

Influence of Host Interleukin-10 Polymorphisms on Development of Traveler's Diarrhea Due to Heat-Labile Enterotoxin-Producing *Escherichia coli* in Travelers from the United States Who Are Visiting Mexico[∇]

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Received 15 February 2008/Returned for modification 22 April 2008/Accepted 8 June 2008

Up to 60% of U.S. visitors to Mexico develop traveler's diarrhea (TD), mostly due to enterotoxigenic *Escherichia coli* (ETEC) strains that produce heat-labile (LT) and/or heat-stable (ST) enterotoxins. Distinct single-nucleotide polymorphisms (SNPs) within the interleukin-10 (IL-10) promoter have been associated with high, intermediate, or low production of IL-10. We conducted a prospective study to investigate the association of SNPs in the IL-10 promoter and the occurrence of TD in ETEC LT-exposed travelers. Sera from U.S. travelers to Mexico collected on arrival and departure were studied for ETEC LT seroconversion by using cholera toxin as the antigen. Pyrosequencing was performed to genotype IL-10 SNPs. Stools from subjects who developed diarrhea were also studied for other enteropathogens. One hundred twenty-one of 569 (21.3%) travelers seroconverted to ETEC LT, and among them 75 (62%) developed diarrhea. Symptomatic seroconversion was more commonly seen in subjects who carried a genotype producing high levels of IL-10; it was seen in 83% of subjects with the GG genotype versus 54% of subjects with the AA genotype at IL-10 gene position -1082 ($P, 0.02$), in 71% of those with the CC genotype versus 33% of those with the TT genotype at position -819 ($P, 0.005$), and in 71% of those with the CC genotype versus 38% of those with the AA genotype at position -592 ($P, 0.02$). Travelers with the GCC haplotype were more likely to have symptomatic seroconversion than those with the ATA haplotype (71% versus 38%; $P, 0.002$). Travelers genetically predisposed to produce high levels of IL-10 were more likely to experience symptomatic ETEC TD.

Traveler's diarrhea (TD) affects up to 60% of short-term U.S. visitors to developing countries (23). Although TD is a self-limited disease with few acute complications, it can result in significant transient discomfort, cause changes in travel plans, and produce temporary incapacitation (25). TD has also been associated with long-term complications, such as postinfectious reactive arthritis, (27) Guillain-Barré neuropathy (22), and postinfectious irritable bowel syndrome (17).

Up to 85% of TD cases are bacterial in origin. In Mexico, enterotoxigenic *Escherichia coli* (ETEC) is the single most commonly identified agent in the stools of travelers with diarrhea, ranging from 19% to 40% of cases (3). ETEC isolates from travelers can produce heat-labile toxin (LT), heat-stable toxin (ST), or both toxins simultaneously (LT/ST). Approximately 43% to 68% of strains isolated from subjects with TD in Mexico produce LT alone or in combination with ST (1, 11, 13). Contact with ETEC LT, as a result of vaccination or after natural infection, is associated with the production of LT-specific antibodies (8, 15) and may serve as an indicator of ETEC LT exposure.

Previous studies carried out in Bangladesh have demonstrated that after adjusting for confounding variables, such as age and prior exposure, ETEC LT can be identified with similar frequencies in the stools of children with diarrhea and healthy controls (7) and that the rates of seroconversion to ETEC LT are similar in children with diarrhea and healthy controls (18). This suggests that the variability in clinical outcomes does not depend only on acquired immunity and involves other factors related to the pathogen or host. Implicated pathogen factors include bacterial load, presence of colonization factors, other virulence determinants, the efficiency of LT production, and coinfection with other pathogens. Variability in susceptibility may also be determined by host factors, such as the presence of host receptors for the pathogen, genetic variability in other molecules that mediate susceptibility, response to injury, and control and clearance of ETEC LT.

Interleukin-10 (IL-10) is an anti-inflammatory cytokine produced by dendritic cells, macrophages, and T-helper (Th) lymphocytes that downregulates the production of inflammatory mediators (26). Three major single-nucleotide polymorphisms (SNPs) in the promoter region of the IL-10 gene (-1082 G/A, -819 C/T, and -592 C/A) determine the level of production of IL-10; the resulting GCC allele has been associated with high IL-10 production, whereas the ATA allele has been associated with low IL-10 production. Polymorphisms in the IL-10 gene

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[∇] Published ahead of print on 25 June 2008.

have been shown to affect the risk for septic shock and its outcomes (21), the incidence of invasive aspergillosis (20), the progression of hepatitis B (6), and paracoccidiodomycosis (4), among other infections.

