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Detection of Antigenically Distinct Rotaviruses from Infants

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Antigenically distinct rotaviruses, i.e., viruses morphologically identical to conventional rotaviruses by electron microscopy, yet lacking the common group antigen(s) detected by an enzyme-linked immunosorbent assay, were found in 2 of 51 fecal samples from Bulgarian infants with rotavirus gastroenteritis. These antigenically distinct viruses contained 11 segments of double-stranded RNA, but they demonstrated a unique RNA migration profile after electrophoresis of the genome RNA in polyacrylamide gels. This report confirms the presence of a new group of rotaviruses in humans. The significance of these viruses is currently unknown, and specific diagnostic tests must be developed for epidemiological studies to determine their role as human and veterinary pathogens and to evaluate their impact on proposed vaccine development programs.

Rotaviruses are the major worldwide cause of viral gastroenteritis in infants and young children. Rotaviruses are members of a genus of the family Reoviridae, and the viral particles are characterized by a 70-nm-diameter double-shelled capsid surrounding an icosahedral core that contains the genome of 11 segments of double-stranded RNA. Rotaviral diarrheal disease is estimated to be responsible for 5,000,000 deaths annually in less developed countries, and rotavirus gastroenteritis is associated with high morbidity in developed countries (6), prompting a major effort to develop a vaccine. The recent demonstration of multiple serotypes of human rotavirus (13), however, suggests that prevention of disease by a vaccine may be more complicated than originally proposed.

Although direct electron microscopy of diarrhetic stool is the classical test for rotavirus detection, immunoassays (complement fixation tests, immunofluorescence assays, and rapid enzyme-linked immunoabsorbent assays [ELISA]) have been developed to facilitate rotavirus detection. These immunoassays have relied on the fact that rotaviruses from both mammalian and avian hosts share a common group antigen(s) (14). Recently, rotaviruses lacking the common antigen(s), antigenically distinct rotaviruses, have been detected from bovine (L. Saif and K. Theil, personal communication), porcine (1, 3, 12), avian (7), and human (8, 10) hosts. These viruses are not detected by immunoassays, and because they are morphologically identical to conventional rotaviruses they cannot be distinguished by electron microscopy. The prevalence and significance of these new groups of rotaviruses remain unclear. The present study reports the detection of antigenically distinct rotaviruses from 2 children (4% of the total samples tested or 7% of those samples with sufficient RNA for electropherotyping) during a survey of 51 Bulgarian infants with diarrhea. This finding suggests that these viruses may be more prevalent than previously believed, and their significance must be evaluated in relationship to proposed vaccine programs and in interpreting ELISA or similar immunoassays now often used as the routine clinical test for rotavirus detection.

MATERIALS AND METHODS

Patients and virus specimens. Stool specimens were obtained from children (ages 1 to 33 months) admitted with diarrhea to the First City Hospital or to the Hospital for Infectious Disease in Sofia, Bulgaria, during the period March 1981 to March 1982. The stool samples were analyzed for rotaviruses by electron microscopy and, in some cases, by ELISA (Rotazyme assay; Abbott Laboratories, North Chicago, Ill.).

Analysis of the viral RNA. The viral RNA was analyzed by polyacrylamide gel electrophoresis. Briefly, a 10% suspension of feces was homogenized twice with an equal volume of Genetron (trichloro-trifluoroethane; Du Pont Co., Wilmington, Del.), and the clarified supernatant was pelleted by ultracentrifugation. The virus-containing pellets were drained and suspended in 30 μl of 0.4 M NaCl solution containing 200 μg of proteinase K (E. Merck, Darmstadt, West Germany) per ml. The suspension was incubated for

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90 min at 37°C, an equal volume of 2× electrophoresis sample buffer (10 mM Tris-hydrochloride [pH 6.8], 16% glycerol, 20% sodium dodecyl sulfate, 10% 2-mercaptoethanol, 0.006% phenol red) was added, and the sample was loaded unto gels. Electrophoresis was performed in 10% polyacrylamide slab gels with the Laemmli discontinuous buffer system (7). Simian rotavirus SA11 RNA was included as an internal reference for comparing the migration patterns of the viral genomes.

