Fatal Gastroenteritis Associated With Coronaviruslike Particles

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- The role of human enteric coronaviruses in infantile gastroenteritis is controversial. We detected coronaviruslike particles in the intestinal contents and within the epithelial cells of the ileum in a 15-month-old infant who had postmortem evidence of severe enteritis. Ultrastructural findings consistent with in vivo coronavirus replication in the human small intestine support a causative role for this agent in gastroenteritis.

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During the past decade, substantial advances in electron microscopic and immunologic techniques have greatly increased our knowledge of viral agents of acute gastroenteritis.1 Rotaviruses are now known to be the single most common cause of diarrhea in infants and young children, and the Norwalk-agent and related viruses are recognized as important causes of epidemic gastroenteritis in school-aged children and young adults. Other noncultivable, putative causes of enteritis, such as calciviruses and enteric adenoviruses, have been seen in feces of ill subjects by use of the electron microscope. We recently found coronaviruslike particles (CVLPs) in the fecal fluid and small intestine of an infant who died of complications of gastroenteritis. Ultrastructural findings demonstrated evidence of in vivo replication of this agent within human intestinal epithelium.

REPORT OF A CASE

A 15-month-old male infant was admitted to Oklahoma Children’s Memorial Hospital, Oklahoma City, in March 1982 in a postictal comatose state. He had been in good health until one week earlier when rhinorrhea had developed. Two days prior to admission, he manifested a nonproductive cough, increased rhinorrhea, and a temperature of 40.6 °C. The family physician diagnosed an upper respiratory tract infection and prescribed ampicillin sodium, diphenhydramine hydrochloride, theophylline, and aspirin. There were no siblings, and neither parent had been ill. The child had no known exposure to other ill persons or animals.

On the evening of admission, the patient appeared well when put to bed. Several hours later, he was found to be cyanotic and gasping, with vomitus on the bedcovers. A 40-minute generalized grand mal seizure was successfully treated in the emergency room with intravenous diazepam and phe- nobarbital sodium. The patient had a temperature of 41.1 °C, systolic BP of 92 mm Hg, and pulse rate of 120 beats per minute. He was unresponsive and had fixed, dilated pupils. He did not appear substantially dehydrated. There was no evidence of upper or lower respiratory tract infection. The abdomen was flat, with active bowel sounds. There were no focal neurologic signs. Initial laboratory data included a normal complete blood cell count, a serum urea nitrogen level of 27 mg/dL, and a serum creatinine level of 1.8 mg/dL. Serum electrolyte levels were as follows: sodium, 138 mEq/L; potassium, 4.2 mEq/L; chloride, 107 mEq/L; and CO2, 10 mEq/L. Initial arterial blood gas measurements showed a pH of 7.13 with a PCO2 of 27 mm Hg and a normal PO2. Chest roentgenograms, computed tomographic brain scan, lumbar puncture, and tox- icologic studies disclosed no abnormalities.

The patient was admitted to the intensive care unit with a diagnosis of postictal anoxic brain damage, possibly secondary to aspiration of gastric contents. He was treated with ventilatory support, with phe- nobarbital for seizure control, and with chloramphenicol and ampicillin for suspected bacterial sepsis. Several hours after admission, the patient passed two large, soft, semiformal stools. There was no return of neurologic responsiveness, and approximately eight hours after admission the patient died after an episode of hypotension and bradycardia. Shortly before his death, the serum urea nitrogen level had risen to 58 mg/dL and the creatinine level to 2.2 mg/dL.

RESULTS

Postmortem Examination

An autopsy was performed within three hours of death. Very dry subcutaneous tissue indicated severe dehydation. Green gastric fluid was present in the trachea and major bronchi. The spleen showed multiple areas of hemorrhagic infarction under the capsule and throughout the parenchyma. There were large volumes of intraluminal fluid in both small and large bowel but no other noteworthy gross gastrointestinal (GI) tract changes. Examination of the CNS disclosed brain-stem herniation associated with severely swollen gyri and narrow sulci.

