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Pathogenic Properties of *Campylobacter jejuni*: Assay and Correlation with Clinical Manifestations

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The pathogenic properties of 20 strains of *Campylobacter jejuni* isolated from persons with clearly defined clinical manifestations were determined. Cell-free broth filtrates were examined for (i) enterotoxin production by Chinese hamster tissue culture assay and an enzyme-linked immunosorbent assay (ELISA) employing GM1 ganglioside and affinity-purified antiserum to *Escherichia coli* heat-labile toxin, (ii) cytotoxin production by Vero and HeLa cell tissue culture lines, and (iii) their ability to cause fluid secretion in rat ligated ileal loops. Viable bacteria were examined for invasive properties by an ELISA with the immunoglobulin fraction of antiserum to Formalin-killed bacteria of an invasive strain, and by their effect on fluid secretion and morphology in rat ligated ileal loops. None of the eight isolates obtained from asymptomatic carriers had any detectable pathogenic properties. All six strains isolated from persons with bloody invasive-type diarrhea elaborated a cytotoxin; their viable bacteria had high titers in the ELISA for invasive properties and caused fluid secretion in ligated ileal loops, although consistent morphologic abnormalities and evidence of mucosal invasion, examined by immunofluorescence techniques, were not detected. All six strains isolated from persons with watery secretory-type diarrhea produced an enterotoxin, one elaborated a cytotoxin, and broth filtrates of all strains caused fluid secretion in ligated ileal loops; viable bacteria had low titers in the ELISA for invasive properties and evoked fluid secretion in ligated loops by means of enterotoxin production. These observations show (i) that a correlation exists between the pathogenic properties of the infective *C. jejuni* strain and gastrointestinal manifestations in the infected host, and (ii) that these pathogenic properties can be identified by in vitro assays, including ELISAs.

Campylobacter jejuni is now recognized to be a common cause of acute diarrheal disease, occurring in sporadic form among persons living in temperate or tropical climates (2, 8, 13, 56), in visitors to developing countries (46, 53, 55), and in epidemic form (37, 43, 47, 58). It is also, together with enterotoxigenic *Escherichia coli* and rotavirus, one of the principal causes of acute diarrheal disease among young children in developing countries (5, 9, 15). Despite this, information is scant regarding the properties of *C. jejuni* that are pathogenic for the intestinal tract, how these properties can be assayed, and how they relate to the development of specific pathophysiologic abnormalities of the intestinal tract and clinical manifestations in infected persons.

Three potentially pathogenic properties have been identified for *C. jejuni*: invasiveness, enterotoxin production, and cytotoxin production.

Invasiveness is compatible with the occurrence of bloody diarrhea, often associated with endoscopic evidence of colitis or bacteremia, in some infected persons (2, 8, 29, 32). A few strains that were tested by the Sereny test have been negative (17, 34). Intestinal infection with *C. jejuni* has been shown to produce inflammatory lesions in the bowel of certain experimental animal models, including young chickens (51, 54, 59), mice (4, 24), hamsters (20), rabbits (10), calves (1), and gnotobiotic dogs (48). In some instances, bacteremia was detected (4, 10, 24, 54), but only a few studies have demonstrated definite invasion of the intestinal mucosa with immunofluorescence techniques or electron microscopy (20, 51, 59).

One third of *C. jejuni* isolates in South India (35) and 75% of strains isolated in Mexico (52) from young children with secretory-type diarrhea produce an enterotoxin that is heat-labile, stimulates cyclic AMP, and is structurally and immunologically related to both cholera toxin and *E. coli* heat-labile toxin (LT) (25–27, 36, 52).

Some *C. jejuni* isolates produce a cytotoxic response in various tissue culture systems, including Vero, HeLa, MRC-5, and Hep-2 cell lines (21, 38, 60). The pathogenic significance of this cytotoxin has not been evaluated.