RESULTS

Identification of antigenically distinct rotaviruses by RNA migration profiles. Rotavirus isolates can be distinguished based on the pattern of electrophoretic migration of the RNA genome segments in polyacrylamide gels. Although current evidence suggests that this method may not be able to differentiate all of the different virus serotypes, it is nevertheless a useful epidemiological tool to analyze outbreaks of rotaviral disease. In the present study of viruses causing diarrhea in children in Bulgaria, sufficient virus to determine the RNA migration profiles was obtained in 29 of 51 samples (Fig. 1). In 27 samples, the RNA profiles were characteristic for conventional human rotaviruses. Two cases (4% of the total or 7% of those with sufficient RNA for analysis) exhibited RNA migration profiles strikingly different from the RNA profiles previously observed from humans (Fig. 1, lanes b and f) and resembled the distribution of the RNA segments reported for an antigenically distinct rotavirus isolated from piglets (1) and humans (9).

The more conventional RNA patterns consisted of 19 "long" profiles, considered characteristic for human subgroup 2 viruses, and 8 "short" profiles, which have been classified as subgroup 1 (5). This distribution of conventional RNA patterns for the 13 months surveyed in this study was not unusual, although the percentage of "short" patterns was slightly greater than in most previous studies.

Characterization of the antigenically distinct rotaviruses. The viruses with the distinct RNA profiles were recovered from diarrheal stool specimens of two male infants admitted to the First City Hospital in Sofia. The clinical symptoms of these children were not standard for rotaviral disease in this population.

The first child, a 5-month-old boy, was admitted to the hospital on 22 January 1981, with a diagnosis of gastroenteritis. The child had diarrhea for 5 days without fever and then was mildly febrile for 3 days. A fecal sample taken on 29 January was negative for rotavirus by electron microscopy, and the child was discharged on 30 January. He was readmitted on 8 February with a diagnosis of tracheobronchitis. The clinical symptoms were a rhinorrhea, cough, and wheezing, but without fever or diarrhea. A fecal sample taken on 12 February contained the antigenically distinct rotavirus. After treatment for bronchopneumonia he was discharged on 22 February.

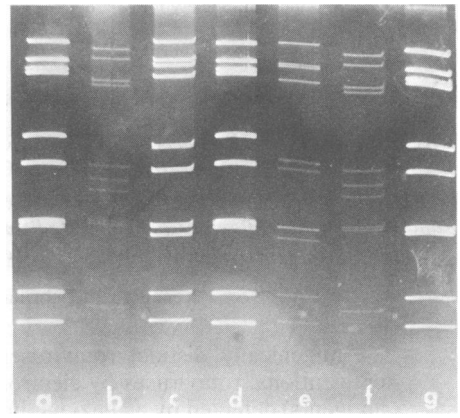

FIG. 1. Comparison of profiles of the 11 rotavirus genome segments after electrophoresis in 10% polyacrylamide gels and staining with ethidium bromide. Patterns observed from the standard simian rotavirus SA11 (lanes a, d, and g) and from fecal samples of Bulgarian children with diarrhea containing the antigenically distinct rotaviruses (lanes b and f) or conventional human rotaviruses (lanes c and e).

The second child, a 4-month-old boy, became ill on 19 February and was admitted on 24 February with diagnoses of gastroenteritis, tonsillitis, malabsorption syndrome, and rickets. Diarrhea continued until 1 March. A fecal sample taken before discharge on March 3 contained the antigenically distinct rotavirus. The child was rehospitalized from 6 to 12 March with gastroenteritis, but no additional fecal samples were analyzed.

