INFECTION AND IMMUNITY, Dec. 1975, p. 1475-1477
Vol. 12, No. 6

Printed in U.S.A.

Antibodies to Heat-Labile *Escherichia coli* Enterotoxin in Apaches in Whiteriver, Arizona

R. BRADLEY SACK,* NORBERT HIRSCHBORN, WILLIAM E. WOODWARD, DAVID A. SACK, AND RICHARD A. CASH

Departments of Medicine, The Johns Hopkins University School of Medicine, and The Baltimore City Hospitals, Baltimore, Maryland 21224; and The Indian Health Service Hospital, Whiteriver, Arizona 85941*

Received for publication 24 June 1975

Antitoxin titers to heat-labile *Escherichia coli* enterotoxin were measured in Apache children hospitalized with acute diarrhea and in Apaches of different age groups without diarrhea in Whiteriver, Ariz. The study suggests that in this locale, exposure to enterotoxigenic *E. coli* is probably widespread and occurs early in life. Antitoxin titer rises after diarrheal disease associated with enterotoxigenic *E. coli* infection, however, were not regularly found.

Enterotoxigenic *Escherichia coli* are now well-recognized etiological agents in the production of diarrheal syndromes ranging in severity from mild travelers' diarrhea (3, 13), to infantile diarrheas (4, 9), to cholera-like disease (8). *E. coli* are capable of elaborating at least two types of enterotoxin, one heat-labile (LT), antigenic, and cholera-like, and the other heat-stable, nonantigenic, and short acting. Both enterotoxins have been implicated in human diarrheal syndromes (8, 9, 10, 11), although LT has been more extensively studied. To date, all LT preparations of *E. coli* studied are immunologically similar and related to cholera enterotoxin (14).

It would be expected that infection with LT-producing *E. coli* would regularly be associated with a rise in antitoxin titers. Indeed, such has been the case in a cholera endemic area, Calcutta (10), and in human volunteers in whom diarrheal disease was produced by orally inoculated organisms (1). No antitoxin titers have been reported, however, in patients with naturally acquired *E. coli* diarrhea from a non-cholera area, and no data are yet available from persons without diarrhea living in such an area. The present paper provides antibody data in these groups of people.

Paired acute and convalescent sera were obtained from 18 Apache children, under 4 years of age, who were admitted with acute watery diarrhea to the Indian Health Service Hospital in Whiteriver, Ariz., during the summer of 1971. These collections were done during part of a larger study of diarrheal disease in this community (9, 15). The protocols were approved by the Committee on Human Research from the National Institutes of Health, the Johns Hopkins University, and the United States Indian Health Service, and by the White Mountain Apache Tribal Council. The clinical courses and treatment of these patients have been described elsewhere (5, 6).

The bacteriological diagnoses, including a search for the presence of enterotoxigenic *E. coli,* have also been described elsewhere (9). In addition to using standard techniques for the isolation of *Salmonella, Shigella,* and enteropathogenic serotypes of *E. coli* (2), the rabbit ileal loop technique, the infant rabbit test, and the adrenal cell tissue culture assay were employed for detection of enterotoxigenic *E. coli* (9).

Single serum specimens were obtained from 237 pediatric and adult patients seen either in the outpatient clinic or in the hospital for diverse causes of a nondiarrheal nature during the summer of 1972.

Antitoxin titers in the sera from patients hospitalized with diarrhea were assayed in the rabbit intestinal loop model (7). All of the single serum specimens were assayed by the adrenal cell tissue culture technique. Based on the neutralization of the *E. coli* LT effect on adrenal cells, this technique was a microtiter modification of one previously described (1). Adrenal cells were grown in microtiter plates (Cooke; 12); twofold dilutions of serum (0.025 ml) were incubated with equal volumes of crude *E. coli* enterotoxin (40 μg/ml) for 1 h at 37 C, added to the tissue culture (each well contained 0.2 ml of tissue culture media), and then incubated at 37 C overnight. The highest dilution of serum which completely neutralized the morphological effects of enterotoxin was considered to be the end point.