The main reason that so little is known about the relationship between specific pathogenic properties of *C. jejuni* isolates and pathophysiologic processes and clinical manifestations in the infected human host has been the unavailability of relatively simple but specific in vitro tests for identifying these properties. Several different techniques have been developed for serotyping *C. jejuni* (30, 45, 50); the application of serotyping, as well as chromosomal restriction endonuclease digest analysis, has proven helpful in epidemiological investigations (12, 22, 44, 45), but these approaches do not define pathogenic properties. Similarly, the technique of determining the response to peroral contamination of various species of experimental animals with strains of *C. jejuni* is cumbersome, it has been found to yield variable responses within the same animal species, and it does not appear to be a sensitive means of discriminating the pathogenic properties of test strains. Sanyal et al., for example, could not detect any correlation between the source of a strain and its pathologic effect in infant chickens (54).

In the present study, we investigated the pathogenic properties of *C. jejuni* strains isolated from persons who had a clearly defined clinical status: they were asymptomatic or had either invasive-type bloody diarrhea or secretory-type watery diarrhea. Cell-free broth filtrates of the isolates were examined for enterotoxin production by means of an en-

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zyme-linked immunosorbent assay (ELISA) and by Chinese hamster ovary (CHO) tissue culture assay, for cytotoxin production with Vero and HeLa tissue culture lines, and for their ability to cause fluid secretion in rat ligated ileal loops. Invasive properties were assayed by testing the effect of viable bacteria in an ELISA with antiserum to killed bacteria of an invasive strain and in rat ligated ileal loops. Our results show that a correlation exists between the presence or absence of specific pathogenic properties of an individual isolate and the clinical status of the infected host, and that these pathogenic properties can be identified by relatively simple in vitro assays.

MATERIALS AND METHODS

Bacterial strains. The 20 strains examined were not consecutively received strains in this laboratory, but rather they were selected for study on the basis of the fact they had been isolated from persons with clearly defined clinical manifestations. They are identified as those isolated from (i) asymptomatic persons, (ii) persons with invasive-type diarrhea who had bloody stools and in whom the presence of colitis (mucosal erythema and friability) was demonstrated by endoscopic examination, and (iii) persons with secretory-type diarrhea who had only copious watery stools. Information concerning the presence or absence of fecal leukocytes was not available in all instances. All strains were fecal isolates, except for strain H1, which was cultured from blood. Four of the 20 strains were negative for hippurate hydrolysis (19), which suggests that they may be *Campylobacter coli* rather then *C. jejuni*, although DNA hybridization studies have shown that some *C. jejuni* strains are hippurate negative (18).

Five strains (labeled C) were isolated from persons in Rochester, N.Y.; 10 strains (labeled V) isolated from persons in South India were kindly provided by V. I. Mathan, Christian Medical College Hospital, Vellore, India; 4 strains (79-102, H1, 79-193, Case) were kindly provided by M. J. Blaser, Denver Veterans Administration Medical Center, Denver, Colo.; and strain INN73-83, isolated from a Mexican child, was kindly provided by G. M. Ruiz-Palacios, Instituto National de la Nutrión, Tlalpan, Mexico.

Bacterial growth. Strains were grown at 42°C for 24 h in GC medium (Difco Laboratories, Detroit, Mich.) supplemented with 1.0% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) under agitated conditions in the presence of 8% CO_2 and, for broth filtrates only, treated for the final 10 min with 2 mg of polymyxin per ml as described before (25). Cell-free broth filtrates were obtained by centrifugation at 13,000 × *g* for 10 min and passage of the supernatant through a 0.22-μm membrane (Millipore Corp., Bedford, Mass.); they were stored at 4°C and assayed within 2 weeks.

