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Distinct Reovirus-Like Agents Associated with Acute Infantile Gastroenteritis

ROMILIO T. ESPEJO,[1]* ERNESTO CALDERON,[2] AND NAPOLEON GONZALEZ[2]

Department of Biotechnology, Instituto de Investigaciones Biomédicas, Universidad Nacional Autonoma de México,[1] and Unidad de Enfermedades Infecciosas y Parasitarias del Hospital del Niño,[2] México City, Mexico

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Human reovirus-like particles were found by electron microscopy in the stools of 25% of 71 infants and young children hospitalized with acute gastroenteritis in Mexico between December 1976 and April 1977. The virus was also identified by the electrophoresis patterns of its ribonucleic acid upon disruption of partially purified particles. This technique is as reliable as electron microscopy but less laborious, and could become a routine diagnostic procedure. The electrophoretic patterns of viral ribonucleic acid from different cases suggest that there are at least two different reovirus-like agents associated with infantile gastroenteritis.

A human reovirus-like (HRVL) agent, also designated orbivirus, rotavirus, duovirus, and infantile gastroenteritis virus, has emerged as a major etiological agent of acute enteritis of infants and young children in many parts of the world (2, 4, 6, 11, 15, 20, 21), but as yet only a limited number of specimens have been examined from areas where mortality rates are particularly high (8, 19). This report contains the results of the first 5 months of a year-long survey of infants and young children admitted to the Hospital del Niño, Mexico City. In Mexico in 1973, mortality rates for enteritis in infants up to 1 year old were 1,200 per 100,000 inhabitants and 200 per 100,000 for children from 1 to 4 years old (5). Preliminary results of this survey are reported here to describe a new technique for detecting this virus by the characteristic electrophoretic pattern of its ribonucleic acid (RNA). This permits the recognition of at least two distinct types of HRVL agents. This procedure seems suitable for a routine diagnostic technique. A difference in RNA electrophoretic patterns between HRVL agents obtained from different cases has been previously mentioned (16).

PATIENTS AND METHODS

Feces were obtained from infants and children under 5 years old on the 1st, 2nd, 3rd, 5th, and 6th days after admittance with acute gastroentertis. On the average, three samples were examined per patient. Control cases were children admitted with respiratory illness who showed no symptoms of gastroenteritis. Stools were stored at −20°C, and 1- to 3-g samples were processed within 1 week and examined by electron microscopy as previously described (4). Electrophoresis of the partially purified virus obtained for electron microsopy or of the precipitate obtained with polyethylene glycol, omitting the sedimentation through sucrose, was performed as follows: 0.025 ml of the sample used for electron microscopy or 0.05 ml of the polyethylene glycol precipitate suspended in 0.3 ml of distilled water was mixed with one-fifth volumes of a solution containing 6% sodium lauryl sulfate, 0.6% 2-mercaptoethanol, and 0.036 M ethylenediaminetetraacetic acid, pH 7.0, and subsequently incubated for 1 h at 55°C to disrupt the virus. The samples were then mixed with glycerol and bromophenol blue and subjected to electrophoresis in cylindrical 2% agarose gels as described (7), except that Loening buffer containing 0.1% sodium lauryl sulfate was used (12). Electrophoresis was carried out at 40 V for 4 to 5 h, and the gels were stained overnight with ethidium bromide and photographed (7). Conditions for slab agarose electrophoresis were as described above, except that the apparatus described by Studier (18) was used and electrophoresis was carried out at 40 V for 8 h.

RESULTS

The characteristic HRVL particles were detected by electron microscopy in the stools of 25% of 71 study patients admitted with acute gastroenteritis. The partially purified samples used for electron microscopy from about one half of the cases were also subjected to electrophoresis in agarose gels after disruption of the virus with sodium lauryl sulfate and mercaptoethanol. Patterns of RNA similar to those reported for purified viral RNA (9, 16) were obtained in most samples from cases in which HRVL particles were observed by electron microscopy. Since identical patterns were obtained with the polyethylene glycol precipitate or the sample used for electron microscopy that had been further purified by sedimentation through sucrose (Fig. 1a), the latter step was omitted in

order to simplify the procedure and make it suitable as a diagnostic technique for a hospital laboratory. Only 6 samples of the 36 found to be positive by electron microscopy failed to show the characteristic RNA pattern when either the polyethylene glycol-precipitable material or the samples further purified for electron microscopy were subjected to electrophoresis. These six samples contained relatively few virus particles and a large amount of nonviral material as judged by electron microscopy. All the samples considered negative by electron microscopy were also negative by electrophoresis. Neither HRVL particles nor viral RNA was detected in the 14 control cases examined, except in one patient who developed diarrhea the day after collection of the positive stool specimen. A similar finding has been previously reported (10); both results suggest that appearance of virus in the stools before the onset of diarrhea may occur frequently.