Abnormal microscopic findings were confined to the lungs, the spleen, and the small intestine and related lymphoid tissue. Proteinaceous material was seen within the bronchi and alveolar ducts. Both lungs showed diffuse interstitial infiltrates of lymphohistiocytic cells and lymphocytes. Multinucleated giant cells or viral inclusions were not observed. Sinusoidal fibrin thrombi and patches of hemorrhagic necrosis were present in the spleen. There was blunting and loss of microvilli in the small intestine, with severe infiltration of lymphocytes and lymphohistiocytic cells throughout the lamina propria (Fig 1). No colitis was microscopically evident.
Fig 1.—Small intestine. Note blunted architecture of mucosal surface and severe lymphocytic infiltration throughout lamina propria (hematoxylin-eosin, original magnification × 200).

Comprehensive light microscopic examination of the CNS and other organs showed no lesions.

**Microbiologic Findings**

Premortem bacterial cultures of blood, CSF, and stool showed no pathogens. Stool cultures were processed by routine methods for bacterial enteric pathogens, including *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Clostridium difficile*. Postmortem cultures of blood, lung, and intestinal contents yielded no bacterial pathogens. Stool contents were negative for rotavirus antigen by enzyme immunoassay. Autopsy samples of stool, intestine, liver, and myocardium were cultured for viruses in primary monkey kidney and in a human fibroblast line (MRC-5 cells). No viruses were isolated.

**Electron Microscopy**

Fecal fluid and sections of small intestine and lung were examined by conventional electron microscopy. The fecal fluid contained numerous pleomorphic, enveloped structures with diameters ranging from 66 to 140 nm. These particles had a peripheral fringellike rim composed of thin projections with bulbous tips. These projections measured 15 to 20 nm in length (Fig 2, left). Ultrastructural examination of the small intestine showed many double-enveloped particles 50 to 60 nm in diameter at the apex of epithelial cells. Some of these particles were within cytoplasmic vesicles, located adjacent to the microvilli of epithelial cells. New daughter particles appeared to be budding off within these vesicles (Fig 2, right). These virions had an envelope 7 nm thick. Electron microscopic examination of lung sections disclosed no viruslike particles or inclusions.

**COMMENT**

The particles in our patient's intestinal fluid are typical of CVLPs previously described in the stools of human beings with gastroenteritis. The size, pleomorphism, presence of an envelope, and demonstration of a "crownlike" fringe surrounding the virion all support this morphologic diagnosis. Of greater interest is our demonstration of smaller, nonfringed viruslike particles within epithelial cells of the distal part of the small bowel. The location, size, shape, and arrangement of these particles within a cytoplasmic vacuole are identical to the published ultrastructural features of canine coronavirus enteritis, of enteritis in infant mice caused by mouse hepatitis virus (also a coronavirus), and of enteritis in newborn calves caused by neonatal calf diarrhea coronavirus. Similar intracellular viruslike particles have been seen on ultrastructural examination of two other coronaviruses cultivated in vitro. Both a human respiratory coronavirus, cultivated in human fetal diploid lung cells, and avian infectious bronchitis virus, cultivated in chorioallantoic membrane, produce double-enveloped particles, 80 to 100 nm in diameter.
diameter, that appear to be budding within cytoplasmic vacuoles. Finally, Roussel et al. published electron micrographs of coronavirus-like particles in the small intestines of human neonates with necrotizing enterocolitis. These virions had an average diameter of 70 nm (range, 60 to 125 nm) and a double envelope and were found within apical cytoplasmic vesicles. These particles appear identical in size and location to those found in the small bowel of our patient.

Coronaviruses are enveloped, pleomorphic RNA viruses with characteristic surface projections; they are a common cause of upper respiratory tract infections, particularly the common cold, in human beings. Several human respiratory tract strains have been successfully cultivated in tracheal organ culture and adapted to growth in cell culture. Animal strains causing enteritis in infant dogs, calves, mice, pigs, and foals have been described, with ultrastructural findings as noted earlier.

Definition of the role of human enteric coronaviruses in human disease has been problematic. Although outbreaks of enteritis associated with CVLPs detected by electron microscopic examination of feces have been reported, such particles have also frequently been observed in the stools of subjects without acute diarrhea. In support of a pathogenetic role for CVLPs in enteritis of human infants, several investigators have recently reported microscopic agglutination of these particles by convalescent serum samples from previously infected infants. Lack of reproducible and productive methods for cultivation of human enteric coronaviruses has hampered the study of their role in intestinal gastroenteritis. Caul and Clarke accomplished limited cultivation of a single isolate in human embryo intestinal organ culture and demonstrated intracytoplasmic antigen by immunofluorescence in this system and in primary human embryonic kidney. A putative isolate from the Paris outbreak of necrotizing enterocolitis was cultured in HRT 18 cells (a well-differentiated human rectal adenocarcinoma cell line). All other attempts to grow CVLPs have been unsuccessful.