In a previous study, we observed high levels of IL-10 in the stools obtained from travelers with diarrhea who were coinfecting with ETEC and norovirus, but not in the stools of travelers for whom norovirus was identified as a single pathogen or who had pathogen-negative diarrhea (14). We hypothesized that in U.S. travelers visiting Mexico and as seen in other infections, genetic variations in the production of IL-10 could play a role in determining the outcome of ETEC infection (16). In this prospective observational cohort study, we examined the influence that SNPs in the IL-10 gene have on the incidence of ETEC LT-associated infection as determined by ETEC-specific seroconversion in U.S. travelers who made short-term visits to Mexico.

MATERIALS AND METHODS

Enrollment and surveillance. The study was conducted in two language schools in Cuernavaca, Mexico, during the summer months of 2005 and 2006.

Travelers with a length of stay of between 11 and 48 days were included in this study. Exclusion criteria precluding participation were: (i) antibiotic use within the previous 2 weeks; (ii) use of antacids, H₂ blockers, or proton pump inhibitors; (iii) use of probiotics; (iv) history of significant underlying enteric, pulmonary, cardiac, or renal disease; (v) seizure disorder; (vi) insulin-dependent diabetes; (vii) human immunodeficiency virus infection or immunosuppressive therapy; (viii) known history of lactose intolerance; and (ix) cholera vaccination in the past 2 years.

Total blood for DNA extraction was collected from the individuals on arrival, and a serum sample was obtained from all participants within 3 days of their arrival in Mexico and at the time of departure. All samples were transported to the laboratories of the University of Texas Health Science Center at Houston and stored at -80°C until being tested.

Participants reported to the clinic daily and completed a weekly diary indicating the number and consistency of bowel movements and the presence of abdominal symptoms, including abdominal pain, excess gas/bloating, nausea, vomiting, urgency, tenesmus, and fever. Appropriate treatment for TD was provided. The study was approved by the committee for the protection of human subjects of the University of Texas Health Science Center at Houston.

Definitions. Acute diarrhea was defined as the passage of three or more unformed stools within a 24-h period plus 1 or more abdominal symptoms of enteric infection. Seroconversion was defined as a fourfold or greater increase in cholera toxin (CT) antibody titers between the arrival and the departure serum samples.

Anti-CT antibody test. Each serum sample was evaluated for the presence of anti-*Vibrio cholerae* CT B-subunit immunoglobulin G antibodies by enzyme-linked immunosorbent assay (ELISA). The diluent for both the sera and the detecting antibody conjugate consisted of phosphate-buffered saline (PBS; 0.01 M, pH 7.2) with 1% skimmed milk. The ELISA washing buffer consisted of PBS with 0.1% polyoxyethylene-sorbitan monolaurate (Tween 20). Microtiter wells (Nunc flat 96 Immulon plates; Thermo, Waltham, MA) were coated with 100 μl of purified *Vibrio cholerae* CT B-subunit (5 $\mu\text{g}/\text{ml}$; Sigma Chemical Co., St. Louis, MO) in sodium bicarbonate buffer (0.1 M, pH 9.0) and incubated overnight at 4°C . The wells were then washed three times using an automated washer (Elx50; BioTek, Winooski, VT), blocked with 5% skim milk in PBS, and incubated for 1 h at 37°C . After a washing step, twofold serial dilutions of sera were added to the appropriate wells and incubated for 1 h at 37°C . After an additional washing step, horseradish peroxidase-conjugated anti-human immunoglobulin G (Sigma Chemical Co., St. Louis, MO) was added to the wells and incubated for 1 h at 37°C . The washing procedure was repeated, and *O*-phenylenediamine-H₂O₂ (Sigma Chemical Co., St. Louis, MO) was added to each well and incubated for an additional 30 min in the dark at room temperature. The reaction was stopped by adding 50 μl of sulfuric acid (2.5 M) to each well. The color intensity was read at the 492-nm wavelength with a microplate reader. The end-point antibody titer was considered to be the highest dilution of the sample that gave an optical density that was higher than the mean value of the optical density of the standard negative control plus 2 times the standard deviation (SD).

An interplate control using a standard high-titer control sample was maintained, keeping every plate within 2 SDs of the optical density reading. The antibody titer dilution range to be tested was determined by measuring the quartile distribution in the first 100 samples.

