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Encephalopathy Associated with Enteroinvasive *Escherichia coli* 0144:NM Infection

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Central nervous system manifestations typically occur with *Shigella* gastroenteritis and also in enteric *Salmonella* and *Campylobacter* infections. To date no association between enteroinvasive *Escherichia coli* infection and neurologic symptoms has been described. Two children with diarrhea caused by *E. coli* 0144:NM had otherwise unexplained encephalopathy manifested by profound stupor in one child and by obtundation and meningismus in the other one. These cases of infection occurred in northern Israel during a period of an unusually high rate of enteric infection caused by this organism. None of the microbiologic properties studied were uniquely attributable to the encephalopathic cases. The two encephalopathic as well as all eight nonencephalopathic isolates studied possessed the 140-MDa invasive plasmid. All 10 isolates examined produced small amounts of cytotoxin by the HeLa cell assay, all were nonmotile, and all had identical antibiograms. Eight of 10 of the isolates had identical plasmid profiles, while 2 isolates (from nonencephalopathic patients) had slightly different plasmid profiles. This is the first report of encephalopathy associated with enteroinvasive *E. coli*.

Enteroinvasive *Escherichia coli* (EIEC) and *Shigella* spp. share multiple properties: clinically, they both cause invasive diarrhea; biochemically, they are very similar; genetically, they share a 140-MDa plasmid essential for invasion (10, 11).

Encephalopathy (signs and symptoms of brain dysfunction) is frequently associated with gastrointestinal infection with *Shigella* spp. and occurs in 12 to 45% of hospital-based studies. The most common manifestation of *Shigella*-associated encephalopathy is seizures, while confusion, lethargy, coma, hallucinations, and other manifestations also occur (1, 3, 5, 8, 12, 15). Encephalopathy is usually reversible, but it may be fulminant and fatal (8, 16). Central nervous system (CNS) manifestations have also been associated with GI *Salmonella* and *Campylobacter* infections (3).

Although *Shigella* cytotoxins, especially Shiga toxin, which is produced mainly by *Shigella dysenteriae* type I, are thought to have neurotoxic properties, this has yet to be proven (2, 18). The mechanisms by which *Salmonella* and *Campylobacter* spp. cause neurologic symptoms are unknown. To date, no association between EIEC infection and neurologic symptoms has been described.

EIEC isolates belong to several serogroups, including O28, O29, O112, O124, O136, O143, O144, O152, O164, O167, and O173 (9). Establishing the presence of the invasive plasmid, however, is a more precise but not readily available method of identifying EIEC. Testing for the ability to invade HeLa cells or guinea pig conjunctivae is cumbersome and also is not readily available (9). Because of the difficulty in EIEC identification, routine stool cultures do not usually screen for these organisms; thus, infections caused by EIEC are often underdiagnosed.

In 1993, two children with EIEC 0144:NM-associated diarrhea were hospitalized with encephalopathy. Their isolates as well as eight EIEC 0144:NM isolates from nonencephalopathic diarrhea patients were studied.

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MATERIALS AND METHODS

Patients. Between May and August 1993, two children presented to the Pediatric Department of the Carmel Medical Center with an acute febrile diarrheal disease caused by EIEC 0144 and with otherwise unexplained encephalopathy. During this period a total of 26 EIEC 0144 isolates were identified in stool samples at the W. Hirsch Regional Microbiology Laboratory (including the two isolates from the patients mentioned above). The physicians caring for these patients were contacted and questioned about gastrointestinal and extraintestinal symptoms, as well as pertinent laboratory data. No other cases of encephalopathy were identified, and 8 of 24 isolates from patients without encephalopathy were chosen randomly for a blinded comparison with the isolates from the patients with encephalopathy.