The ultrastructural findings in our patient, particularly those suggesting in vivo replication of CVLPs in intestinal epithelium, support an enteric coronavirus being the cause of our patient’s intestinal disease. The intracellular location of the CVLPs suggests that the virions in the intestinal lumen were produced by local enteric multiplication, rather than merely representing ingested viruses from the respiratory tract. Comprehensive histopathologic and microbiologic examinations failed to demonstrate any other viral or bacterial pathogens in this patient.

An important lesson from this case is the occurrence of pathologically severe enteritis and fluid loss in a patient who had not experienced remarkable GI tract symptoms. Fatal infection attributed to human enteric coronaviruses has been reported previously in young infants, including cases of fatal necrotizing enterocolitis and fatal enteritis in neonates. In the latter outbreak, several infants with fatal outcomes appeared to have sepsis rather than a primary GI tract process. Fulminant fatal viral gastroenteritis, complicated in some cases by aspiration of vomitus or seizures, has been well described in young, previously healthy children infected with rotaviruses. The possibility of severe intraluminal fluid loss or pulmonary aspiration in the patient without antecedent GI tract symptoms must be considered in the young child whose clinical appearance resembles that of our patient. Investigation of the cause of such an illness should include the application of electron microscopy to search for novel noncultivable enteric viruses. Our findings in this case support the role of enteric coronaviruses as one potential cause of such fulminating illness.
References


Book Review


Raven Press, a leader in publishing good neurology texts, has done it again. These two monographs, the second and third volumes of the International Review of Child Neurology Series, are excellent and timely reviews of two neurological disorders not recently reviewed elsewhere. The volumes are sponsored by the International Child Neurological Association, which is devoted to providing an outlet for the exchange of professional ideas and to advancing neurological sciences in infancy and childhood. These official publications of the association have definitely achieved that goal. The authorship is broad, representing a superb field of child neurology in the United States and throughout the world.

Brain Tumors in Children includes sections on principles of diagnosis and treatment, management, and complications. In this single volume new concepts of epidemiology, neuroradiologic diagnosis, surgical excision, radiation therapy, chemotherapy, and the long-term effects of radiation and chemotherapy are discussed. Case examples and algorithms are used appropriately to illustrate current approaches in diagnosis and management. Controversies, such as total removal of craniopharyngiomas and therapy of optic nerve gliomas, are discussed freely. The editors, leaders in efforts to establish a Childhood Brain Tumor Registry in the United States, note sadly that only 50% of children with brain tumors reach university or cancer treatment centers, making trials of new therapy difficult. For this reason, they urge that children with brain tumors be referred to university cancer centers affiliated with large cooperative study groups. The results of current brain tumor therapy certainly justify their recommendation.

Dr. Gamstorp and Sarnat note in the preface to Progressive Spinal Muscular Atrophies that they hope their book will be regarded as "of historical interest only in the future, because the etiology of this terrible affliction of children and its treatment remain obscure." Despite this caveat, they have edited an excellent "state of the art" monograph on the historical aspects, genetics, clinical features, pathology, electrophysiologic abnormalities, rehabilitation, and future research strategies for "Lou Gehrig's disease" in children. Dr. Gamstorp's scholarly chapters reflect her wealth of clinical experience, combined with a sensitive and scholarly approach to diagnosis and management. The use of pulse echo ultrasonic imaging and computed tomography scanning are reviewed as potentially convenient, noninvasive tools for diagnosing these disorders. Chapters on rehabilitation and psychological counseling will be of interest to practicing pediatricians who will be involved in following up these children. The value of parent groups, rarely discussed in neurological texts, is also reviewed. Dr. Sarnat's concluding chapter on research strategies highlights the studies of fetal motor neuroblasts, the "dying-back" phenomena of retrograde axonal degeneration, and the transplantation of healthy motor neurons.

For excellent state of the art reviews on these grim neurological problems in children, the pediatrician is advised to consult these two monographs. Pediatric neurologists should add them to their libraries.

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