Genotyping by PCR and pyrosequencing. DNA from participants was extracted by using a PureGene DNA isolation protocol (Gentra Systems) per the manufacturer's instructions. The presence of SNPs in the IL-10 promoter gene regions (positions -592 , -819 , and -1082) was determined by using pyrosequencing methods. SNP information for the human IL-10 gene was obtained from the National Center for Biotechnology Information (NCBI) database. The sequencing primers for SNP genotyping were designed by using SNP Primer Design software (Biotage AB, Sweden). The probe sequences used were as follows (critical SNPs are in bold and underlined): for promoter position -1082 , we used **TCTTTGGGAG/AGGGGAAGTA**; in the case of the promoter SNP localized in position -819 , we used **GGTGATGTAAC/TATCTCTGTG**; and in the case of the SNP in the -592 position, we used **CCCCGCTGTCA/CTGTAGGAA**. For the pyrosequencing step, biotin-labeled PCR fragments were generated by using a published protocol with 5'-GACTGGCTTCCTACAG as the pyrosequencing primer (28). Biotin-labeled DNA fragments were then captured on streptavidin-coated magnetic beads (DynaBeads M280; Dynal AS, Oslo, Norway). The pyrosequencing reactions were performed by using a pyrosequencer with a PSQTM 96 SNP reagent kit (Biotage AB, Sweden).

Microbiology examination. In cases of diarrhea, a stool sample was collected and transported in coolers containing ice to the laboratory. Stool specimens were subjected to microbiologic analysis in a field laboratory in Mexico. Five individual *E. coli*-like colonies were stored in peptone, and an aliquot of stool from each subject was immediately stored at -20°C until processed. Stool samples were examined for enteric protozoal parasites, including *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium* species, by use of a commercial enzyme immunoassay (Alexon, Sunnyvale, CA). Cultures for enteric bacteria were completed by using six standard media: MacConkey, Tergitol, Hektoen enteric, *Yersinia*, thiosulfate-citrate-bile salts-sucrose, and *Campylobacter* agar plates. The presence of diarrheagenic *E. coli* (ETEC, enteroaggregative *Escherichia coli* [EAEC], enteropathogenic *E. coli* [EPEC], enterohemorrhagic *E. coli* [EHEC], and enteroinvasive *E. coli* [EIEC]) was detected by performing PCR directly from stools using specific probes (14a). Stools were evaluated for the presence of mucus, fecal leukocytes, and occult blood by conventional methods.

Statistical analysis. Calculations were done by using SPSS version 14.0 and NCSS 2007 software. Differences in group characteristics were analyzed by the Pearson chi-square and Fisher's exact test (for cells with less than five elements). Linear variables were compared by using Student's *t* test, and geometric variables were compared by means of the Wilcoxon rank sum test. A multiple logistic regression model was used for the analysis of risk factors and TD. Significance was set at a value of 0.05 for subsequent entry into a forward stepwise Wald regression, with adjustment for each independent variable.

RESULTS

Six hundred forty-two students were enrolled in the study over 2 years. Seventy-three subjects (11.4%) were lost to follow-up; 25 (3.9%) did not provide a departure blood sample, and 48 (7.5%) did not come back to the clinic for follow-up. Thus, a total of 569 participants completed the study. As shown in Table 1, 403 students were female (70.8%), 516 were Caucasian (90.6%), and 57 were of Hispanic ethnicity (10.1%); the mean age at arrival was 35 years (SD, 14.4), and the mean length of stay in Mexico was 20 days (SD, 8.3). Two hundred fifty-five (44.8%) participants developed diarrhea while in Mexico. There were no significant differences in rates of diarrhea related to gender, race, or ethnicity; however, participants who developed diarrhea were younger (33 versus 36 years; *P*, 0.01) and stayed longer in Mexico (21 versus 19 days; *P*, 0.02) than those who remained asymptomatic during their trip. Those individuals who developed diarrhea while in Mexico had lower LT antibody geometric mean titers on arrival, although the difference was not statistically significant (28.75 versus 20.55; *P*, 0.07). One hundred twenty-one (21%) participants had a fourfold increase in LT-specific titers after their stay in

