ASTROVIRUS GASTROENTERITIS

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I. INTRODUCTION

Acute infectious gastroenteritis is one of the major causes of death in the world (1). It also produces an enormous burden of disease-related morbidity. In the United States, mortality is limited but morbidity is substantial (1). It is only in the past two decades that viral agents of acute gastroenteritis have been discovered and defined for the first time and have joined bacteria and parasites as medically important pathogens. Indeed, it is now recognized that viruses cause approximately 30 to 40 percent of cases of acute infectious gastroenteritis in the United States, considerably more than known cases produced by bacteria and parasites (with the cause of about 40 percent of presumed cases of infectious gastroenteritis still unknown) (1). The purpose of this article is to summarize current concepts regarding the medical importance of astrovirus, one of the four families of viruses now known to cause human viral gastroenteritis.

II. BACKGROUND

There are four major categories of human gastroenteritis viruses recognized today: rotavirus, enteric adenovirus (types 40 and 41), calicivirus (including Norwalk virus and its relatives), and astrovirus. Their important medical and virological features are summarized in Table 1. Additional details are referenced (1).

Astrovirus was first recognized by electron microscopic examination of diarrheal stool samples from infants (2). 27 to 32 nm diameter round viral-like particles were observed that possessed a distinctive ultrastructure, exhibiting a five or occasionally six pointed star-like appearance on their surface and a continuous outer margin that formed a distinct rim (3). Because some particles had a star-shape on their surface, they were called astroviruses. These particles were later con-

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TABLE 1
Medical and Virological Features of Human Gastroenteritis Viruses

Virus	Medical Characteristics	Virological Characteristics	Medical Importance Demonstrated
Rotavirus (Group A)	Major cause of severe dehydrating diarrhea in infants and young children	70 nm diameter, double-shelled wheel-like capsid, double stranded segmented RNA	Yes
Enteric Adenovirus (Types 40, 41)	Endemic diarrhea of infants and young children	75 nm diameter, fastidious growth in culture, double stranded DNA	Yes
Calicivirus (Includes Norwalk virus and other small round structured viruses (SRSV))	Epidemics of acute diarrhea and vomiting in older children and adults, often food or waterborne	27 to 38 nm diameter, round virus, single structural protein, single stranded RNA	Yes
Astrovirus	Watery endemic diarrhea of children, in day care centers, some disease outbreaks, role in HIV-related diarrhea	27 to 32 nm diameter, classical structure with round surface and pointed star, several structural proteins, single stranded RNA	Yes

firmed as actual viruses. For 15 years, their detection in diarrheal stools could only be accomplished by electron microscopy, until our description in 1990 of an astroviral antigen detection enzyme-immunoassay (EIA) that employed virus-specific monoclonal antibodies (4). Thus, it was only in the 1990's that the medical importance of astrovirus became established. This is because the antigen-detection EIA permitted for the first time large scale epidemiological and clinical studies of astrovirus infections that the very cumbersome electron microscopic technique did not allow previously. Most of the findings summarized below in this article have been made possible by use of the new rapid detection techniques for astroviruses.

III. BIOLOGICAL CHARACTERISTICS

As described above, astroviruses have distinct ultrastructural features that distinguish them by electron microscopy from other 27 nm to 38 nm sized round viruses (3). They also contain a positive-sense, single stranded RNA genome of an approximate size of 7.2 kb (5). Several astrovirus structural proteins are described, including a 87 kDa protein that reacts with monoclonal antibody specific for viral capsids (5). There are also three smaller structural proteins of approx-

imately 30 kDa size (6). Sequence analysis of a portion of the viral genome and its protein product is consistent with classification of astroviruses as a new family of RNA viruses, the Astroviridae (7), as are the structural protein and replication strategy characteristics of the virus.

Astroviruses can be cultivated in cell cultures treated with trypsin (8, 9). There are seven serotypes (9, 10). We prepared a monoclonal antibody that reacts with a group-specific antigen shared by all astrovirus serotypes (9). When we incorporate the monoclonal antibody into an EIA format, it detects all astroviral serotypes in stool (4), thereby providing the framework for our understanding of the epidemiology and medical importance of astrovirus infection.