Assays for enterotoxin. Unconcentrated broth filtrates were tested (i) in the CHO assay by a modification (11) of the techniques of Guerrant et al. (16), except that 96-well microtiter plates (Costar Inc., Cambridge, Mass.) were used. All samples were tested in duplicate with working volumes of 100 μl per well. Morphologic alteration (elongation) of ≥50% of the cells in a well was considered a positive response. (ii) In an ELISA in which GM1 ganglioside (Supelco Inc., Bellafonte, Pa.) was used for the solid phase and alkaline phosphate-conjugated affinity purified goat antiserum to LT was the antibody. The amount of *C. jejuni* enterotoxin present was quantitated by comparing the response of the broth filtrates to that of pure LT in these assays, and the results are expressed as nanograms of *C. jejuni* enterotoxin per milligram of protein as described before (25).

Assays for cytotoxin. The Vero cell assay was performed by a modification of the method of Konowalchuk et al. (28). Freshly trypsinized cells were diluted 1:5 in Eagle minimal essential medium plus 2% fetal calf serum; 100-μl samples were placed in 96-well microtiter plates to which were added serial 2-fold dilutions of broth filtrates concentrated 10-fold by passage through a YM-10 ultrafiltration membrane (Amicon Corp., Danvers, Mass.). The plates were incubated for 18 h at 36°C in 5% CO_2 and then fixed with methanol and stained with Giemsa stain. The results are reported as the last dilution that showed ≥50% rounding of cells.

The HeLa cell assay was performed by the dye-release method of Gentry and Dalrymple (14). Serial 10-fold dilutions of unconcentrated broth filtrates were made with GC medium, 25-μl samples were placed in microtiter wells, to which were added 100-μl sample of freshly trypsinized cells (2.4×10^5 cells per ml). The plates were incubated for 18 h at 36°C in 5% CO_2. The cells were then fixed with Formalin and stained with crystal violet stain, and the 50% cell-detachment dilution was determined.

Rat ligated ileal loops. Samples (0.5 ml) of the unconcentrated broth filtrate were instilled for 16 h into single 10-cm-long ligated ileal loops of fasting 175- to 200-g Sprague-Dawley rats as described previously (25). The results reported for each broth filtrate are the mean ± standard error of the mean for the volume/length ratio expressed in microliters of fluid per centimeter of intestine in three or four rats. The volume/length ratio in 10 rats given either saline or growth medium was consistently <50 μl/cm.

Tests for invasiveness. (i) ELISA. Strains were tested by ELISA with antiserum to Formalin-killed bacteria of *C. jejuni* strain 79-102, which was isolated from a person with bloody invasive-type diarrhea. A 2-ml sample of bacteria was mixed with 2 ml of Freund complete adjuvant, and 0.5 ml of this mixture was given both subcutaneously and intramuscularly in four quadrants to a New Zealand White rabbit, followed by four boosters with Freund incomplete adjuvant given at the same sites at 2-week intervals. The immunoglobulin fraction of antiserum collected 2 weeks after the last immunization was obtained by passing it through a CM Affi-Gel Blue column (Bio-Rad Laboratories, Richmond, Calif.), followed by ammonium sulfate precipitation of the optically identified, unbound elution fractions by procedures defined by the manufacturer (Bio-Rad bulletin 1092). The protein concentration of the pooled eluates was determined by the method of Lowry et al. (33) and adjusted to 2 mg/ml.

Each test strain was grown as described above, washed once with ELISA coating buffer, resuspended at 1/10 the original volume in this buffer, and used as the solid phase to which was added serial twofold dilutions of an initial concentration of 0.1 mg of the immunoglobulin fraction (corresponding to an initial dilution of 1:20) as the first antibody and alkaline phosphatase-conjugated goat anti-rabbit IgG as the second antibody. Optical densities were determined with a model EL 307 ELISA reader (Bio-Tek Instruments, Burlington, Vt.), and the final dilution which yielded an optical density of ≥0.200 was determined.