TABLE 1. Demographic characteristics of U.S. students participating in a prospective study of the occurrence of diarrhea during short-term stay in Mexico

| Characteristic | Students | | <i>P</i> ^a |
|--|--|-------------------------------|-----------------------|
| | healthy during travel (<i>n</i> = 314) | TD cases (<i>n</i> = 255) | |
| Gender [no. (%) of subjects] | | | |
| Male | 90 (54.2) | 76 (45.8) | 0.8 |
| Female | 224 (55.6) | 179 (44.4) | |
| Race [no. (%) of subjects] | | | |
| White | 283 (54.8) | 233 (45.2) | 0.5 |
| African American | 21 (56.8) | 16 (43.2) | 0.8 |
| Asian ^b | 7 (77.8) | 2 (22.2) | 0.3 |
| Indian American ^b | 1 (50) | 1 (50) | 0.6 |
| Pacific Islander ^b | 1 (100) | 0 (0) | 0.9 |
| Other ^b | 1 (25) | 3 (75) | 0.5 |
| Ethnicity [no. (%) of subjects] | | | |
| Non-Hispanic | 279 (54.5) | 233 (45.5) | 0.3 |
| Hispanic | 35 (61.4) | 22 (38.6) | |
| Age ^c (yr; mean ± SD) | 36 ± 14.6 | 33 ± 13.9 | 0.01 |
| Length of stay ^c (days; mean ± SD) | 19 ± 8.1 | 21 ± 8.4 | 0.02 |
| Geometric mean titer of LT on arrival ^d | 28.75 | 20.55 | 0.07 |
| LT seroconversion | 46 (14.6) | 75 (29.4) | <0.0001 |

^a Comparison of TD case data to healthy control data.

^b Fisher's exact test was used to compare the data for healthy subjects to the data for TD cases for this group.

^c By Student's *t* test.

^d By Wilcoxon rank sum test.

Mexico, and travelers who developed diarrhea were more likely to seroconvert than subjects who remained healthy during travel (29.4% versus 14.6%; *P* < 0.0001).

Among the 255 participants who developed diarrhea, 173 (67.8%) provided a stool sample for microbiological analysis when ill. As shown in Table 2, 119 (69%) samples were positive for diarrheagenic *E. coli* by means of PCR; ETEC (ST or LT) was identified in 60 (35%) cases, EAEC was identified in 68 (39%) cases, 12 (7%) cases were found to be positive for EIEC, 49 (28%) cases were positive for EPEC, and EHEC was found in 24 (14%) cases. Other bacterial pathogens found by means of stool culture included five (3%) cases with *Salmonella* sp. and one (1%) case with *Plesiomonas* sp. All samples were negative for *Campylobacter* or *Shigella*. Parasites were found by ELISA in 2.9% of the travelers; three (2%) had *Cryptosporidium* sp. and two (1%) had *Giardia* sp. None of the samples tested positive for *Entamoeba histolytica*.

In terms of seroconversion, we observed that ETEC was more likely to be identified in the stools of travelers who seroconverted (53% versus 27%; *P*, 0.001), particularly when ETEC LT (29% versus 10%; *P*, 0.003) or ETEC LT/ST (20% versus 10%; *P*, 0.056) was found in the stools.

We then analyzed the effect that SNPs in IL-10 alleles had on diarrhea among ETEC LT seroconverters. As shown in Table 3, the relative risk (RR) for the development of TD was significantly higher in subjects with any of the alleles associated with high levels of IL-10 production. Participants possessing the -1082 G allele had a 1.24-fold increased risk of developing TD compared to those with the allele associated with low IL-10 production (95% confidence interval [CI], 1.02 to 1.51; *P*, 0.03). Participants with the -819 C allele had a 1.53-fold increased risk of TD (95% CI, 1.19 to 1.97; *P* < 0.0001), and

TABLE 2. Etiology of TD and seroconversion to ETEC LT in U.S. travelers to Mexico