The predominant RNA pattern observed was that shown in Fig. 1. The top band observed in this and the following figures did not seem to be of viral origin since it appeared in samples without HRVL particles and also in samples from control cases. Nine distinct viral RNA bands could be observed, with an overall pattern similar to those previously reported for the HRVL agent isolated in other areas (9, 16). This

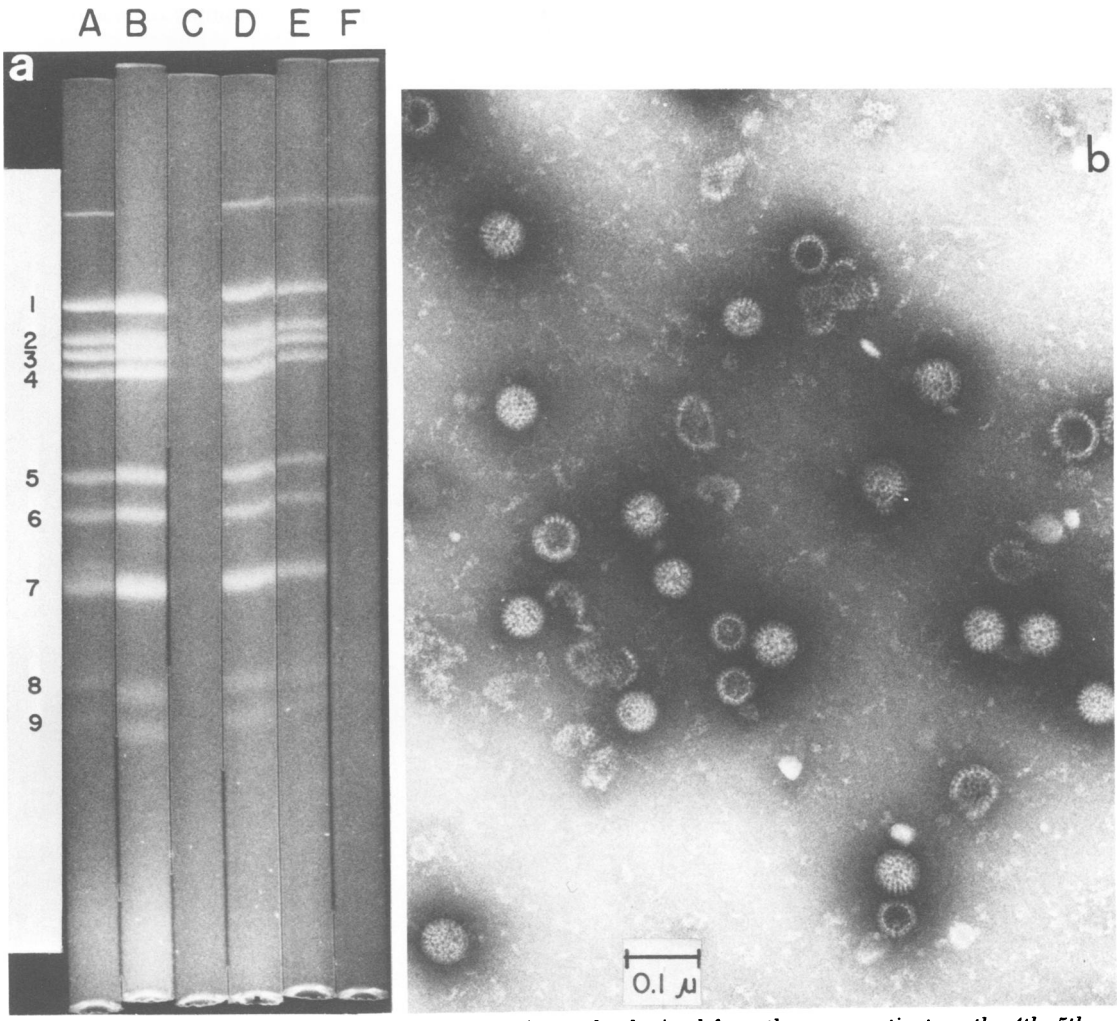

FIG. 1. *(a) Electrophoresis of RNA from virus in stools obtained from the same patient on the 4th, 5th, and 9th days after the onset of symptoms, partially purified by treatment with fluorocarbon and precipitation with polyethylene glycol (gels A, B, and C, respectively) and after subsequent sedimentation through sucrose as described for electron microscopy (gels D, E, and F, respectively). (b) Electron micrograph of the sample used for electrophoresis in gel E.*

