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Acute Viral Gastroenteritis

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Two virus-like particles have been found to be associated with acute viral gastroenteritis. One, a 270-nm parvovirus-like particle (the Norwalk agent), was visualized and recognized by immune electron microscopy in an infectious stool filtrate derived from a community outbreak of nonbacterial gastroenteritis. It appears to be one of the etiologic agents of a usually self-limited form of gastroenteritis which commonly occurs in community outbreaks. The other, a 70-nm reovirus-like particle, has been observed by electron microscopy in specimens from infants and children with severe diarrhea in many parts of the world. The reovirus-like particle may emerge as a major etiologic agent of diarrhea of infants and young children.

Significant advances have recently been made in elucidating the etiologic agents of acute infectious nonbacterial gastroenteritis. This disease affects a broad segment of the population and was the second most common disease experience in the Cleveland family study which embraced a period of approximately 10 yr and included approximately 25,000 illnesses (10,11). We will begin by discussing one form of acute gastroenteritis which characteristically occurs in epidemics and has been given various descriptive names such as viral diarrhea, epidemic diarrhea and vomiting, winter vomiting disease, epidemic nausea and vomiting, and acute infectious nonbacterial gastroenteritis (6). This is a usually self-limited disease which occurs often in outbreaks and which is characterized by a spectrum of clinical symptoms which may include nausea, vomiting, diarrhea, abdominal pain, malaise, low-grade fever, or a combination thereof. It usually is self-limited with clinical features usually lasting 24–48 hr and appears to have a characteristic seasonal pattern occurring most frequently from September to March (6).

This form of acute infectious nonbacterial gastroenteritis was successfully transmitted to volunteers in the 1940’s and 1950’s and again in the early 1970’s but all attempts to definitely cultivate and characterize an etiologic agent have been unsuccessful (6,8,12,13). In the 1971 study by Dolin et al. (12), the disease was successfully transmitted to volunteers using material derived from a rectal swab specimen obtained from a secondary case of gastroenteritis which occurred during an outbreak in Norwalk, Ohio (1). A filtrate made from a volunteer’s stool specimen produced the disease in addi-

1 These studies were done in collaboration with Drs. R. G. Wyatt, H. W. Kim, R. M. Chanock, R. H. Parrott, T. S. Thornhill, R. Dolin, J. L. Gerin, W. J. Rodriguez, S. Ross, Mr. A. R. Kalica, and Mr. W. L. Cline.

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tional volunteers, and in addition, filtrates derived from stools from the second volunteer passage again successfully induced the characteristic disease in volunteers (6,12,13). However, in spite of the availability of known infectious stool filtrates and recent advances in cell and organ culture technology, an etiologic agent could not be isolated from any of these filtrates in cell cultures, and such attempts in intestinal organ cultures were inconclusive (6,12,13).

We, therefore, utilized the technique of immune electron microscopy (IEM) (2,16,17) in an attempt to detect these fastidious agents of acute viral gastroenteritis (18). In this technique, specific antibody aggregates the virus particles into small or large groups, thereby enabling their recognition. In addition, in extreme antibody excess or when particles are few in number, the antibody will coat single particles so that they are readily recognizable also. We used a convalescent serum from a volunteer who developed gastroenteritis after Norwalk filtrate challenge in the hope that the specific antibody would enable the detection and identification of an otherwise unidentifiable low-titered fastidious agent. We had previously utilized this technique to detect a fastidious coronavirus in organ culture harvests derived from a nasal-nasopharyngeal washing from an adult with an acute upper respiratory illness (19).

Utilizing this technique, we examined a known infectious stool filtrate derived from a stool from a volunteer who had developed gastroenteritis after oral administration of a stool filtrate derived from a volunteer who became ill after receiving the original inoculum from the Norwalk outbreak (18). In this technique the stool filtrate was incubated with a dilution of convalescent serum, centrifuged, the supernatant fluid discarded, the pellet suspended in distilled water, then stained with phosphotungatic acid, and placed on a Formvar carbon-coated grid for examination by electron microscopy. In this manner, we visualized aggregates of viral particles which were coated with antibody and which were not randomly distributed but were present as groups; these particles resembled the picorna- or parvoviruses morphologically. The Norwalk particle measured approximately 27 nm in its shortest diameter and 32 nm in its longest in a preparation in which particles were not incubated with serum (18).

We devised a rating system of 0−4+ based on the quantity of antibody coating the particles and found that five of six volunteers who developed illness after Norwalk filtrate challenge demonstrated evidence of infection as determined by testing 1:5 dilutions of acute and convalescent sera by IEM. An example of a significant seroresponse as determined by IEM with a single volunteer’s paired sera from a later IEM study is shown in Fig. 1 (17). Figure 2 shows a particularly well-defined aggregate of Norwalk particles after incubation with a volunteer’s prechallenge serum and further preparation for EM; the latter serum was rated 1+ for antibody to the Norwalk particle (18). In addition, we found that three of five individuals from the original Norwalk outbreak also developed serologic evidence of infection as determined by testing acute and convalescent sera by IEM. These, and
FIG. 1 (A). An aggregate observed after incubation of 0.8 ml of the Norwalk stool filtrate with 0.2 ml of a 1:5 dilution of a volunteer's prechallenge serum and further preparation for electron microscopy. This volunteer developed gastroenteritis after challenge with a second-passage Norwalk filtrate which had been heated for 30 min at 60°C (13). The quantity of antibody on the particles in this aggregate was rated 1-2-2+ and this prechallenge serum was given an over-all rating of 1-2+. The bar = 100 nm and applies to Figs. 1 B and C also. B and C. (B) A single particle and (C) three single particles observed after incubating 0.8 ml of the Norwalk stool filtrate with 0.2 ml of a 1:5 dilution of the same volunteer's convalescent serum and further preparation for electron microscopy. These particles are heavily coated with antibody. The quantity of antibody on these particles was rated 4+ and the serum was given an over-all rating 4+ also. The difference in the quantity of antibody coating the particles in the prechallenge and postchallenge sera is clearly evident. After: Kapikian, A. Z., Feinstone, S. M., Purcell, R. H., Wyatt, R. G., Thornhill, T. S., Kalica, A. R., and Chanock, R. M., in "Perspectives in Virology," Vol. IX (in press).