| Pathogen | No. (%) of subjects with indicated status | | | <i>P</i> ^b |
|------------------------------|---|--|---|-----------------------|
| | TD (<i>n</i> = 173) | No seroconversion (<i>n</i> = 124) | Seroconversion ^a (<i>n</i> = 49) | |
| Diarrheagenic <i>E. coli</i> | 119 (69) | 86 (69) | 33 (67) | 0.8 |
| ETEC | 60 (35) | 34 (27) | 26 (53) | 0.001 |
| ETEC LT | 27 (16) | 13 (10) | 14 (29) | 0.003 |
| ETEC ST | 11 (6) | 9 (7) | 2 (4) | 0.4 |
| ETEC LT/ST | 22 (13) | 12 (10) | 10 (20) | 0.056 |
| EAEC | 68 (39) | 52 (42) | 16 (32) | 0.3 |
| EIEC | 12 (7) | 9 (7) | 3 (6) | 0.8 |
| EPEC | 49 (28) | 36 (29) | 13 (26) | 0.7 |
| EHEC | 24 (14) | 17 (14) | 7 (14) | 0.9 |
| <i>Salmonella</i> spp. | 5 (3) | 4 (3) | 1 (2) | 0.7 |
| <i>Shigella</i> spp. | 0 (0) | 0 (0) | 0 (0) | |
| <i>Campylobacter</i> spp. | 0 (0) | 0 (0) | 0 (0) | |
| <i>Plesiomonas</i> spp. | 1 (1) | 1 (1) | 0 (0) | 0.5 |
| <i>Cryptosporidium</i> spp. | 3 (2) | 2 (2) | 1 (2) | 0.8 |
| <i>Giardia lamblia</i> | 2 (1) | 2 (2) | 0 (0) | 0.4 |
| <i>Entamoeba histolytica</i> | 0 (0) | 0 (0) | 0 (0) | |
| No pathogen identified | 51 (29) | 35 (28) | 16 (32) | 0.6 |

^a ≥Fourfold rise in antibodies to ETEC LT.

^b Comparison of seroconverter data to nonseroconverter data.

participants with the -592 C allele had a 1.44-fold increased risk for TD (95% CI, 1.12 to 1.84; *P*, 0.004).

An analysis of the effect that SNPs in IL-10 haplotypes had on the risk of acquiring symptomatic diarrhea in subjects who seroconverted to ETEC LT is shown in Table 4. Participants with the high-IL-10 GCC haplotype had a significant higher risk for developing TD (71 versus 57%, RR, 1.85; 95% CI, 1.08 to 3.17; *P*, 0.02) than those with the ATA haplotype.

In order to determine the relative contribution of the SNPs to diarrhea in the seroconverter group and to exclude confounding variables, we performed a multiple logistic regression analysis. The independent variables included in the model consisted of factors known to influence susceptibility to TD, such as gender, ethnicity, race, age on arrival, length of stay, and the three SNPs of the haplotype associated with a high, intermediate, or low level of IL-10 production. In this model, TD was considered the dependent variable. In the results of this multivariate analysis, the odds of having TD were significantly higher for participants having one of the SNPs associated with high IL-10 production (-819 C/C) (odds ratio, 6.8; *P*, 0.009).

DISCUSSION

Controversy exists around the pathogenic role that ETEC LT has as an agent of diarrhea. The results of several case-controlled studies have demonstrated similar rates of infection in cases and controls. However, most studies have been conducted in areas where ETEC is endemic; in our study of U.S. travelers with little previous exposure to ETEC LT, we found a correlation between seroconversion and diarrheal disease. It is of interest that in this study, a relationship was found between symptomatic ETEC LT and IL-10 alleles associated with high IL-10 production. Since LT is both antigenic and immunomodulatory at mucosal surfaces (9), we hypothesize that our observations could be related to the immunomodulatory properties of this molecule. LT is composed of two subunits, the enzymatically active A subunit and the B subunit. The B subunit binds to GM1 ganglioside on the surface of mammalian

TABLE 3. Analysis of alleles of subjects with and without TD among subjects showing seroconversion to ETEC LT

| IL-10 gene position | Status of subjects | High IL-10 producers | | Low IL-10 producers | | RR (95% CI) | P | | |
|---------------------|--------------------|----------------------|-----|---------------------|---|-------------|----|------------------|---------|
| | | No. with allele | % | No. with allele | % | | | | |
| -1082 | No diarrhea | G | 32 | 30 | A | 60 | 44 | 1.24 (1.02-1.51) | 0.03 |
| | TD | | 73 | 70 | | 77 | 56 | | |
| -819 | No diarrhea | C | 57 | 32 | T | 35 | 56 | 1.53 (1.19-1.97) | <0.0001 |
| | TD | | 122 | 68 | | 28 | 44 | | |
| -592 | No diarrhea | C | 59 | 33 | A | 33 | 53 | 1.44 (1.12-1.84) | 0.004 |
| | TD | | 121 | 67 | | 29 | 47 | | |

