

Detection by Immune Electron Microscopy of 27-nm Viral Particles Associated with Community-Acquired Diarrhea in Children

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The proportion of diarrheal illnesses of unknown origin that were associated with small round virus (SRV, 23–38 nm) particles among children <2 years old attending an outpatient clinic in Baltimore was determined. During a 9-month period, stool specimens from 188 patients with acute diarrhea and 108 healthy age-matched control children were examined for enteric bacterial pathogens, protozoa, enteric adenovirus, and rotavirus. An enteropathogen was identified in 75 patients (40%) and in 21 controls (20%). A random sample of specimens without an identifiable pathogen was then examined for SRV particles by immune electron microscopy (IEM) using commercial human gamma globulin. Viruses of 26–30 nm diameter that were not enteroviruses were detected in specimens from 9 (12.5%) of the 72 patients and 1 (1.8%) of the 53 control subjects ($P < .04$). Of 6 patients with available acute and convalescent sera, 4 demonstrated a significant immune response when tested by IEM. All patients experienced a mild, self-limited (1–3 days) illness. These findings suggest that SRV may be endemic in the Baltimore community and may result in clinically significant diarrheal illnesses.

Studies of the etiology of diarrhea in the USA and elsewhere associate 40%–60% of episodes with an infectious agent [1–3]. The relative importance of each individual agent differs with geographic location, and many episodes remain undiagnosed. Since its description in 1973, rotavirus has emerged as the most common cause of pediatric diarrhea in the USA [4, 5]. Currently, research is being carried out to establish the epidemiologic and clinical importance of other viral agents that have been associated with gastroenteritis—enteric adenovirus, enteric coronavirus, Norwalk and Norwalk-like viruses, calicivirus, astrovirus, and other small round viruses (SRV).

Data describing the epidemiology of Norwalk virus and most other SRV have been obtained largely from studies of outbreaks of gastroenteritis [6–8] and seroprevalence surveys [9, 10]. Norwalk virus was implicated in 31 of 74 outbreaks of acute nonbacterial gastroenteritis investigated by the Centers for Disease Control from 1976 to 1980, establishing that it is an important pathogen in epidemic diarrhea [11].

In developed countries immunity against this agent is acquired gradually during childhood and at a higher rate during adulthood, reaching a seroprevalence of 50%–70% by the fifth

decade of life. In contrast, in developing countries infection with Norwalk virus occurs early in life, and by 10 years of age 70%–90% of individuals have antibodies [9]. Specific incidence rates in infants and toddlers for illness associated with Norwalk virus and other SRV that cause diarrhea are not available, and the importance of these agents as pathogens during early childhood is not clearly defined.

Subjects and Methods

From 1 November 1984 to 31 July 1985 we conducted a prospective case-control study at the Community Pediatric Center at the University of Maryland in Baltimore to provide data on the etiology and incidence of acute diarrhea (<14 days duration) among outpatients [1]. Children <2 years old presenting with diarrhea were enrolled, along with healthy age-matched controls making well-child-care visits to the clinic. Clinical history, physical examination data, and a history of diarrhea in household contacts were recorded for each patient.

Whole stool samples or rectal swabs were obtained, tested for the presence of bacterial enteropathogens, protozoa, rotavirus and enteric adenovirus by methods described elsewhere [1], and stored at -20°C in phosphate-buffered saline (10% wt/vol, pH 7.4) until use. Acute (at the time of the visit) and convalescent (10–21 days later) sera were obtained from children with diarrhea. A sample of specimens negative for all of these agents was selected using a random numbers table and screened by immune electron microscopy (IEM) [12] using commercial human gamma globulin (Sandoglobulin, Sandoz, East Hanover, NJ). For these purposes specimens from patients and controls were analyzed under a code to which the examiner was blinded. Specimens positive for any spherical viral particles that measured 23–38 nm in diameter were analyzed for the presence of enterovirus by standard techniques (tissue culture and inoculation in suckling mice).

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When available, IEM was done with acute and convalescent sera from the same patient to investigate the presence of an immune response to the identified agent. The examiner performing IEM, who was blinded as to acute or convalescent status of serum, rated specimens for the relative amount of antibody coating the particles and the formation of viral aggregates as described elsewhere [12]. Antibody coating was rated on a scale of 0 to 4+. A 1+ change in antibody rating between paired serum samples was defined as an immune response [12].

Results

During the 9-month study, 188 patients with acute diarrhea and 108 age-matched controls were enrolled (~50% of those eligible). An enteric pathogen was identified in 75 patients (40%) and 21 controls (20%). As reported elsewhere in detail, rotavirus followed by enteric adenovirus were the most common agents associated with diarrhea [1]. When we examined by electron microscopy a random sample of specimens from 72 patients and 53 controls in whom no pathogen was identified, SRV that were not enteroviruses were detected in 9 (12.5%) of 72 patients and 1 (1.8%) of 53 controls (Fisher's exact test, $P < .04$) (table 1). In 3 other subjects (2 patients and 1 control), all of whom had received an oral polio vaccine within 4 weeks of collection of the sample, poliovirus was identified. Acute and convalescent sera were available from 6 of the 9 patients with nonculturable SRV; of these 6, 4 showed a significant immune response when tested by IEM.