In both assays, a single crude antigen prepa-

ration from *E. coli* 408-3 (serotype O78:H12) was used as the enterotoxin standard. This was prepared by dialysis and lyophilization of culture filtrates, as previously described (14). Swiss Serum Institute cholera antiserum, which has been arbitrarily assigned the value of 1,000 U of anti-*E. coli* enterotoxin per ml (7), was used as the standard in the adrenal cell assay. A rabbit antiserum containing 295 U/ml, when compared to this standard (7), was used as the working standard in the ileal loop assay. Titers of unknown sera were based on a direct comparison with the standard antitoxin assayed simultaneously.

The results of the antitoxin titrations in children with diarrheal disease are given in Table 1. None of the three patients harboring enterotoxigenic *E. coli* developed fourfold or greater titer rises, although in one the acute titer was relatively high. Of the other 15 patients, three developed greater than fourfold rises in titer and another two greater than twofold titer rises, although no enterotoxigenic *E. coli* had been isolated from their gastrointestinal specimens.

TABLE 1. *Serum antitoxin titers against E. coli LT enterotoxin in Apache children with acute diarrheal disease*

Patients	Acute	Convalescent	Interval (days)
Harboring enterotoxigenic *E. coli*			
17974[a]	42[b]	83	6
19054	7	4	10
19217	5	6	5
Harboring *S. flexneri*			
18879	<6	29[c]	11
18241	13	10	10
18841	<12	<12	25
17497	9	22	7
Harboring no known enteric pathogens			
19094	5	8	8
18879	12	49[c]	9
18899	15	17	7
19182	20	6	13
19013	11	10	7
18729	16	5	11
18501	<1	22[c]	21
19056	8	5	11
18896	43	12	17
17718	9	27	8
17999	25	>48	13

[a] Patient number.
[b] Given as units per milliliter.
[c] Greater than fourfold difference between titers.

Although the number of patients is small, these results suggest that children with enterotoxigenic *E. coli*-mediated diarrhea do not uniformly develop antitoxin titer rises, an observation which differs from previous studies (1, 10). This may be due to a relatively small antigen challenge or the relatively short interval of the convalescent specimen collection. The few children who did develop fourfold or greater titer rises did not have enterotoxigenic *E. coli* isolated from their stool specimens, which suggests that the bacteriological techniques employed were relatively insensitive. There is also the possibility that other as yet unidentified enterotoxigenic bacteria infected these patients; at present the only known cross-reactive antigen in these assays is *Vibrio cholerae* enterotoxin.

Antitoxin titers from single serum specimens unrelated to diarrheal disease are summarized in Fig. 1. Mean titers increased until age 2 years and remained essentially the same thereafter. The lowest mean titer, seen in children less than 1 year old, differed significantly from the titers of the 1 year olds ($P < 0.001$) and all the remaining groups ($P < 0.001$). The mean titers in the 1 year olds were also significantly less than in the 2 year olds ($P < 0.001$) and in all other age groups ($P < 0.01$).

These seroepidemiological data suggest that exposure to enterotoxigenic *E. coli* is fairly widespread and occurs early in life. Previous clinical epidemiological studies in this locality by Woodward et al. (15) have shown that diarrheal diseases occur with highest frequency in children under 2 years old. Although severe diarrhea in adults in this population was unusual (15), one elderly Apache woman was hospitalized with severe enterotoxigenic *E. coli*-

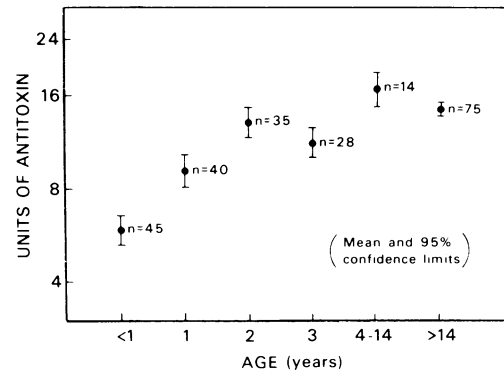

FIG. 1. *Antitoxin titers against E. coli LT enterotoxin in Apaches in Whiteriver, Ariz., grouped according to age. Antitoxin titers are given as units per milliliter.*

associated diarrhea during the course of this study (9). The observation that serum antitoxin titers remain at the same level in adults also suggests that continued exposure may be occurring throughout life.