cells and allows for the introduction of the A subunit into the host cell. The A subunit in turn initiates a cascade of events that result in watery diarrhea. However, once the B subunit binds to GM1, a separate series of events takes place which leads to the transcription of genes involved in modulating the host inflammatory responses. LT suppresses lipopolysaccharide and gamma interferon-induced tumor necrosis factor alpha and IL-12 production but enhances IL-10 secretion by macrophages (19). Dose-dependent stimulation of IL-10, IL-6, and tumor necrosis factor alpha by the binding of LT to GM1 receptors has also been demonstrated, causing a downregulation in the Th1 response and a shift toward a Th2 response (24). It is plausible that during the initial stages of infection, ETEC LT uses its ability to induce an IL-10-mediated anti-inflammatory state to limit its own clearance by the host prior to the formation of effective neutralizing antibodies. In this context, subjects who are genetically predisposed to produce high levels of IL-10 would be more likely to experience symptomatic ETEC LT diarrhea that is watery and noninflammatory, and subjects with low IL-10 would mount an inflammatory response that would contain infection early on or result in rapid clearance of the enteropathogen. Future analysis of stools for fecal markers of inflammation and cytokines in the context of IL-10 SNPs may assist in further elucidating the role that the high-IL-10 genotypes have in ETEC LT-associated diarrhea.

Our findings also contrast with data from the IL-10-knock-out mouse studies, in which a chronic enterocolitis mimicking inflammatory bowel disease occurs after exposure to bacteria. It is thought that IL-10^{-/-} mice are susceptible to continued inflammation as a result of interactions with endogenous gut bacteria at the bowel wall (2) and that in the absence of IL-10 there is an unchecked proinflammatory response to luminal organisms. There is evidence to suggest that this heightened

inflammatory response may be pathogen specific, as demonstrated by the variable phenotypes of enterocolitis that IL-10^{-/-} mice experience when challenged with different commensal bacteria. Kim et al. have found that severe enterocolitis can be induced with *Enterococcus faecalis*, while milder enterocolitis is seen with *E. coli*, and no inflammation is caused in the mice challenged with *Pseudomonas fluorescens* or those that are germ free (12). Also as a contrast to the results in this report, we have previously shown that subjects with a heightened proinflammatory state by virtue of polymorphisms in the IL-8 gene promoter that result in high IL-8 levels in the gut experience TD due to EAEC, an organism that is more likely to be associated with intestinal inflammation than ETEC LT (10). The association of high IL-10 with ETEC LT and high IL-8 with EAEC suggests that distinct pathways are involved in host responses to diverse enteropathogens.

This study has several limitations. First, not all travelers with diarrhea provided a stool specimen. We also found a high frequency of coinfections with bacterial agents. These confounders may limit the power to correlate the relationships of the different genotypes with individual agents of diarrhea. Second, the study design did not include a control group that could be monitored for asymptomatic colonization to further confirm the association of the genotypes producing low levels of IL-10 with the pathogens of diarrhea. Third, the exposure to agents of diarrhea may have not been uniform, as travelers demonstrate variable degrees of adherence to the avoidance of risky food items. The microbiologic assessment of all food items consumed by travelers would have been impractical and cost prohibitive. Finally, the contribution that this polymorphism makes to the risk of bacterial diarrhea in travelers is modest, with a 1.2- to 1.4-fold increase in the likelihood of diarrheal illness as a whole. It is likely that in addition to unidentified environmental and immune factors, other genes contribute to the risk of acquiring TD.

LT has been shown to modulate the mucosal immune response in part by enhancing IL-10 production, and this may explain why individuals genetically predisposed to produce higher levels of IL-10 are more likely to experience diarrhea when exposed to ETEC LT. This observation could have important future applications, such as for determining which individuals are more likely to become ill with TD, and may prove a useful tool for targeted prophylaxis of susceptible travelers. Further understanding of ETEC LT as an immune modulator

TABLE 4. Influence of host IL-10 haplotype on TD occurrence in U.S. travelers to Mexico

| Status of subjects | No. (%) of subjects with ^a : | | |
|--------------------|---|----------------------------------|-------------------------|
| | High-IL-10 GCC haplotype | Intermediate-IL-10 ACC haplotype | Low-IL-10 ATA haplotype |
| No diarrhea | 18 (29) | 20 (43) | 8 (62) |
| TD | 44 (71) | 26 (57) | 5 (38) |

^a For high (GCC) versus low (ATA) IL-10 haplotypes, the RR is 1.85 (95% CI, 1.08-3.17) (P = 0.02).

and its relationship to the various IL-10 genotypes could also have important ramifications for vaccine trials.

ACKNOWLEDGMENTS

We are indebted to Judy Guillen, Gabriela Meza, Lily Carlin, and the administration and staff of Universidad Internacional in Cuernavaca, Morelos, Mexico, for their assistance with this project.