other findings as well, suggested that the 27-nm particle was the etiologic agent of Norwalk gastroenteritis (18).

Additional studies by Thornhill et al. (24) in our laboratory, in which the technique of IEM was used to determine the shedding patterns of the Norwalk particle have provided additional evidence to support this view. In these studies, a total of 77 stool filtrates were examined by IEM using a serum which contained a high level of antibody; the particle was visualized in stool filtrates derived from stools of 11 of 23 volunteers who developed illness after challenge with Norwalk outbreak-derived stool filtrates. It was striking that the Norwalk particle was not observed in any of the 12 specimens obtained prior to the onset of illness, in 26 of 54 obtained during the first 72 hr after the
onset of illness, and in only two of 11 obtained after 72 hr following the onset of illness. It was also found that the maximal shedding of the particle quantitatively occurred at the onset of experimental illness and shortly thereafter.

We also found that a 1:5 dilution of an aliquot from a preparation of commercial immune serum globulin contained antibody to the Norwalk particle indicating that antibody to this or a related agent must occur more than rarely in the general population (17). It appears, however, from cross-challenge studies by Wyatt et al. (25) in our laboratory and from our IEM studies (17,18) that there are other gastroenteritis-producing viral agents which may represent additional serotypes not related antigenically to the Norwalk agent. It is noteworthy that the Norwalk agent appears to be parvovirus-like on the basis of its morphology, ether, acid, and relative heat stability, and buoyant density in cesium chloride (13,20).
Fig. 3. Reovirus-like particles observed in a stool filtrate prepared from a stool from an infant with acute gastroenteritis. An 0.8-ml quantity of this filtrate was incubated only with 0.2 ml of phosphate-buffered saline prior to further preparation for electron microscopy. The particles had a definite capsomere structure and appeared to have a double-shelled capsid. Occasionally "empty" particles were seen. The bar = 100 nm. After: Kapikian, A. Z., Kim, H. W., Wyatt, R. G., Rodriguez, W. J., Ross, S., Cline, W. L., Parrott, R. M., and Chanock, R. M. 1974. Science 185, 1049–1053 (1974).
We expanded our gastroenteritis studies to include infants and young children admitted to Children's Hospital of the District of Columbia with acute gastroenteritis in an attempt to determine the etiologic agents of this severe disease in this young age group (21). Previous studies from Australia, England, and Canada have suggested that an orbivirus- or reovirus-like agent might be an important etiologic agent of this disease (3-5,7,14). We examined stool filtrates prepared from stools obtained during January, February, and March of 1974 from 21 infants and children 2 to 20 mo of age who had acute gastroenteritis manifested by diarrhea with or without vomiting, by IEM and in some instances conventional EM as well. These children all had gastroenteritis severe enough to warrant hospitalization. We found characteristic reovirus-like particles in stool filtrates prepared from stools from 13 (62%) of the 21 patients (21). These particles had such a distinct morphological appearance and in general were so numerous in this study, that when stool filtrates were examined for the presence of reovirus-like particles by both conventional and IEM, they were positive by both methods. They measured approximately 70 nm in diameter and resembled the reoviruses morphologically (Fig. 3). We were able to demonstrate serologic evidence of infection by IEM, and in addition were able to develop a complement-fixation test using a stool filtrate as antigen and by this test system demonstrated serologic evidence of infection also (21).

In additional serologic studies (21) by the CF technique we found that the reovirus-like agent was not related to reovirus types 1, 2, and 3, and to those orbiviruses for which sera were available; it was noteworthy that the reovirus-like agent was found to be related to two animal viruses, the epizootic diarrhea of infant mice virus and the Nebraska calf diarrhea virus both of which resemble the reovirus morphologically. Its relationship to neonatal calf diarrhea virus has also recently been found in England (15).

It appears that the parvovirus-like Norwalk particle and the reovirus-like agent are etiologic agents of acute viral gastroenteritis. The reovirus-like agent may emerge as a major etiologic agent of infantile diarrhea (3-5,7,9,14,21-23). Preliminary surveys of sera from adults from various parts of the U. S. have shown that CF antibody to this or a related agent is quite common indicating that this agent is relatively ubiquitous (21). In a recent study by Wyatt et al. from our laboratory, the reovirus-like agent has been propagated in human fetal intestinal organ cultures, and in addition a serologic test has been developed utilizing indirect immunofluorescence (26). Another major step in the further study of these gastroenteritis agents will be attained when they are successfully propagated in a readily available cell culture system so that widespread epidemiologic studies can be carried out in order to elucidate their relative importance as agents of viral gastroenteritis. Until this is done, studies with available techniques should continue to yield valuable information.

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