(ii) Rat ligated ileal loops. Test strains were grown as described above, centrifuged, and reconstituted in growth medium to a 15-fold concentration which contained 10^8 viable organisms per ml; 0.1-ml samples were instilled for 16 h into single ligated loops in three or four rats, and the

TABLE 1. Assay of pathogenic properties of *C. jejuni*

Type of diarrhea	Strain	Hippurate	Cell-free broth filtrates					Viable bacteria	
			Enterotoxin[a]		Cytotoxin[b]		Rat loops[c]	ELISA[d]	Rat loops[c]
			ELISA	CHO	Vero	HeLa			
None	C006	+	0	0	0	0	28 ± 14	40	18 ± 11
None	V136	+	0	0	0	0	10 ± 10	160	17 ± 17
None	V79	+	0	0	0	0	15 ± 8	40	17 ± 9
None	V86	+	0	0	0	0	7 ± 7	20	18 ± 9
None	V145	+	0	0	0	0	11 ± 5	80	3 ± 3
None	V1	−	0	0	0	0	13 ± 13	40	20 ± 10
None	V59	−	0	0	0	0	32 ± 10	160	24 ± 6
None	V4	−	0	0	0	0	15 ± 15	20	25 ± 12
Invasive	79-102	+	0	0	6.3	631	10 ± 10	2,560	222 ± 6
Invasive	H1	+	0	0	0.4	63	22 ± 13	5,120	159 ± 13
Invasive	C009	+	0	0	0.4	6	28 ± 3	1,280	166 ± 13
Invasive	79-193	+	0	0	1.6	32	15 ± 15	1,280	196 ± 4
Invasive	Case	+	0	0	1.6	50	10 ± 6	2,560	210 ± 9
Invasive	C018	+	0	0	0	16	17 ± 7	5,120	105 ± 5
Secretory	C003	+	5.1	4.4	0	0	289 ± 1	20	267 ± 5
Secretory	C004	+	14.9	8.3	0	0	295 ± 14	80	270 ± 10
Secretory	V48	+	22.4	23.0	0	0	263 ± 4	80	262 ± 2
Secretory	V39	+	20.9	46.4	0	0	251 ± 5	80	279 ± 12
Secretory	V58	−	22.2	21.3	0	0	265 ± 15	40	321 ± 1
Secretory	INN 73-83	+	58.2	44.7	1.6	1,995	321 ± 1	160	276 ± 4

[a] Nonograms of enterotoxin per milligram of protein as determined by comparing the response of the broth filtrates to that of *E. coli* LT in (i) an ELISA that used GM1 as the solid phase and affinity pure antiserum to LT as the antibody, and (ii) the Chinese hamster ovary (CHO) tissue culture assay.

[b] Values are the reciprocals of the maximum dilutions that yielded rounding of ≥50% Vero cells or 50% cell death, as determined by the dye release method, of Hela cells.

[c] Fluid secretion expressed as mean ± standard error of the mean volume/length ratio in microliters per centimeter.

[d] Values are the reciprocals of the maximum dilutions of antibody that yielded an optical density of ≥0.200 in an ELISA that used viable bacteria for the solid phase and the immunoglobulin fraction of rabbit antiserum to Formalin-killed bacteria of invasive strain 79-102 as the antibody.

amount of fluid secretion was determined. Swiss roll preparations of the intestinal loops were fixed in 10% buffered Formalin and embedded in paraffin, and 4-μm sections were stained with hematoxylin and eosin and with Warthin-Starry stain for bacteria. These sections and 8 to 10-μm frozen sections were also exposed for 2 h at 36°C to a 1:10 dilution of the immunoglobulin fraction of rabbit antiserum to killed bacteria of *C. jejuni* invasive strain 79-102, followed by incubation for 1 h at 36°C with a 1:20 dilution of goat anti-rabbit immunoglobulin G conjugated with fluorescein isothiocyanate.

Immunization procedures. Rats were given primary immunization intraperitoneally with 200 μg of LT B subunit of Freund complete adjuvant followed by two peroral boosters of 1 mg of LT B subunit, given 2 h after the peroral administration of 50 mg of cimetidine (Tagamet; Smith Kline and French Laboratories, Carolina, P.R.) per kg of body weight, at 4-day intervals. They were challenged by the instillation of viable bacteria 4 to 6 days after the final booster immunization as described previously (27).

