NOTES

Intestinal Secretory Immunoglobulin A Response to Enteroaggregative Escherichia coli in Travelers with Diarrhea

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We examined stool samples from travelers for secretory immunoglobulin A (sIgA) to enteroaggregative Escherichia coli (EAEC) during episodes of acute diarrhea. Ten paired samples from 10 patients with diarrhea caused by EAEC were examined for the presence of specific sIgA by dot blot and Western blot immunoassays. Five samples were positive by dot blotting, and two samples were positive by Western blotting.

Enteraggregative Escherichia coli (EAEC) strains are distinct E. coli strains that adhere to HEp-2 cells in an aggregative pattern (11, 15). These strains have been incriminated as causative agents in persistent diarrhea in children (1), adult travelers (2, 8), and AIDS patients with chronic diarrhea (7, 10, 12, 16). EAEC strains have been recovered from healthy individuals, suggesting that some strains may not be pathogenic (11). The pathogenesis of EAEC is not fully understood. EAEC can bind to human colonic mucosa (5, 19), with formation of a thick mucus layer and production of intestinal inflammation (13).

Bacterial enteropathogens can induce an intestinal immune response after an episode of diarrhea (3, 17). This is an indirect way to confirm the enteropathogenicity of a particular microorganism. Our aim in the study described here was to study the intestinal immune response to EAEC strains among travelers with diarrhea.

Our study population consisted of U.S. adult travelers with acute diarrhea acquired during a stay in Guadalajara, Mexico, from June to September 1998. Enteric parasitic and bacterial pathogens were sought by previously published methods (8). We collected two stool samples from each of the patients enrolled in a clinical trial: one was collected on the day of presentation to the clinic with diarrhea, before treatment was started, and the second sample was obtained 5 days later, after completion of a course of antimicrobial therapy. The 5-day period should be sufficiently long to allow a mucosal antibody response. We studied 10 samples from travelers in whom EAEC was found to be the sole pathogen. A stool sample from a healthy individual without diarrhea was used as a control. Strain JM221 (3) and homologous and heterologous organisms from the other nine patients were used as a source of antigens in this study. A nonaggregative-nonpathogenic E. coli strain isolated from a healthy individual without diarrhea was used as the control antigen.

We used a previously published method for the HEp-2 cell adherence assay (15). Secretory immunoglobulin A (sIgA) was extracted from stool samples with trichlorotrifluoroethane (10). Successful extraction was confirmed for the presence of sIgA by a dot blot technique in which 1 μl of the stool extract was used as antigen and was blotted onto the nitrocellulose paper. The sIgA was then detected with peroxidase-labeled anti-human sIgA as described for a previously published dot blot assay (17).

Dot blot and Western blot assays were carried out on the basis of a previously published method (17). Crude EAEC extract was prepared by boiling a bacterial suspension in electrophoretic sample buffer containing 2-mercaptoethanol, and then outer membrane protein (OMP) extraction was performed. The crude EAEC extract or OMP fraction at approximately 2 to 4 mg of protein/ml in electrophoresis sample buffer was subjected to sodium dodecyl sulfate (SDS)-polyacrylamide electrophoresis by the method described by Laemmli (6). The protein components within the polyacrylamide slab gels were transferred to nitrocellulose sheets by the method of Towbin et al. (14).

The sIgA in five of the paired stool samples bound to the respective homologous E. coli strain (Table 1) when the samples were screened for an immune response by a dot blot method. Three patients had sIgA to the homologous EAEC strain only at 5 days after clinic presentation but not on the day of presentation. The two other patients (patient 14031 and 14085) had sIgA directed to the homologous EAEC on the day of presentation as well as at 5 days after presentation for their diarrheal illness (Table 1). It is possible that these two patients may already have had antibodies to EAEC prior to the current episode of illness or they may have rapidly developed an intestinal immune response early in the illness.

Samples with sIgA to the homologous strain also had sIgA directed to strain 37054. Two of the stool extract samples showed sIgA reactions to JM221 strain. None of the 10 paired samples reacted to a normal nonaggregative control E. coli strain (Table 1).