Bacteriology. Stool samples for culture were plated onto MacConkey, Salmonella-Shigella, and selective Campylobacter media for 24 h (7, 14). Lactose-positive and nonmotile lactose-negative colonies were screened by direct agglutination with a polyvalent EIEC antiserum (Central Microbiology Laboratory, Ministry of Health, Jerusalem, Israel) containing the following anti-O antibodies: 20 ac, 112 ac, 124, 136, 143, 144, 152, 153, 159, and 164. Agglutinating *E. coli* isolates which were lysine decarboxylase and mucate negative were considered EIEC and were delineated further by monovalent O-antigen-specific antisera and H-antigen-specific antisera, as well as by standard disk diffusion assessment of antibiotic susceptibilities (13). Blood was cultured aerobically and anaerobically (BACTEC NR-660; Becton Dickinson and Co., Sparks, Md.). Cerebrospinal fluid was plated onto blood, chocolate, and MacConkey agar plates, inoculated into thioglycolate broth, and cultured aerobically.

Identification of invasive plasmid. The presence of the 140-MDa plasmid was demonstrated by PCR detection of the specific *virF* and *ipaH* loci of this plasmid in DNA extracts of the *E. coli* 0144 isolates. Previously described PCR protocols with two different pairs of primers, complementary to the *virF* and *ipaH* loci of the large *Shigella* virulence plasmid, were used (17, 20). The amplified DNA fragments were separated by electrophoresis on a 2% agarose gel, stained with ethidium bromide, and detected under UV light (17, 20).

Plasmid and chromosomal DNA analysis. (i) Plasmid profile. Plasmid DNA was extracted with the Magic TM Mini-prep DNA Purification System (Promega, Madison, Wis.), digested with restriction enzymes (*Hin*dIII [MBI Fermentas, St. Leon Rot, Germany] and *Pvu*II and *Sal*I [Promega]), separated by electrophoresis on a 1.4% agarose gel, and visualized under UV light after staining with ethidium bromide (6).

(ii) RFLP analysis. Restriction fragment length polymorphism (RFLP) analysis of the *E. coli* 0144:NM isolates was carried out as reported previously (19):

About 2 μg of purified chromosomal DNA was digested with the restriction endonucleases *Eco*RI, *Sma*I, *Kpn*I, and *Pvu*II (International Biotechnologies, Inc., New Haven, Conn.). The restriction fragments were separated by electrophoresis on a 0.8% agarose gel, transferred to GeneScreen Plus membranes (DuPont, New England Nuclear), and hybridized. The ^{32}P-labeled 2.5-kb *Eco*RI-*Hin*dIII restriction fragment of the pKK_{3535} plasmid, encoding the 16S rRNA and part of the 23S rRNA, was used as a probe. Bacteriophage lambda DNA digested with *Hin*dIII was used as a molecular mass marker on each gel. The hybridization banding patterns of the strains isolated from encephalopathic and nonencephalopathic patients were analyzed.

Cytotoxicity assay. EIEC strains were grown in syncase broth for 48 h with shaking at 200 rpm, lysed by sonication, and filter sterilized as described previously (2). Cytotoxicity was quantitatively determined on HeLa cells by neutral-red uptake assay (4): 96-well plates (Nunc, Roskilde, Denmark) were inoculated with 100 μl of serial dilutions of bacterial sonicates, and HeLa cells were then added at 5×10^4 per well in 100 μl. After overnight incubation at 37°C with 5% CO_2, the viability of the cells was determined spectrophotometrically (550 nm) after incubation with neutral red (2 h, 37°C), washing away excess dye, and extracting the neutral red that was taken up by the cells with Sorenson's citrate-ethanol mixture.

RESULTS

Encephalopathic patients. (i) Patient 1. A previously healthy 6-year-old male was admitted with obtundation after 1 day of fever, watery diarrhea, and vomiting. On physical examination, his temperature was 39°C, the pulse was 118 beats per min, respiration was 24 breaths per min, and blood pressure was 100/60 mm Hg. He was disoriented and slept if undisturbed. Clinically, he was not significantly dehydrated, his abdomen was minimally tender without peritoneal signs, his neurologic examination including fundoscopy was nonfocal, and he was normal other than his mental state and marked nuchal rigidity.

The results of laboratory studies were as follows: hemoglobin, 10.9 g/dl; hematocrit, 34%; leukocytes, 11,540/mm^3 with 7% bands, 75% polymorphonuclear leukocytes, 16% lymphocytes, and 2% monocytes; Na, 132 meq/liter; K, 4.4 meq/liter; glucose, 134 mg/dl; urea, 25 mg/dl; creatinine, 0.5 mg/dl; lumbar puncture, 3 mononuclear leukocytes and 0 erythrocytes; glucose, 91 mg/dl; protein, 25 mg/dl; and negative Gram stain. A stool smear did not contain leukocytes and was negative when tested for occult blood.

