interferon response (WBGIR) against the B subunit of CT as a surrogate of the ETEC LT immune response on arrival to Mexico and prior to departure. Briefly, 10 ml of venous blood was collected in a heparinized tube from each participant and 1 ml of whole blood was cultured per well of a 24-well ultra-low attachment plate. The whole blood was then stimulated with either polystyrene tissue culture plate (Corning, NY) within 2 h after blood was cultured per well of a 24-well ultra-low attachment plate. Briefly, 10 ml of venous blood was collected in a heparinized tube from each participant and 1 ml of whole blood was cultured per well of a 24-well ultra-low attachment polystyrene tissue culture plate (Corning, NY) within 2 h after collection. The whole blood was then stimulated with either 100 μl sterile phosphate-buffered saline (PBS; negative control), 100 μl of 1 μg/ml phytohemagglutinin-L (positive control), or 100 μl of 5 μg/ml CT subunit B. The tissue culture plates were incubated for 24 h at 37°C with 5% CO₂ and 100% humidity. Plasma was then harvested and the gamma interferon concentration was determined by using a commercially available enzyme immunoassay according to the manufacturer’s instructions (Quantiferon, Valencia, CA). A positive gamma interferon response was defined as >0.5 IU/ml after 24 h of antigen stimulation.

Nine (52.3%) travelers had positive WBGIR on arrival to Mexico. Six participants had a previous travel history to Mexico. Only one recalled suffering from TD during a prior trip to Mexico. Previous travel history to Mexico within the prior year was not associated with a positive WBGIR (3/9 versus 3/8; \( P = 0.04 \)). Of interest, individuals with negative WBGIR were more likely to experience TD (\( P = 0.05 \)). Five out of the eight (62.5%) travelers with negative WBGIR at arrival developed TD. In contrast, only one out of nine (11.1%) travelers with positive WBGIR at arrival developed TD (Table 2).

In the present study, we found that individuals possessing a Th-1 cellular immune response against ETEC LT as evidenced by a WBGIR to the B subunit of CT were less likely to develop diarrhea when traveling to an area where ETEC LT is endemic than individuals without such a response. This report is the first prospective study describing an association between the cellular immune response to the CT B subunit and TD during natural exposure. The small sample size did not allow us to determine if the WBGIR to CT B was protective of ETEC LT diarrhea, but it appeared to correlate with protection against TD overall. This protective effect of immunity to LT against TD is consistent with previous observations of vaccines with an LT component that provided protection against other enteropathogens (3, 7), presumably by preventing the LT-mediated conditioning of the intestinal epithelium that renders it more susceptible to infection (9).

We report that there are no conflicts of interest related to this work.

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We report that there are no conflicts of interest related to this work.

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**TABLE 2. WBGIR to CT B among 17 students traveling to Mexico and followed for 3 weeks after arrival**

| Health status group | Mean arrival WBGIR | Arrival CT B/mitogen ratio | No. (%) of prearrival responders | No. (%) of nonresponders | Mean departure WBGIR | No. (%) who converted to positive WBGIR |
|---------------------|--------------------|---------------------------|----------------------------------|--------------------------|----------------------|--------------------------------------|
| Healthy during travel | 1.14 ± 1.275 | 49.8 ± 49.1 | 8 (72.7) | 3 (27.3) | 1.034 ± 1.292 | 2 (66.7) |
| Developed classic TD | 0.410 ± 0.597 | 4.0 ± 5.7 | 1 (16.7) | 5 (83.3) | 0.290 ± 0.299 | 2 (40.0) |
| Total | 0.883 ± 1.121 | 33.6 ± 45.0 | 9 (52.9) | 4 (47.1) | 0.568 ± 0.882 | 4 (50.0) |
| \( P \) value | 0.04 | 0.02 | 0.05 | NS | NS | NS |

a The whole-blood gamma interferon response to CT.  
b Calculated as follows: [(CT B stimulation result – negative control result)/mitogen stimulation result) × 100.  
c Change in WBGIR to CT B among subjects with no response at arrival.  
d WBGIR result among nonresponders.  
e Based on one-sided nonparametric comparisons.  
f Percent based on 11 healthy students.  
g Percent based on the total number of students (\( n = 17 \)).
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