The identified SRV were 26–30 nm in diameter; they typically had an irregular surface and formed small aggregates. None had the characteristic morphology of astrovirus or calicivirus.

The clinical manifestations were characterized by a brief self-limited illness with symptoms that included low-grade fever, vomiting, and diarrhea (table 2). In two cases a household contact had experienced diarrhea in the previous 2 weeks.

Discussion

The term "small round virus" is used to refer to spherical virus particles of 23–38 nm in diameter as observed in stool specimens by electron microscopy, and includes different viral agents that share the property of being excreted in stool, presumably as a result of intestinal replication. These viruses

Table 1. Results of immune electron microscopy screening of stool specimens with human gamma globulin.

Virus	Patients (n = 72)	Controls (n = 53)
Adenovirus	13	6
Small round virus (26–30 nm)	9	1
Coronavirus	0	2
Poliovirus*	2	1

* Polioviruses were identified when all the samples positive for small round viruses were inoculated into cell culture.

Table 2. Clinical symptoms of patients who had small round viruses (26–30 nm) observed in a fecal specimen.

Pa-tient	Age (mo)	Fever	Vomiting	Diarrhea			Immune response
				Duration (d)	Blood	Mucus	
1	2	+	+	1	–	+	–
2	2	+	–	2	–	+	–
3	4	+	+	2	–	+	ND
4	8	–	+	1	–	–	+
5	9	+	+	3	–	+	ND
6	10	+	–	2	–	+	ND
7	12	+	–	1	–	+	+
8	13	–	+	2	–	–	+
9	14	+	+	1	–	–	+

NOTE. Immune response was tested by immune electron microscopy with patients' acute and convalescent sera. ND = not done.

can be classified morphologically according to the structure of their surfaces as featureless (enterovirus, parvovirus, and Ditchling, cockle, and Parramatta agents) or structured (astrovirus, calicivirus, Norwalk virus, and Montgomery County, Hawaii, Snow Mountain, and Taunton agents) [13].

Among the featureless viruses, enteroviruses can occasionally cause diarrhea, but few reports implicate these agents in outbreaks of gastroenteritis. Although parvoviruses are known diarrheal pathogens in animals, diarrhea is only an occasional feature of erythema infectiosum caused by the B19 virus [14], thus far the only human virus officially classified as a parvovirus. However, several other unclassified SRV that resemble parvovirus morphologically (Ditchling, cockle, and Parramatta) have been involved in gastroenteritis outbreaks.

On the other hand, all the SRV that have a structured surface or ragged edge have been implicated in gastroenteritis outbreaks. These viruses may belong to a common family and represent different antigenic types. Recent studies have shown that Norwalk virus and Snow Mountain agent are antigenically related [15]; further, analysis of their structural proteins suggests that they belong to the family Caliciviridae [16–18].

In addition to morphologic differences, enteroviruses can be distinguished from the other SRV by being readily propagated in cell culture and suckling mice. Accordingly, we were able to identify poliovirus in three of the patients that excreted SRV. As performed in our study, IEM with sera of unknown specificity did not permit classification of the remaining viruses into one of the SRV groups already described [10]. Also, storage of the stool samples at -20°C and the potential viral coating with antibody during IEM may have altered the morphology, making it difficult to differentiate the characteristic surface structures of calicivirus and astrovirus [13]. Furthermore, due to the limited amount of sample available from each individual, cross-analysis of sera and stool specimens from different patients to demonstrate antigenic similarity was not possible.

The presence of SRV in stool specimens is not unusual.

When observed in the stools of infants these viruses have not always been associated with gastrointestinal symptoms. In the present study, however, the identification of SRV at a significantly higher frequency in patients than in controls and the demonstration of an immune response in four subjects is strong evidence that a causative relationship exists with diarrheal illness.

Our preliminary results over the 9-month study period suggest that SRV account for 5%–7% of diarrheal illnesses in outpatients <2 years old in a Baltimore inner city population. Taking into consideration that the Community Pediatric Center has 2234 active patients <2 years of age and 403 cases of diarrhea were seen among that population in 1985, and assuming that the proportion of diarrheal episodes caused by SRV does not change significantly during the year, we estimate an incidence of one episode of SRV diarrhea requiring medical care per 100 children per year.

The clinical manifestations were characterized by mild and self-limited gastrointestinal symptoms, findings that are similar to those reported in patients during outbreaks with Norwalk virus, calicivirus, astrovirus, and other SRV [6–8, 10]. Along with the well-characterized role of enterovirulent SRV in gastroenteritis epidemics, our data suggest that these viruses may have an endemic presence in the community and may account for a measurable portion of the episodes of diarrhea in children.

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