Twenty-four hours after admission the patient was oriented and feeling better, having been treated with parenteral fluids alone. No bacterial, viral, or parasitic pathogen other than EIEC 0144:NM grew or was identified from the patient's stool samples. One cerebrospinal fluid culture and two blood cultures were negative.

(ii) Patient 2. A previously healthy 8-year-old female was admitted with a 1-day-history of fever to 39°C and dysenteric diarrhea. Within hours after the onset of diarrhea, she became increasingly apathetic and somnolent. On admission she was stuporous, having no contact with her surroundings and responding only to pain. Her parents insisted that no medicines, conventional or traditional, were given. Vital signs were a pulse of 88 beats per min, respiration of 20 breaths per min, blood pressure of 100/70 mm Hg, and a temperature of 37.2°C. Clinically, she was minimally dehydrated and her abdomen was nontender with increased peristalsis. The remainder of her physical examination was normal except for her mental status: she responded only to pain by withdrawal. She did not have meningeal signs, fundoscopy was normal, and there were no focal findings; deep tendon reflexes were normal, and a Babinski sign was absent.

The results of laboratory studies were as follows: hemoglobin, 11.5 g/dl; hematocrit, 35%; leukocytes, 12,430/mm^3 with 5% band forms, 80% polymorphonuclear leukocytes, 11% lymphocytes, and 4% monocytes; venous pH, 7.28, with a base excess of −9.4; Na, 130 meq/liter; K, 3.4 meq/liter; urea, 14 mg/dl; creatinine, 0.6 mg/dl; and glucose, 59 mg/dl. Liver function tests were within normal limits. A urine toxicology screen was negative.

She did not respond clinically to intravenous glucose, and when a stool examination showed numerous polymorphonuclear leukocytes as well as erythrocytes, a presumptive diagnosis of encephalopathic shigellosis was made. Treatment with intravenous fluids and 50 mg of ceftriaxone per kg of body weight per day was initiated (pending stool and blood culture results), but a lumbar puncture was not performed. Within 48 h her fever and diarrhea improved considerably, and her mental status returned to normal.

Two blood cultures were negative, and the only pathogen (bacterial, parasitic, or viral) identified in her stool was EIEC 0144:NM. Of note is the fact that her mother and brother had a concomitant diarrheal illness but were not encephalopathic. Their stools were not cultured.

Nonencephalopathic patients. Of the 8 EIEC 0144-infected patients randomly chosen from among the 24 nonencephelopathic patients, 2 were children and 6 were adults (age range, 6 to 46 years), 2 were male, 6 were female, 3 had dysenteric diarrhea, and 5 had watery stools. Two of the eight patients were febrile, and one of the eight patients (a 21-year-old female) required parenteral fluids in an emergency room.

Laboratory studies. (i) Lactose fermentation. All isolates were lactose negative.

(ii) Antibiotic susceptibility testing. All isolates were susceptible to chloramphenicol, tetracycline, ampicillin, amoxicillin-calvulanic acid, trimethoprim-sulfamethoxazole, ciprofloxacin, nalidixic acid, and ceftriaxone.

(iii) Serology. Ten of 10 of the 0144 isolates were nonmotile by H typing; thus, all isolates were EIEC serotype 0144:NM.

(iv) Invasive plasmid. Ten of 10 EIEC 0144:NM isolates amplified *virf* and *ipa*H primers, indicative of the presence of the 140-MDa *Shigella* invasive plasmid.

(v) Plasmid profile. Ten of 10 EIEC 0144:NM isolates had a single identical, small, 20-kb plasmid when their DNA was digested by *Hin*dIII. After digestion with *Sal*I, two isolates (from nonencephalopathic patients) had an identical, second, very small, 2-kb plasmid.

(vi) RFLP. Analysis of the 0144:NM isolates from encephalopathic and nonencephalopathic patients after digestion of chromosomal DNA with four restriction enzymes revealed the same unique pattern.