RESULTS

Strains from asymptomatic persons. None of the broth filtrates of these eight strains produced an enterotoxin, had a cytotoxic effect in Vero or HeLa cells, or caused fluid secretion in rat ligated ileal loops (Table 1). Titers for viable bacteria in the ELISA for invasive properties were all ≤1:160, and the instillation of these bacteria did not evoke fluid secretion, show evidence of invasion, or cause structural abnormalities in rat ligated ileal loops.

Strains from persons with invasive-type diarrhea. None of the strains from persons with invasive-type diarrhea produced enterotoxin, but all six elaborated a cytotoxin detected in low titers of concentrated broth filtrates by Vero cells and in somewhat higher titers of unconcentrated broth filtrates by HeLa cells (Table 1). We have previously reported that the cytotonic effect of *C. jejuni* enterotoxin in CHO cells can be neutralized by incubation with antiserum to LT (25, 26). The application of the same procedures to broth filtrates of these strains showed that antiserum to LT does not modify their cytotoxic effect in Vero cells. None of the broth filtrates of these strains evoked a fluid response in rat ligated ileal loops.

The titers for viable bacteria of these strains in the ELISA for invasive properties were all ≥1:1,280. The instillation of viable bacteria of each strain evoked fluid secretion in rat ligated ileal loops. The mean ± standard error of the mean fluid secretion of 186 ± 10 μl/cm for this entire group was significantly ($P < 0.001$ by Student's t test for two independent means) greater than the value of 18 ± 4 μl/cm for the group given viable strains from asymptomatic persons. Histologic sections of rat ileal loops from this group showed no consistently present structural abnormalities of either the enterocytes or the lamina propria. Sections processed with the Warthin-Starry stain showed occasional bacteria within the enterocytes of some loops, and immunofluorescence studies of frozen sections identified scattered *C. jejuni* within enterocytes in some loops, but none within the lamina propria (Fig. 1).

Strains from persons with secretory-type diarrhea. All six strains from persons with secretory-type diarrhea produced varied quantities of enterotoxin (Table 1). The broth filtrate of one strain also had a cytotoxic effect in both Vero and

