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Antigenic Similarity of Heat-Labile Enterotoxins from Diverse Strains of *Escherichia coli*

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With use of the rabbit intestinal loop model, heat-labile enterotoxins from 21 *Escherichia coli* strains isolated from a wide spectrum of patients with diarrheal diseases were all neutralized to high titer by two antisera prepared against enterotoxins of either *E. coli* or *Vibrio cholerae*. These findings suggest marked immunological similarity among heat-labile enterotoxins from a heterogenous group of *E. coli*.

Vibrio cholerae enterotoxin and the heat-labile enterotoxins (LTs) of *Escherichia coli* have been found to be similar physiologically (2, 9, 16), immunologically (12, 26), and biochemically (1). The enterotoxins produced by either of the two serotypes of *V. cholerae* (Inaba and Ogawa) are identical (7); similarly, the crude LTs produced by different *E. coli* serotypes are known to be at least immunologically related (12, 26). Whereas enterotoxin production is controlled by the chromosomal deoxyribonucleic acid of *V. cholerae* (27), enterotoxins of *E. coli* are genetically controlled by plasmids (13, 24, 25). Because of the assumed closely related antigenic structure of *E. coli* LTs, it has become the practice (3, 15, 22, 23) to use a single LT preparation to test for antitoxins that develop during infections caused by a variety of serotypes of enterotoxigenic *E. coli* (ETEC). Only a relatively few human strains of ETEC have been closely studied, however; these are mostly from the Indian subcontinent (4-6, 10-12, 26).

The present study was undertaken to test the hypothesis that LTs produced by *E. coli* of different serotypes, isolated from either children or adults with diarrheal disease in widely separated geographic areas, are immunologically similar.

MATERIALS AND METHODS

Bacterial cultures. Twenty-one LT-producing *E. coli* isolated from patients who had diarrheal illness between 1968 and 1975 were studied. Some of the organisms produced heat-stable enterotoxin (ST) in addition to LT. Cultures were either maintained in nutrient agar stabs at room temperature or lyophilized. Serotyping was kindly done by F. Ørskov and I. Ørskov at the World Health Organization Collaborative Center for Reference and Research on Escherichia, Copenhagen (17).

Enterotoxin preparations. Crude LT preparations from each of the strains were prepared in Syncase by methods previously described (18, 26). These dialyzed, lyophilized preparations were assayed in the 18-h rabbit intestinal loop model (14, 26) to determine the mean effective dose (ED_{50}) as a function of dry weight. The slopes of the dose-response curves were similar to those previously described (18, 19, 26).

Antisera. Two antisera were used to neutralize the biological activity of these preparations. The antisera were: (i) purified equine cholera antitoxin prepared at the Swiss Serum Institute (SSVI) and supplied by the National Institutes of Health, and (ii) anti-*E. coli* serum prepared in rabbits against a single LT preparation of strain 408-3 (O78:H12), as previously described (18). The capacity of each antiserum to neutralize each of the LT preparations was determined in the rabbit intestinal loop model. Twofold dilutions of serum were mixed with 3 ED_{50} of LT and shaken gently (60 shakes/min) for 1 h at 37°C. The antigen-antibody mixtures were then injected into rabbit intestinal loops (26), and fluid accumulation was read at 18 h. The end point of the titration was the highest dilution of serum that neutralized the fluid accumulation response by more than 50%. The titration curves were steep, and the end point usually represented complete neutralization. The geometric mean of at least two neutralization tests for each assay was calculated. (In most instances, three tests were done for each determination.)

RESULTS

Thirteen of the ETEC were from adults and eight were from children; one porcine strain is given for comparison. There were 11 different known "O" serogroups, one rough strain, and three untypable strains represented. Eight strains were from the Indian subcontinent and have been widely used in a number of laboratories (10407, 408-3, 339, 411-5). One was from the Middle East, one from Europe, eight from the United States, and three from Mexico. Nine were from patients hospitalized with nonspe-

cific diarrhea (21), and four were from adults with traveler's diarrhea (15). Eleven strains produced both LT and ST; 10 produced LT only. The pig strain has been well characterized previously (16).

The results of the neutralizations are summarized in Table 1. Both anti-cholera and anti-*E. coli* neutralized each of the LT preparations to high, though somewhat variable, titers. The anti-cholera serum neutralized all but one of the LT preparations at higher dilutions than did the anti-*E. coli* antiserum, by an average factor of 5 (range, 0.97 to 12.6). Not shown on the table are neutralization titers of these sera assayed in identical fashion against crude *V. cholerae* enterotoxin, National Institutes of Health lot 001. The SSVI serum had a titer of 25,000, and the rabbit antiserum had a titer of 40, a strikingly different factor difference of 625 (26).