(vii) Cytotoxicity. All 10 EIEC isolates showed low-level cytotoxicity (to dilutions of 1:16 to 1:64). There was no difference between the two encephalopathy-associated isolates and the other isolates.

DISCUSSION

Although CNS manifestations occur with invasive bacterial enteric infections caused by *Shigella, Salmonella*, and *Campylobacter* spp., EIEC has not been associated with CNS symptoms. Given the similarities between EIEC and *Shigella* spp., it is not surprising that encephalopathy was associated with the two cases of EIEC 0144:NM infection described here.

The pathogenesis of enteric infection-associated encephalopathy is not well understood. It has been extensively studied in patients with shigellosis. Shiga toxin, the main toxic product of *S. dysenteriae*, has been considered neurotoxic (2, 18). However, Shiga toxin's action on the nervous system cannot explain all cases of encephalopathy since neurologic symptoms occur in patients with *Shigella flexneri* and *Shigella sonnei* infections. These bacteria do not usually produce Shiga toxin and lack the structural genes encoding its production (1, 2). The closely

related Shiga-like toxins produced by *E. coli* and other cytotoxins may play a role, mainly by causing endothelial cell damage in the brain (2, 3). Recently, synergy between Shiga toxin and *Shigella* lipopolysaccharide has been described in the induction of neurologic symptoms in mice (21). In addition, severe brain edema has been documented in a patient with *Shigella*-associated encephalopathy (15). Those investigators suggested that brain edema during shigellosis is often undiagnosed and may be related to hyponatremia. Although direct CNS involvement with *Shigella* species is possible, it is rare and cannot explain the majority of cases of *Shigella*-associated encephalopathy (3). The pathogenesis of *Salmonella*- and *Campylobacter*-associated encephalopathy has not been elucidated.

The W. Hirsch Regional Microbiology Laboratory serves a large proportion of the western Galilee population (approximately 700,000 people). Each year about 26,000 bacterial stool samples are processed. The classical microbiologic techniques described above are used to identify a number of EIEC serotypes. Between 1983 and 1992, an average of 36 EIEC isolates per year (range, 2 to 69 isolates) were isolated. EIEC 0144 was isolated only three times during this period, in 1989. In 1993, 26 of 31 EIEC strains isolated were 0144:NM; 17 of 26 strains were isolated during June. All were sporadic and were neither geographically nor otherwise epidemiologically related.

Molecular analyses of EIEC isolates from both of the encephalopathic patients and all eight nonencephalopathic patients were identical, in that all isolates possessed the invasive plasmid and all had the same chromosomal DNA pattern by RFLP analysis. The presence of an additional small plasmid in two of eight isolates from nonencephalopathic patients is not relevant as far as encephalopathic potential is concerned.

The cases of infection described here indicate that EIEC can be associated with CNS symptoms. The pathogenesis of encephalopathy caused by EIEC specifically and other invasive enteric pathogens in general is not understood.

Of interest is the fact that the 8-year-old girl with encephalopathic EIEC infection in 1993 presented with an identical clinical picture 1 year later. This time, *S. sonnei* was isolated from her stool. Her blood ammonia level, lumbar puncture, and fluid and electrolyte status were normal. Thus, other than shigellosis, no other logical explanation for her encephalopathy was found. Once again, other members of her family had diarrhea concurrently, this time caused by *S. sonnei*, although none had CNS symptoms. This strongly suggests that host factors probably play an important role in the pathogenesis of the encephalopathy associated with invasive diarrheal disease and probably act in concert with certain bacterial traits.