This work was supported by the following sources: NIH grant R01 AI54948-01, NIH Clinical and Translational Sciences Award (CTSA) UL1 RR024148, and NIH grant DK56338, which funds the Texas Gulf Coast Digestive Diseases Center.

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

REFERENCES

- Adachi, J. A., C. D. Ericsson, Z. D. Jiang, M. W. DuPont, S. R. Pallegar, and H. L. DuPont. 2002. Natural history of enteroaggregative and enterotoxigenic *Escherichia coli* infection among US travelers to Guadalajara, Mexico. *J. Infect. Dis.* **185**:1681–1683.
- Berg, D. J., N. Davidson, R. Kühn, W. Müller, S. Menon, G. Holland, L. Thompson-Snipes, M. W. Leach, and D. Rennick. 1996. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. *J. Clin. Investig.* **98**:1010–1020.
- Bouckenooghe, A. R., Z. D. Jiang, F. J. De La Cabada, C. D. Ericsson, and H. L. DuPont. 2002. Enterotoxigenic *Escherichia coli* as cause of diarrhea among Mexican adults and US travelers in Mexico. *J. Travel Med.* **9**:137–140.
- Bozzi, A., P. P. Pereira, B. S. Reis, M. I. Goulart, M. C. Pereira, E. P. Pedroso, M. F. Leite, and A. M. Goes. 2006. Interleukin-10 and tumor necrosis factor- α single nucleotide gene polymorphism frequency in paracoccidioidomycosis. *Hum. Immunol.* **67**:931–939.
- Reference deleted.
- Cheong, J. Y., S. W. Cho, I. L. Hwang, S. K. Yoon, J. H. Lee, C. S. Park, J. E. Lee, K. B. Hahm, and J. H. Kim. 2006. Association between chronic hepatitis B virus infection and interleukin-10, tumor necrosis factor- α gene promoter polymorphisms. *J. Gastroenterol. Hepatol.* **21**:1163–1169.
- Clemens, J. D., A. M. Svennerholm, J. R. Harris, S. Huda, M. Rao, P. K. Neogy, M. R. Khan, M. Ansaruzzaman, S. Rahaman, and F. Ahmed. 1990. Seroepidemiologic evaluation of anti-toxic and anti-colonization factor immunity against infections by LT-producing *Escherichia coli* in rural Bangladesh. *J. Infect. Dis.* **162**:448–453.
- Clemens, J., S. Savarino, R. Abu-Elyazeed, M. Safwat, M. Rao, T. Wierzbza, A. M. Svennerholm, J. Holmgren, R. Frenck, E. Park, and A. Naficy. 2004. Development of pathogenicity-driven definitions of outcomes for a field trial of a killed oral vaccine against enterotoxigenic *Escherichia coli* in Egypt: application of an evidence-based method. *J. Infect. Dis.* **189**:2299–2307.
- Elson, C. J., and W. Ealding. 1984. Generalised systemic and mucosal immunity in mice after mucosal stimulation with cholera toxin. *J. Immunol.* **132**:2736–2743.
- Jiang, Z. D., P. C. Okhuysen, D. C. Guo, R. He, T. M. King, H. L. DuPont, and D. M. Milewicz. 2003. Genetic susceptibility to enteroaggregative *Escherichia coli* diarrhea: polymorphism in the interleukin-8 promoter region. *J. Infect. Dis.* **188**:506–511.
- Jiang, Z. D., J. J. Mathewson, C. D. Ericsson, A. M. Svennerholm, C. Pulido, and H. L. DuPont. 2000. Characterization of enterotoxigenic *Escherichia coli* strains in patients with travelers' diarrhea acquired in Guadalajara, Mexico, 1992–1997. *J. Infect. Dis.* **181**:779–782.
- Kim, S. C., S. L. Tonkonogy, C. A. Albright, J. Tsang, E. J. Balish, J. Braun, M. M. Huycke, and R. B. Sartor. 2005. Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. *Gastroenterology* **128**:891–906.
- Ko, G., C. Garcia, Z. D. Jiang, P. C. Okhuysen, J. Belkind-Gerson, R. I. Glass, and H. L. DuPont. 2005. Noroviruses as a cause of traveler's diarrhea among students from the United States visiting Mexico. *J. Clin. Microbiol.* **43**:6126–6129.