The five stool extract samples that were positive for EAEC antibodies by dot blotting were examined for sIgA binding to EAEC crude antigens and the OMP fraction of strain 37054 by the Western blot method. Strain 37054 was used because it has the epitopes also present on the homologous strains. Two of
the five samples were demonstrated to have sIgA to EAEC antigens in the Western blot studies (Table 2 and Fig. 1). Sample 37034p reacted predominantly to the 15-kDa antigen in both the crude antigen preparation and the OMP fraction. Sample 47807p recognized multiple antigens of approximately 20, 45, and 85 kDa in the crude antigen preparation and antigens 20 of 45 kDa in the OMP fraction.

We found a lower positivity rate by the Western blot assay than by the dot blot assay (Table 1). This discordance suggests that some of the EAEC antigens may have been denatured during antigen preparation or in the process of electrophoresis.

The SDS in the polyacrylamide gel preparation, the 2-mercaptoethanol in sample buffer, and the boiling process during sample preparation prior to loading of the sample onto the gel would cause denaturation of these antigens (4, 18). The other possibility is that important epitopes of EAEC may be glycolipids or polysaccharides instead of protein antigens.

The findings in our study suggest that strains of EAEC commonly induce an immune response in patients with traveler's diarrhea. The data provided here offer indirect evidence that the EAEC strains isolated from the patients are pathogenic. Additional studies with a larger sample size and also with additional samples collected over time should be developed.

Dot blot and Western blot techniques can be used to detect specific serum and sIgA intestinal antibody responses to virulent enteric pathogens (18). The finding of 5 of 10 paired samples with a detectable immune response to EAEC may underestimate the actual rate of the immune response in patients with diarrhea caused by EAEC. A more sensitive and reliable assay that allows the quantitative detection of low-level specific sIgA antibodies to EAEC, such as enzyme-linked immunosorbent assay, should be examined in the future.

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TABLE 1. Dot blotting results for stool extract specimen

| Specimen | Reactivity to: | Homologous strain | Strain 37054 | Strain JM221 | Control E. coli strain |
|----------|----------------|------------------|-------------|-------------|-----------------------|
|          |                |                  |             |             |                       |
| 47802a   | –              | –                | –           | –           | –                     |
| 47802p   | –              | –                | –           | –           | –                     |
| 47807a   | –              | –                | –           | –           | –                     |
| 47807p   | +              | +                | –           | –           | –                     |
| 37034a   | –              | –                | –           | –           | –                     |
| 37034p   | +              | +                | –           | –           | –                     |
| 37054a   | –              | –                | –           | –           | –                     |
| 37054p   | +              | +                | –           | –           | –                     |
| 37067a   | –              | –                | –           | –           | –                     |
| 37067p   | –              | –                | –           | –           | –                     |
| 14031a   | +              | +                | –           | –           | –                     |
| 14031p   | –              | –                | –           | –           | –                     |
| 14034a   | –              | –                | –           | –           | –                     |
| 14034p   | –              | –                | –           | –           | –                     |
| 14085a   | +              | +                | –           | –           | –                     |
| 14085p   | +              | +                | –           | –           | –                     |
| 14099a   | –              | –                | –           | –           | –                     |
| 14099p   | –              | –                | –           | –           | –                     |
| Stool extract, from healthy subject | – | – | – | – | – |

* a, fecal sample obtained at time of presentation with diarrhea; b, fecal sample obtained 5 days after presentation.

TABLE 2. Reactivity of sIgA from five patients who tested positive for crude antigen by dot blot method and OMP fraction of strain 37054 by Western blot method

| Specimen | Reactivity to antigens of 37054 strain [antigen size(s) (kDa)] |
|----------|-------------------------------------------------------------|
|          | Crude antigen | OMP fraction |
| 47807p   | + (20, 45, 85) | + (20, 45) |
| 37054p   | –              | –           |
| 37034p   | + (15)         | + (15)      |
| 14085p   | –              | –           |
| 14099p   | –              | –           |

* p, fecal sample obtained 5 days after presentation.
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