Tubular Structures Associated with Acute Nonbacterial Gastroenteritis in Young Children

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We clearly demonstrated a fine morphology of the tubular structures antigenically related to the reovirus-like particles associated with acute nonbacterial gastroenteritis in young children. The tubular structures with a caplike structure on each end seemed to be complete forms, 75 to 80 nm in width and approximately 1,000 nm in length.

Tubular structures have been observed both within infected cells and in negatively stained suspensions of several orbiviruses, such as bluetongue, epizootic hemorrhagic disease of deer, Tribec, and African horse sickness viruses (6, 7). In 1974 it was reported (3) that a tubular structure was found in a clump of human reovirus-like particles aggregated by convalescent serum and that this was presumably an aggregate of capsid protein. Tubular structures have since been detected in negatively stained preparations of human, bovine, murine, and equine reovirus-like particles (1, 4, 8), but a fine morphology of the tubular structures seems not to have been described.

In this paper we clearly demonstrate a fine morphology of tubular structures from fecal extracts of young children with acute nonbacterial gastroenteritis by negative-contrast electron microscopy. Furthermore, we confirm, by using immunoelectron microscopy, that the tubular structures are antigenically related to the reovirus-like particles.

MATERIALS AND METHODS

Clinical specimens. Feces were obtained from 102 young children of 2 to 61 months of age who had been hospitalized in Taniuchi Hospital for Sick Children, Osaka, Japan, with acute nonbacterial gastroenteritis during December 1975 and January and February 1976. Feces were also obtained from 13 young children who did not have gastroenteritis. The age range of the control group was similar to the group with gastroenteritis. All feces from patients and the control group were stored at −20°C until extraction.

Preparation of fecal extracts for electron microscopic studies. Fecal extracts for electron microscopic studies were prepared by the method described previously (2) with some modifications. Suspensions of feces (about 20%, vol/vol) were made in phosphate-buffered saline and centrifuged at 1,500 rpm for 10 min to remove large debris. The supernatants were then centrifuged at 7,000 rpm for 30 min in a Sorvall SS-34 rotor to deposit bacteria and debris. The supernatant fluids were then centrifuged at 35,000 rpm for 2 h in a Hitachi RP40 rotor, and the deposits were suspended in 0.1 to 0.2 ml of distilled water containing 0.2% (wt/vol) sucrose and used immediately for electron microscopic studies.

Negative-contrast electron microscopy and immunoelectron microscopy. A drop of the sample was placed for 1 min on a 400-mesh collodion-coated copper grid covered with a carbon film, the excess was drawn off with a piece of filter paper, a drop of the contrasting agent (2% phosphotungstate, pH 7.0, or 2% uranyl acetate, pH 4.2) was added to the sample, and the excess liquid was quickly removed with a piece of filter paper. After drying, the grid was examined in a JEOL JEM-100B electron microscope at a magnification of 40,000, using an operating voltage of 80 kV. Immunoelectron microscopy was performed by the method described previously (3).

RESULTS

Reovirus-like particles were observed in fecal extracts of 60 of 102 young children with acute nonbacterial gastroenteritis collected in the acute phase, but not in fecal extracts from 14 infants in the convalescent stage and 13 infants without gastroenteritis (unpublished data). They seem to be similar to the reovirus-like particles detected by numerous other investigators in many parts of the world (5).

Tubular structures were observed in 3 of 60 fecal extracts (positive for reovirus-like particles) of young children with acute nonbacterial gastroenteritis. The appearance of the negatively stained tubular structures is shown in both Fig. 1 and Fig. 2. The diameter of the tubular structures was 75 to 80 nm, similar to that of the reovirus-like particles, and the length of the tubular structures varied (Fig. 1 and 2). The tubular structures shown in Fig. 1 appeared to be similar to those described previously (3, 4). The tubular structures with a cap-

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like structure (Fig. 2, arrow) on each end were also observed in the same negatively stained preparation. The tubular structures with cap-like structures seemed to be complete forms, 75 to 80 nm in width and approximately 1,000 nm in length. The surfaces of the tubular structures were composed of hexagonally arrayed subunits, which appeared to be hexagonal in outline. Each subunit was about 15 nm in diameter with a central hole of approximately 5 nm (Fig. 1). Furthermore, similar hexagonally arrayed subunits were seen on the capsid surface of complete reovirus-like particles (Fig. 3, arrow).

On the other hand, the tubular structures were agglutinated by convalescent serum. At the same time the complete reovirus-like particles and coreless particles were also agglutinated by convalescent serum, as shown in an immunoelectron micrograph (Fig. 4).
Fig. 3. Electron micrograph of reovirus-like particles. A complete virus particle 80 nm in diameter with hexagonally arrayed subunits on its surface and coreless particles 65 to 70 nm in diameter. Negative staining was with 2% phosphotungstate at pH 7.0. ×200,000.

Fig. 4. Immunoelectron micrograph of tubular structures, complete reovirus-like particles, and coreless particles agglutinated by convalescent serum. Negative staining was with 2% phosphotungstate at pH 7.0. ×120,000.
DISCUSSION

In negatively stained preparations the tubular structures were observed in 3 out of 60 fecal extracts of young children with acute nonbacterial gastroenteritis. The tubular structures usually observed are shown in Fig. 1. They are constant in width similar to that of the reovirus-like particles, but they vary in length and appear to be similar to tubular structures described by previous workers (3, 4). Furthermore, tubular structures with a caplike structure on each end were also observed (Fig. 2). They are about 1,000 nm in length and seem to be complete forms. Therefore, both the tubular structures shown in Fig. 1 and those described previously (3, 4) may be fragments of the complete form.

Hexagonally arrayed subunits similar to those found on the surfaces of the tubular structures were observed on the capsid surfaces of complete reovirus-like particles (Fig. 3, arrow). It was further confirmed, by immunoelectron microscopy (Fig. 4) as described previously (3, 4), that the tubular structures were antigenically related to the reovirus-like particles. These findings suggest that the hexagonally arrayed subunits composing the surfaces of the tubular structures may represent virus subunits.

In this study we have clearly demonstrated a fine morphology of the tubular structures antigenically related to reovirus-like particles, but the role of the tubular structures in virus maturation does not yet seem to be determined. However, we should like to support the suggestion that the tubular structures are formed by aberrant assembly of viral capsid material, as proposed previously (4).

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LITERATURE CITED