- Ko, G., Z. D. Jiang, P. C. Okhuysen, and H. L. DuPont. 2006. Fecal cytokines and markers of intestinal inflammation in international travelers with diarrhea due to noroviruses. *J. Med. Virol.* **78**:825–828.
- Lopez-Saucedo, C., J. F. Cerna, N. Villegas-Sepulveda, R., Thompson, F. R. Velazquez, J. Torres, P. I. Tarr, and T. Estradu-Garcia. 2003. Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic *Escherichia coli*. *Emerg. Infect. Dis.* **9**:127–131.
- Mattila, L., A. Siitonen, H. Kyrönseppä, I. Simula, P. Oksanen, M. Stenvik, P. Salo, H. Peltola, et al. 1992. Seasonal variation in etiology of travelers' diarrhea. *J. Infect. Dis.* **165**:385–388.
- Mohamed, J. A., H. L. DuPont, Z. D. Jiang, J. Belkind-Gerson, J. F. Figueroa, L. Y. Armitige, A. Tsai, P. Nair, F. J. Martinez-Sandoval, D. C. Guo, P. Hayes, and P. C. Okhuysen. 2007. A novel single-nucleotide polymorphism in the lactoferrin gene is associated with susceptibility to diarrhea in North American travelers to Mexico. *Clin. Infect. Dis.* **44**:945–952.
- Okhuysen, P. C., Z. D. Jiang, L. Carlin, C. Forbes, and H. L. DuPont. 2004. Post-diarrhea chronic intestinal symptoms and irritable bowel syndrome in North American travelers to Mexico. *Am. J. Gastroenterol.* **99**:1774–1778.
- Rao, M. R., T. F. Wierzbza, S. J. Savarino, R. Abu-Elyazeed, N. El-Ghoreb, E. R. Hall, A. Naficy, I. Abdel-Messih, R. W. Frenck, Jr., A. M. Svennerholm, and J. D. Clemens. 2005. Serologic correlates of protection against enterotoxigenic *Escherichia coli* diarrhea. *J. Infect. Dis.* **191**:562–570.
- Ryan, E. J., E. McNeela, M. Pizza, R. Rappuoli, L. O'Neill, and K. H. Mills. 2000. Modulation of innate and acquired immune responses by *Escherichia coli* heat-labile toxin: distinct pro- and anti-inflammatory effects of the non-toxic AB complex and the enzyme activity. *J. Immunol.* **165**:5750–5759.
- Sainz, J., L. Hassan, E. Perez, A. Romero, A. Moratalla, E. López-Fernández, S. Oyonarte, and M. Jurado. 2007. Interleukin-10 promoter polymorphism as risk factor to develop invasive pulmonary aspergillosis. *Immunol. Lett.* **109**:76–82.
- Schaaf, B. M., F. Boehmke, H. Esnaashari, U. Seitzer, H. Kothe, M. Maass, P. Zabel, and K. Dalhoff. 2003. Pneumococcal septic shock is associated with the interleukin-10-1082 gene promoter polymorphism. *Am. J. Respir. Crit. Care Med.* **168**:476–480.
- Sinha, S., K. N. Prasad, D. Jain, C. M. Pandey, S. Jha, and S. Pradhan. 2007. Preceding infections and anti-ganglioside antibodies in patients with Guillain-Barré syndrome: a single centre prospective case-control study. *Clin. Microbiol. Infect.* **13**:334–337.
- Steffen, R. 2005. Epidemiology of travelers' diarrhea. *Clin. Infect. Dis.* **41**:S536–S540.
- Turcanu, V., T. R. Hirst, and N. A. Williams. 2002. Modulation of human monocytes by *Escherichia coli* heat-labile enterotoxin B-subunit; altered cytokine production and its functional consequences. *Immunology* **106**:316–325.
- von Sonnenburg, F., N. Tornieporth, P. Waiyaki, B. Lowe, L. F. Peruski, Jr., H. L. DuPont, J. J. Mathewson, and R. Steffen. 2000. Risk and aetiology of diarrhoea at various tourist destinations. *Lancet* **356**:133–134.
- Wang, P., P. Wu, M. I. Siegel, R. W. Egan, and M. M. Billah. 1995. Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J. Biol. Chem.* **270**:9558–9563.
- Yates, J. A., and L. C. Stetz. 2006. Reiter's syndrome (reactive arthritis) and travelers' diarrhea. *J. Travel Med.* **13**:54–56.
- Zhang, Z., W. Liu, X. Jia, Y. Gao, K. Hemminki, and M. Linholm. 2004. Use of pyrosequencing to detect clinically relevant polymorphisms of genes in basal cell carcinoma. *Clin. Chim. Acta* **342**:137–143.