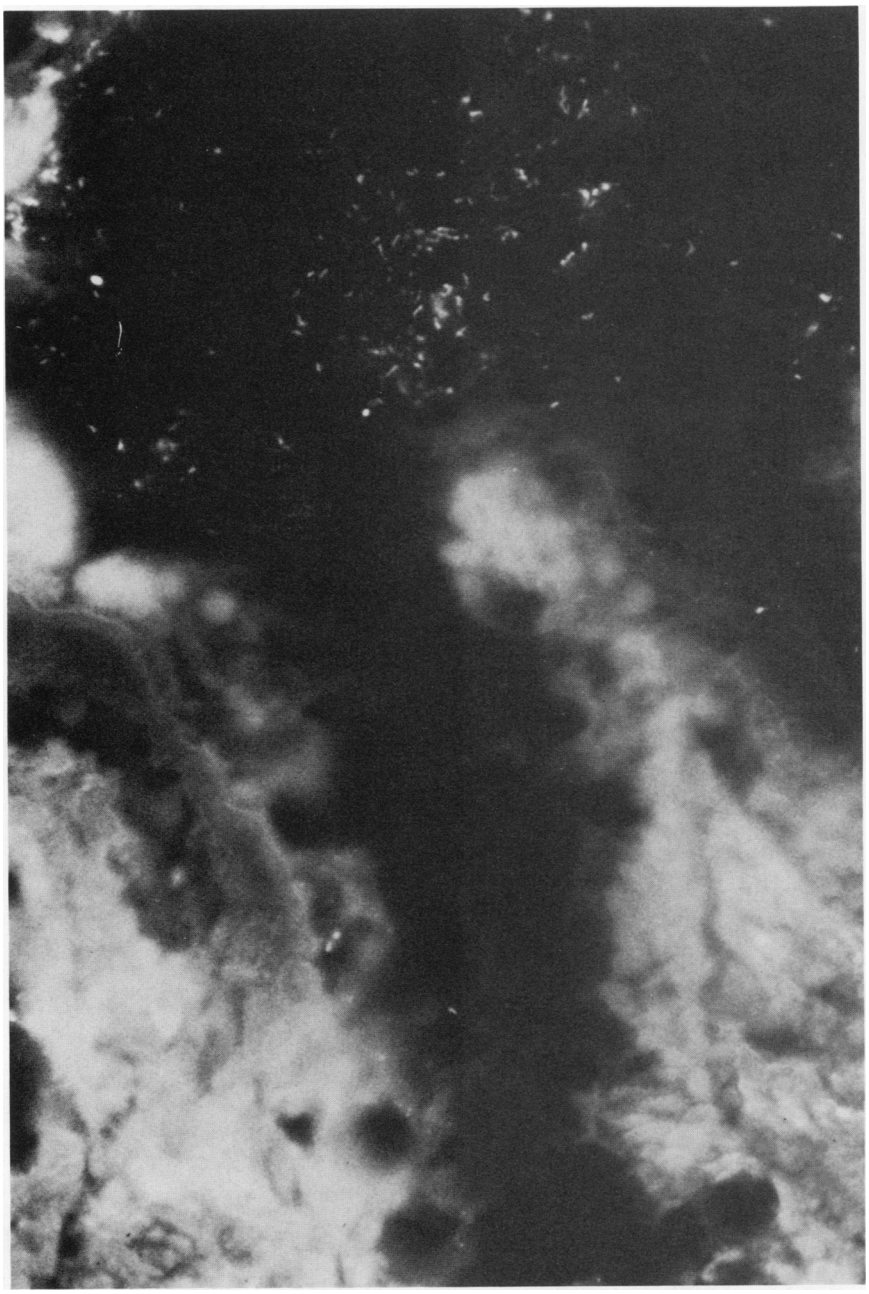

FIG. 1. Indirect immunofluorescence of a frozen section of rat ligated ileal loop that was exposed for 16 h to *C. jejuni* invasive strain 79-193. Villus structure is normal and *C. jejuni*, identified by their reaction with an immunoglobulin preparation of antiserum to Formalin-killed bacteria of an invasive strain, are located mostly within the lumen with a few scattered within enterocytes, but none in the lamina propria.

HeLa cells. Broth filtrates of all strains evoked fluid secretion in rat ligated ileal loops.

The titers for viable bacteria in the ELISA for invasive properties were all ≤1:160. The instillation of viable bacteria of these strains consistently caused fluid secretion. The mean ± standard error of the mean fluid secretion for this entire group, 276 ± 6 μl/cm, was significantly ($P < 0.001$) greater than the value of 186 ± 10 μl/cm for the group of rats given viable bacteria of the invasive strains. No morphologic abnormalities or evidence of bacterial invasion were seen in histologic sections of rat ligated ileal loops in this group.

Pathogenesis of fluid secretion. Since viable bacteria of both enterotoxigenic and invasive strains caused fluid secretion in rat ligated ileal loops, we sought to determine whether separate pathophysiologic mechanisms could be established for this by determining the response to immunization with *E. coli* LT B subunit. Fluid secretion was significantly ($P < 0.001$) reduced by 53% less than the value for similarly challenged unimmunized controls in rats challenged with enterotoxigenic strain INN 73-83, but it was unaffected in rats challenged with invasive strain 79-102 (Table 2).