REFERENCES

1. **Ashkenazi, S., G. Bellah and T. G. Cleary.** 1989. Hallucinations as an initial manifestations of childhood shigellosis. J. Pediatr. **114:**95–96.
2. **Ashkenazi, S., K. R. Cleary, L. K. Pickering, B. E. Murray, and T. G. Cleary.** 1990. The association of Shiga toxin and other cytotoxins with the neurologic manifestations of shigellosis. J. Infect. Dis. **161:**961–965.
3. **Ashkenazi, S., and M. Dan.** 1994. Extraintestinal manifestations of gastrointestinal tract infections. Semin. Pediatr. Infect. Dis. **5:**210–221.
4. **Ashkenazi, S., Y. Yuhas, S. Even-Tov, E. Kaminsky, and Y. L. Danon.** 1994. The effect of Shiga toxin and sonicates of *Shigella* isolates from children with neurologic manifestation on neuroblastoma cell lines. Isr. J. Med. Sci. **30:** 604–610.
5. **Barret-Connor, E., and J. D. Connor.** 1970. Extraintestinal manifestations of shigellosis. Am. J. Gastroenterol. **53:**234–245.
6. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1523.
7. **Farmer, J. J., III, and M. J. Kelly.** 1991. *Enterobacteriaceae*, p. 360–383. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
8. **Goren, A., S. Freier, and J. H. Passwell.** 1992. Lethal toxic encephalopathy due to childhood shigellosis in a developed country. Pediatrics **89:**1189–1192.
9. **Guerrant, R. L.** 1995. Inflammatory enteritides, p. 987–998. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Principles and practice of infectious diseases, 4th ed. Churchill Livingstone, New York.
10. **Hale, T. L., P. J. Sansonetti, P. A. Schad, S. Austin, and S. B. Formal.** 1983. Characterization of virulence plasmids and plasmid-associated outer membrane proteins in *Shigella flexneri, Shigella sonnei*, and *Escherichia coli*. Infect. Immun. **40:**340–350.
11. **Harris, J. R., I. K. Wachsmuth, B. R. Davis, and M. L. Cohen.** 1982. High molecular weight plasmid correlates with *Escherichia coli* invasiveness. Infect. Immun. **37:**1295–1298.
12. **Mulligan, K., S. Nelson, H. S. Friedman, and P. I. Andrews.** 1992. Shigellosis-associated encephalopathy. Pediatr. Infect. Dis. J. **11:**889–890.
13. **National Committee for Clinical Laboratory Standards.** 1994. Performance standards for antimicrobial susceptibility testing. Fourth informational supplement. NCCLS document M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
14. **Penner, J. L.** 1991. Campylobacter, Helicobacter and related spiral bacteria, p. 402–409. *In* A. Balows, W. J. Hausler, K. L. Herrmann, H. D. Isenberg and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
15. **Perles, Z., J. Bar-Ziv, and E. Granot.** 1995. Brain edema: an underdiagnosed complication of *Shigella* infection. Pediatr. Infect. Dis. J. **14:**1114–1115.
16. **Sandyk, R., and M. J. W. Brennan.** 1983. Fulminating encephalopathy associated with *Shigella flexneri* infection. Arch. Dis. Child. **58:**70–71.
17. **Sethabutr, O., M. Venkatesan, G. S. Murphy, B. Eampokalap, C. H. Hoge, and P. Echeverria.** 1993. Detection of *Shigella* and enteroinvasive *Escherichia coli* by amplification of the invasion plasmid antigen H DNA sequence in patients with dysentry. J. Infect. Dis. **167:**458–461.
18. **Wiley, R. G., A. Donahue-Rolfe, and G. T. Keusch.** 1985. Axonally transported *Shigella* cytotoxin is neurotoxic. J. Neuropathol. Exp. Neurol. **44:**489–506.
19. **Yavzori, M., D. Cohen, and H. Bercovier.** 1992. Molecular epidemiology of *Shigella* infections in Israel. Epidemiol. Infect. **109:**273–282.
20. **Yavzori, M., D. Cohen, R. Wasserlauf, R. Ambar, G. Rechavi, and S. Ashkenazi.** 1994. Identification of *Shigella* species in stool specimens by DNA amplification of different loci of the *Shigella* virulence plasmid. Eur. J. Clin. Microbiol. Infect. Dis. **13:**232–237.
21. **Yuhas, Y., A. Weizman, G. Dinari, and S. Ashkenazi.** 1995. An animal model for the study of neurotoxicity of bacterial products and application of the model to demonstrate that Shiga toxin and lipopolysaccharide cooperate in inducing neurologic disorders. J. Infect. Dis. **171:**1244–1249.