These neutralization titers should not be confused with standard antitoxin units previously assigned to these antisera. SSVI has been assigned the value of 1,000 U/ml against *E. coli* 408-3 LT (18), and the rabbit *E. coli* antiserum assayed at 280 U/ml against *E. coli* 408-3 LT (18); this difference in titers (a factor of 3.6) is also similar to that described from different enterotoxin preparations in this study.

The biological activity of the LT preparations also varied over a 20-fold range. There were no significant differences, however, between (i) strains from children versus adults, (ii) strains producing LT only versus strains producing both LT and ST, (iii) strains from different geographical areas, or (iv) strains isolated from adults with cholera-like illness or traveler's diarrhea.

DISCUSSION

This study corroborates previous information that LTs from the majority of (if not all) ETEC are immunologically closely related. Since this antibody titration is based solely on neutralization of biologically active enterotoxin, the presence of naturally occurring toxoids or different amounts of enterotoxin subunits might cause considerable variation in the titer. This variation is not incompatible, however, with the possibility that the LTs may all be identical. These findings support the use of a single antigen to

TABLE 1. *Neutralization of E. coli LTs by antiserum prepared against enterotoxins of E. coli or V. cholerae*

Strain	Serotype	Adult (A) or child (C)	Geography	Clinical illness	Toxin(s)	LT ED_{50}	Neutralizing antitoxin titer[a]	
							Anti-*E. coli* rabbit serum	SSVI anti-cholera serum
10407	O78:H11	A	Dacca, Bangladesh	CLI[b]	LT-ST	0.096	640	2500
408-3	O78:H12	A	Calcutta, India	CLI	LT-ST	0.125	783	2,000
339	O15:H11	A	Calcutta, India	CLI	LT-ST	0.082	160	625
J3-2	O85:H7	A	Calcutta, India	CLI	LT-ST	0.15	320	1,580
106-3	O78:H11	A	Calcutta, India	CLI	LT	0.200	640	625
411-5	O126:H12	A	Calcutta, India	CLI	LT	0.053	393	1,986
29487	O128:H21	A	Dacca, Bangladesh	CLI	LT-ST	0.75	1,416	5,000
1105-F	O6:H16	A	Calcutta, India	CLI	LT	0.54	500	3,784
I-439	O114:H−	A	Sana'a, Yemen	CLI	LT-ST	0.074	500	2,500
P-3	O8:H9	A	Baltimore[c]	TD[d]	LT	0.28	393	625
G-14850	O6:H16	C	Topolčany, Czechoslovakia	NSG[e]	LT	1.05	62.5	ND[f]
Deh-3	(O109):H21	C	Arizona	NSG	LT	0.20	1,000	ND
Joh-4	O64:H−	C	Arizona	NSG	LT	0.302	500	6,295
Lee-1	X	C	Arizona	NSG	LT-ST	0.31	314	1,574
Eth-6	O25:H−	C	Arizona	NSG	LT	0.165	198	1,574
Coo-10	O25:H−	C	Arizona	NSG	LT	0.06	314	993
Cos-6	X	C	Arizona	NSG	LT-ST	0.14	706	3,140
Wcy-4	X	C	Arizona	NSG	LT-ST	0.35	250	1,574
206 C-2	Rough	A	Mexico City, Mexico	TD	LT	0.60	500	3,148
412 C-1	O6:H16	A	Mexico City, Mexico	TD	LT-ST	0.10	500	1,766
260 C-1	O6:H16	A	Mexico City, Mexico	TD	LT-ST	0.057	250	1,250
P-263[g]	O8:K87,K88a,b,:H19				LT	0.20	160	1,250

[a] Reciprocal of geometric mean dilution of serum that neutralized 3 ED_{50} of LT.
[b] Cholera-like illness.
[c] Recently returned from Pakistan.
[d] Traveler's diarrhea.
[e] Nonspecific gastroenteritis.
[f] ND, Not done.
[g] Porcine strain.

measure antibody directed against LT produced by diverse isolates of ETEC.

The observation that the biological activity of the different LT preparations is similar, regardless of the origin of the strain, suggests that the differences in severity of diarrheal illness seen in patients may be largely a function of the human host, or possibly other non-LT bacterial factors, rather than that of the LT itself.

It should be pointed out that this study was begun at a time before the development of the tissue culture assays. Although the rabbit loop model is cumbersome and time-consuming, we believed it worthwhile to complete the study with a single assay. There is every reason to suspect that results from other assay systems would be similar (3, 22).

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