pattern differs, however, from those reported by Schnagl and Holmes (16) and Kalica et al. (9) in that (i) what they call components 2 and 3 were in our experiments well resolved, and (ii) components 7, 8, and 9 of Kalica et al. (9) were not resolved into two bands. Further purification of the virus by isopycnic centrifugation in CsCl (9) did not change this pattern except for the disappearance or large diminution of the top band, which was probably a contaminant, as pointed out above.

The RNA patterns from 6 of the 18 HRVL-positive cases differed from that shown in Fig. 1 in the relative migration of band 2, even though the HRVL particles were indistinguishable from those having the predominant RNA pattern (Fig. 2b). This difference is illustrated in Fig. 2a, which shows the RNA pattern obtained for viruses partially purified as described for electron microscopy from the stools of different patients and subjected to electrophoresis in the same slab agarose gel (the gel was cut so as to fit into our ultraviolet lamp). The viruses in wells 2, 4, 7, and 9 had the RNA pattern previously shown in Fig. 1a, which we shall call 2l (component 2 large), whereas those in wells 1, 3, 5, 6, and 10 showed the pattern 2s (component 2 small). Well 8 contained virus from the sample in well 1 further purified by isopycnic centrifugation in CsCl (9). Bands 8 and 9 failed to show in most of the samples. These bands, comprising the smaller viral RNA molecules, required a high concentration of virus because they made up a small proportion of the total viral RNA.

Since the difference observed may have been due to the presence of compounds or enzymes in some of the stools which, by combining with or acting on some of the RNA molecules, might