Identification of pathogenic properties by ELISAs. The procedures used for both ELISAs involved testing serial dilutions of materials, broth filtrates for enterotoxigenicity

and antibody for invasive properties. Since this approach is too cumbersome for general clinical application, we determined whether these ELISAs can detect pathogenic properties equally well with fixed standard conditions. Unconcentrated broth filtrates were tested for enterotoxigenicity in an ELISA that used 1 μg of alkaline phosphatase-conjugated, affinity-purified rabbit antiserum to LT, and 10-fold concentrates of viable bacteria were tested for invasive properties in an ELISA that used 16.6 μg of the immunoglobulin fraction of rabbit antiserum to killed bacterial cells of invasive strain 79-102. These ELISAs detected the pathogenic properties that had been identified by the other approaches in every instance (Fig. 2).

DISCUSSION

Using the clinical status of the person from whom the particular strain of *C. jejuni* was isolated as our reference point, we found a correlation between pathogenic properties of specific isolates and the clinical status of the infected host. None of the strains isolated from asymptomatic persons had any detectable pathogenic properties. All of the isolates from persons with bloody diarrhea produced a cytotoxin, and their viable bacteria showed evidence of invasive properties as detected both by ELISA and their secretory effect in rat ligated ileal loops. All strains isolated from persons with secretory-type diarrhea produced the enterotoxin; one also elaborated the cytotoxin, but none showed invasive properties. We recognize that the groups studied were small and that the clinical manifestations of *C. jejuni* infection are not always as clearly cut as in the subjects selected for inclusion in this study. It is quite possible, therefore, that more extended studies of this nature will reveal a less consistent correlation, and also that more isolates will be identified which possess multiple pathogenic properties, such as the single strain that we found that produces both enterotoxin and cytotoxin. Ruiz-Palacios et al., for example, have described a strain isolated from a person with bloody invasive-type diarrhea that both produces enterotoxin and is invasive in young chickens (51, 52).

Other factors appear to be relevant to the clinical response of the host in addition to the pathogenic property of the infecting strain of *C. jejuni*. The isolation rate of *C. jejuni* in the Indian subcontinent is approximately the same (between 15 and 25%) among children with acute diarrhea and those who are asymptomatic (5, 15, 49). The prevalence of enterotoxigenic strains has been found to be the same, about one-third, among isolates obtained both from sick and asymptomatic Indian children (35), although the prevalence of enterotoxigenic strains has been found to be much greater among isolates from Mexican children with acute diarrhea than among asymptomatic carriers (52). These observations suggest that factors such as host resistance also play a role in determining the clinical response to infection with pathogenic strains of *C. jejuni*. This concept is furthered by the recent observation by Blaser et al. that levels of specific serum antibody to *C. jejuni* are higher in Bangladeshi children than in Americans of similar age (3).

TABLE 2. Effect of immunization with LT B subunit on fluid secretion in rats challenged with viable bacteria of enterotoxigenic or invasive strains

Strain	Property	Fluid secretion[a]	
		Unimmunized	Immunized
INN-73-83	Enterotoxigenic	321 ± 1	131 ± 5 (53)[b]
79-102	Invasive	222 ± 6	226 ± 2 (0)

[a] Mean ± standard error of the mean fluid secretion in microliters per centimeter.

[b] Numbers within parentheses indicate the percent reduced secretion in immunized rats as compared with the value in unimmunized controls.

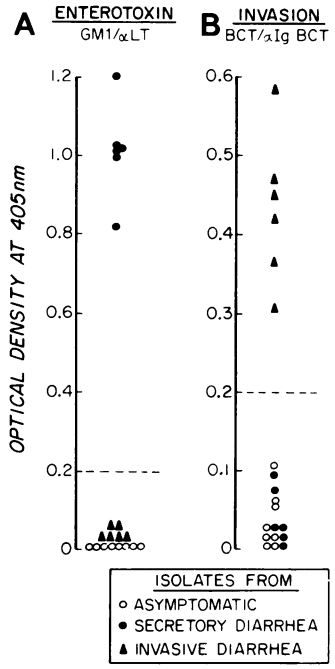

FIG. 2. ELISAs for pathogenic properties. (A) Assay for the presence of enterotoxin in broth filtrates. GM1 ganglioside was the solid phase, and 1 μg of affinity purified antiserum to *E. coli* LT was the antibody. (B) Assay for invasive properties. Viable bacteria were the solid phase, and 16.6 μg of the immunoglobulin fraction of antiserum to Formalin-killed bacteria of an invasive strain was the antibody. Values with an optical density of ≥0.200 are considered positive in each assay.