This work was supported by Public Health Service contract 71-2260 from the National Institute of Arthritis, Metabolism and Digestive Diseases, by Public Health Service grant 5 R22 AI07625 from the National Institute of Allergy and Infectious Diseases, and by U. S. Army contract DADA 17-73-C-3055.

The technical assistance of Jean Froehlich and Robert Borchardt is gratefully acknowledged. Acknowledgment is made to the Gerontology Research Center, National Institute on Aging, for facilities extended under its Guest Scientist Program.

LITERATURE CITED

1. Donta, S. T., D. A. Sack, R. B. Wallace, H. L. Dupont, and R. B. Sack. 1974. Tissue-culture assay of antibodies to heat-labile *Escherichia coli* enterotoxins. N. Engl. J. Med. 291:117-121.
2. Edwards, P. R., and W. H. Ewing. 1972. Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
3. Gorbach, S. L., B. H. Kean, D. G. Evans, D. J. Evans, Jr., and D. Bessudo. 1975. Travelers' diarrhea and toxigenic *Escherichia coli*. N. Engl. J. Med. 292: 933-936.
4. Gorbach, S. L., and C. M. Khurana. 1972. Toxigenic *Escherichia coli*. A cause of infantile diarrhea in Chicago. N. Engl. J. Med. 287:791-795.
5. Hirschhorn, N., R. A. Cash, W. E. Woodward, and G. H. Spivey. 1972. Oral fluid therapy of Apache children with acute infectious diarrhea. Lancet ii:15-18.
6. Hirschhorn, N., B. J. McCarthy, B. Ranney, M. A. Hirschhorn, S. T. Woodward, A. Lacapa, R. A. Cash, and W. E. Woodward. 1973. Ad libitum oral glucose-electrolyte therapy for acute diarrhea in Apache children. J. Pediatr. 83:562-571.
7. Sack, R. B. 1973. Immunization with *Escherichia coli* enterotoxin protects against homologous enterotoxin challenge. Infect. Immun. 8:641-644.
8. Sack, R. B., S. L. Gorbach, J. G. Banwell, B. Jacobs, B. D. Chatterjee, and R. C. Mitra. 1971. Enterotoxigenic *Escherichia coli* isolated from patients with severe cholera-like disease. J. Infect. Dis. 123:378-385.
9. Sack, R. B., N. Hirschhorn, I. Brownlee, R. A. Cash, W. E. Woodward, and D. A. Sack, 1975. Enterotoxigenic *Escherichia coli*-associated diarrheal disease in Apache children. N. Engl. J. Med. 292: 1041-1045.
10. Sack, R. B., B. Jacobs, and R. Mitra. 1974. Antitoxin responses to infections with enterotoxigenic *Escherichia coli*. J. Infect. Dis. 129:330-335.
11. Sack, D. A., M. H. Merson, J. G. Wells, R. B. Sack, and G. K. Morris. 1975. Human diarrhea associated with heat-stable enterotoxin producing strains of *Escherichia coli*. 1975. Lancet ii:239-241.
12. Sack, D. A., and R. B. Sack. 1975. Test for enterotoxigenic *Escherichia coli* using Y1 adrenal cells in miniculture. Infect. Immun. 11:334-336.
13. Shore, E. G., A. G. Dean, K. J. Holik, and B. R. Davis. 1974. Enterotoxin-producing *Escherichia coli* and diarrheal disease in adult travelers: a prospective study. J. Infect. Dis. 129:577-582.
14. Smith, N. W., and R. B. Sack. 1973. Immunologic cross-reactions of enterotoxins from *Escherichia coli* and *Vibrio cholera*. J. Infect. Dis. 127:164-170.
15. Woodward, W. E., N. Hirschhorn, R. B. Sack, R. A. Cash, I. Brownlee, G. H. Chickadonz, L. K. Evans, R. H. Shepard, and R. C. Woodward. 1974. Acute diarrhea on an Apache Indian reservation. Am. J. Epidemiol. 99:281-290.