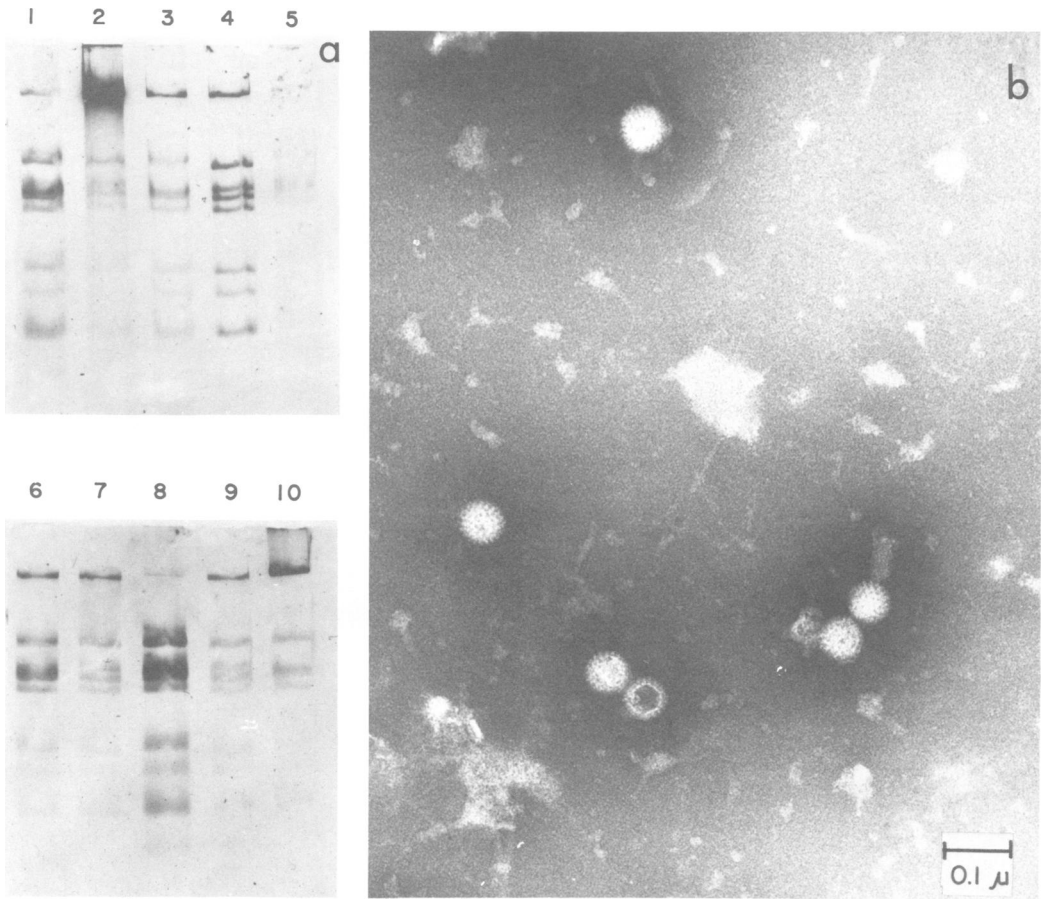

FIG. 2. *(a) Slab gel electrophoresis RNA from partially purified virus obtained from different patients. The patterns in wells 2, 4, 7, and 9 differ from those in wells 1, 3, 5, 6, and 10 in the mobility of band 2 (Fig. 1). Well 8 contained virus from the sample in well 1 further purified by isopycnic centrifugation. (A photographic reversal is shown.) (b) Electron micrograph of virus present in the sample used for electrophoresis in well 3.*

change their electrophoretic mobility, stools from different cases that had been shown to contain HRVL particles with different RNA patterns were processed individually, mixed, and subsequently subjected to electrophoresis. Component 2 from samples showing patterns 2l and 2s could indeed be resolved into two bands when both samples were mixed and subsequently processed and analyzed (Fig. 3), suggesting that the difference in RNA patterns is due to an intrinsic difference between the viruses and that there are at least two different types of HRVL virus.

FIG. 3. *Electrophoresis of HRVL agent RNA partially purified from stools of different patients (gels A and C) and from a mixture of stools from both patients (gel B). (A photographic reversal is shown.)*

DISCUSSION

The preliminary results on the frequency of gastroenteritis caused by the HRVL agent presented here suggest that it is similar to the HRVL agent found in other parts of the world (2, 4, 11, 15, 20, 21). Its seasonal variation, its influence on mortality rate, and the possible protection provided by colostrum are aspects we want to examine at the completion of this survey.

The procedure reported here for detection of the HRVL agent by electrophoresis of its RNA may become a diagnostic technique as reliable as electron microscopy. The lack of a requirement for relatively sophisticated equipment such as the ultracentrifuge and electron microscope makes it feasible for use in a hospital laboratory. The possibility of distinguishing different HRVL virus classes by their RNA pattern may also help in understanding the epidemiology of this virus.

Important considerations regarding the use of viral RNA detection as a diagnostic technique are its sensitivity and specificity. Samples showing at least the four bands corresponding to the larger viral RNAs have been considered positive. It seems that the specificity of the technique by this criterion is very high. Regarding sensitivity, it seems that viral RNA detection is not as sensitive as electron microscopy; in fact, about 20% of the samples found positive by electron microscopy failed to show viral RNA after electrophoresis. A better evaluation of this technique will be obtained at the end of this survey. The minimum amount of viruses detectable is presently under study. Calculations based on the estimated amount of viruses in the stool (4), the minimum detectable amount of RNA equal to 20 ng as reported for DNA (7), and electrophoresis of purified virus are in disagreement.

The genome of the HRVL agent is composed of 11 segments that can be separated into eight or nine distinct bands by electrophoresis in polyacrylamide gels (9, 16), or into nine bands on agarose gels as reported here. The existence of HRVL agent isolates differing in their RNA composition has been previously mentioned (16). The possible existence of several HRVL virus serotypes has also been suggested by results of immunoelectron microscopy (8) and hemagglutination inhibition (17). The existence of several HRVL virus types could hinder the development of a vaccine, especially if gross differences such as those suggested by RNA electrophoretic patterns occur. Since the HRVL agent can infect piglets (1), calves (13), and monkeys (23), the possibility of "new" strains arising as the result of genetic recombination with animal reovirus-like agents as observed with influenza viruses (22) should be considered.

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ADDENDUM

After this manuscript was submitted for publication, similar observations were published regarding differences in the size of RNA segments between different isolates or types in calf rotavirus (E. Verly and J. Cohen, J. Gen. Virol. **35**:583–586, 1977) and in reovirus (R. F. Ramig, R. K. Cross, and B. N. Fields, J. Virol. **22**:726–733, 1977).

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