The presence of rotaviruses indistinguishable from the conventional ones by electron microscopy in these stool samples was confirmed by electron microscopy at Baylor College of Medicine. The stool samples and control stool specimens that contained the same number of virus particles (10^6/ml as estimated by electron microscopy) and exhibited conventional RNA migration profiles with similar intensity of the RNA bands were also examined by ELISA. The results of the ELISA test were interpreted by using a visual color scale and by reading the optical density with a Beckman spectrophotometer after termination of the reaction with 1 M HCl. The samples containing antigenically distinct viruses were negative by the visual scale and had an optical density at 492 nm of 0.013, whereas those containing conventional rotaviruses were 4+ by the visual scale and had an optical density at 492 nm of 2.027.

DISCUSSION

The criteria for classification of a virus as a rotavirus include size, characteristic morphology, and a genome of 11 segments of double-stranded RNA. Recently, Rodger et al. (10) reported a rotavirus-like agent associated with diarrhea in an infant in Australia. They identified 10 bands of RNA by electrophoretic analysis of the viral genome, but, based on the intensity of the bands after staining with ethidium bromide, suggested that the eighth band contained two comigrating segments. Unfortunately, no additional sample was available for further analysis. With the exception that our gel system clearly resolved 11 RNA bands, the RNA profiles of our two viruses are strikingly similar to the one reported in Australia and to the pattern of one ELISA-negative rotavirus sample isolated in France (9). The RNA profile shown here also resembles that reported for the antigenically distinct rotavirus isolated from piglets in the United States, but it is different from the antigenically distinct viruses isolated from calves in the United States (L. Saif, personal communication), piglets in England (3), or from avian species (8). Although the relationship between these different isolates of antigenically distinct rotaviruses from humans and animals remains to be determined, it should be noted that the known isolates from animals do not share common antigens with each other or with the conventional rotaviruses. It therefore seems that the rotavirus genus contains several (possibly many) distinct groups that can be distinguished by serology and nucleic acid sequences (S. Pedley, J. C. Bridger, and M. A. McCrae, *in* D. H. L. Bishop, ed., *Double-Stranded RNA Viruses*, in press). We support the suggestion that these antigenically distinct rotaviruses be called group B, group C, etc., rotaviruses to distinguish them from the previously known viruses, which would be known as group A rotaviruses (Pedley et al., in press). In this scheme, viruses in each group would share common antigens.

The prevalence of this new rotavirus group is unknown. The two previous reports (9, 10) suggested that the prevalence of these agents is rare, but it must be noted that currently these viruses are only detected and characterized when samples are analyzed by a combination of tests including electron microscopy, an immunoassay, and RNA profiles. It is important to include RNA profiles because it is unknown whether antigenically distinct rotaviruses exist that exhibit more conventional RNA patterns. Evaluation of the recent reports comparing ELISA tests (that would only detect conventional rotaviruses) and direct electron microscopic procedures (that would detect both conventional and antigenically distinct rotaviruses) also yields some data on the possible prevalence of these agents (2, 4, 11). These studies have suggested that ELISA is a useful diagnostic aid, especially in laboratories lacking electron microscopic facilities. Although one study reported complete concordance of electron microscopy and ELISA results (2), most studies report a percentage of samples (up to 12%) that are negative by ELISA and positive by electron microscopy, and these samples could represent the new rotavirus group(s). Antigenically distinct viruses may be more prevalent than previously considered, and they may have escaped diagnosis, if these new agents are excreted in amounts that are at the limits of detection by electron microscopy or if they are associated with unusual or subclinical illness as suggested by this and the previous reports on these agents in humans (9, 10).

Because antigenically distinct rotaviruses have been recognized only recently, their role as agents of viral gastroenteritis is unknown. It is important to determine whether they cycle with conventional rotaviruses, whether they have been prevalent in the past, and what their future distribution might be. Since the antigenically distinct rotaviruses cannot be detected by the available ELISA tests, physicians and researchers should be cautious in interpreting ELISA-negative results in children with gastroenteritis. Development of specific diagnostic tests is required for epidemiological analyses to determine their role as human and veterinary pathogens and their impact on proposed vaccine development programs.

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