The ELISA that we used to test for invasive properties of *C. jejuni* was based on a report by Pál et al., who found that virulent, invasive strains of *E. coli* and *Shigella* spp. can be differentiated from noninvasive strains by an ELISA that uses viable bacteria as the solid phase and crude absorbed antiserum to killed bacteria of an invasive strain of *E. coli* as the antibody (42). These observations led to their suggestion that virulent invasive strains of these bacterial species have a similar or identical antigen(s) which is absent from noninvasive ones. We found that a similar ELISA with the immunoglobulin fraction of antiserum to killed bacteria of a *C. jejuni* invasive strain can also differentiate between invasive and noninvasive strains of *C. jejuni*. The identity of the cell surface antigen responsible for this is unknown; although a number of antigenic outer membrane proteins have been identified for *C. jejuni* (6, 7, 31), to date none of these has been evaluated for its relation with invasive properties.

The response of rat ligated ileal loops to the instillation of viable strains of *C. jejuni* also proved to be helpful in differentiating between nonpathogenic and invasive strains, but not between invasive and enterotoxigenic strains, since both caused fluid secretion. Our observations in rats immunized with the LT B subunit clearly showed that the process resulting in fluid secretion caused by the invasive strains is unrelated to that resulting from enterotoxin production, but the precise mechanism by which the invasive strains cause

fluid secretion in this animal model is obscure. We could detect no consistent evidence of bacterial invasion of the mucosa, and the patchy structural abnormalities seen in some loops did not appear to be sufficiently severe or consistent to account for the fluid secretion. Whether this is a reflection of the animal model or of the short duration of exposure to *C. jejuni* is uncertain; other investigators have observed definite evidence of mucosal invasion only after peroral contamination in the chicken and hamster animal models (20, 51, 59).

The role of the *C. jejuni* cytotoxin in pathophysiology is uncertain. Other laboratories have reported that this property can be demonstrated in several tissue culture lines (21, 38, 60); our observations suggest that HeLa cells are more suseptible than Vero cells, although the difference may have been a function of different number of cells used for each assay. In the case of other intestinal pathogens, some enteropathogenic strains of *E. coli*, which are considered to be the putative cause of acute secretory diarrhea, also produce a Vero cytotoxin (28), as do strains of *E. coli* O:157, which cause colitis (23, 41). O'Brien et al. have presented evidence that these cytotoxins are similar to, or identical with, Shiga toxin (39, 40). Two findings make us skeptical that the *C. jejuni* cytotoxin, which in the present study was produced primarily by invasive strains, is of pathophysiologic significance. First, titers for this cytotoxin were low; by comparison, the titer of a crude broth filtrate of *Clostridium difficile*, which was used as a positive control in the Vero and HeLa cell assays, was the range of 1:500,000. Second, whereas broth filtrates that contained enterotoxin consistently caused fluid secretion in ligated ileal loops, those containing cytotoxin never did so.

The isolation of *C. jejuni* from persons with acute diarrheal disease is generally regarded as presumptive evidence that this organism was the causative agent. Our findings indicate, however, that some human fecal isolates are not pathogenic. In view of the fact that acute diarrhea sometimes has a polymicrobial etiology (57), it may be that in some instances the *C. jejuni* isolated is nonpathogenic and not the actual causative agent. This can be clarified by demonstrating that the *C. jejuni* isolate is pathogenic by means of ELISAs for enterotoxin production and invasive properties.

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