IV. CLINICAL SETTINGS

As our understanding of astrovirus infections deepens, it is apparent that they occur in several important clinical settings which are described below:

A. Endemic Childhood Diarrhea

Early electron microscopical studies detected astroviruses in stools of infants and young children with endemic diarrhea (11–13). However, these reports consisted of small numbers of patients, and there was a lack of controlled studies. Thus, astrovirus infection had yet to be firmly established as a cause of endemic pediatric diarrhea or, for that matter, any other setting for human gastroenteritis.

We studied 3150 children with and without gastroenteritis, who attended an outpatient clinic in Bangkok, Thailand (14). Stool samples were obtained from mostly age-matched children with and children without gastroenteritis in two one-year studies, three years apart. All stool samples were tested by our monoclonal antibody-based EIA test for astrovirus, as well as by similar assay format tests for rotavirus and enteric adenovirus, and stools were also tested for bacterial and parasitic pathogens. In the first study (1985–1986), 84% of children were less than two years old and 55% less than one year old. In the second study (1989), all children were less than 6 months of age.

As shown in Table 2, astroviruses were detected in 8.6% of children with gastroenteritis in the first study and in 2.0% of children without gastroenteritis. In the second study, the rates were 8.6% and 2.1%, respectively. Enteric adenoviruses were found in 2.6% of children with gastroenteritis for both studies combined and in 0.5% of controls, whereas rotaviruses were detected in 19% of those with gastroenteritis

TABLE 2
Frequency of Astrovirus Infection in Two Studies in Age-Matched Children with and Children without Gastroenteritis

Group	No. Tested		No. (%) Positive for Astroviruses*	
	Study 1	Study 2	Study 1	Study 2
With Gastroenteritis	1111	580	96 (8.6)	50 (8.6)
Without Gastroenteritis	947	512	19 (2.0)	11 (2.1)

* In both studies the children with gastroenteritis had significantly more astrovirus infections ($P < 0.001$).

Reprinted from reference (14).

and in 1.0% of controls. A stepwise logistic regression analysis was applied in all patients to data on all viral, bacterial and parasitic pathogens simultaneously to validate that astrovirus infection was independently associated with gastroenteritis in both studies. These two controlled studies in Thailand provided conclusive epidemiologic data to indicate that astroviruses are etiologic agents of gastroenteritis. Clinical findings observed in this study and their relevance to the medical importance of astrovirus infections are outlined below in the section "Clinical Findings."

Astroviruses were detected by the monoclonal EIA test in 7.3% of young children with diarrhea in rural Guatemala studied over a two year period and in 2.4% of well controls (15). These findings were therefore similar to those in Thailand. A small study of diarrhea in pediatric outpatients in Baltimore revealed 5 of 184 children (2.7%) with astrovirus infection compared to 2 of 144 (1.4%) of controls (16). In reported endemic diarrhea cases from Japan, 8.5% of 165 patients (from whom no other bacterial or viral agent was identified) were infected with astrovirus (17). Overall, we can conclude tentatively, based on reported studies, that about two to ten per cent of endemic pediatric gastroenteritis cases are caused by astrovirus infection.

B. Day Care Center Outbreaks

Two studies of gastroenteritis in day care settings implicate astrovirus as an important pathogen. Using the monoclonal antibody-based EIA test (18), a prospective study of children in day care in Maricopa County, Arizona revealed astrovirus in 4% (21/524) of children with diarrhea compared to less than 1% (1/138) of those without symptoms, ($p < 0.05$). The Arizona study also revealed that the incidence of astrovirus-associated diarrhea was comparable to that of the other

enteric viruses studied (rotavirus, enteric adenovirus and calicivirus), and, therefore, by inference astrovirus represents a substantial percentage of the viral gastroenteritis observed in day care settings.

A second prospective study was performed with children experiencing outbreaks of gastroenteritis in day care centers in Houston, Texas. Astrovirus was detected in 6 (7%) of 81 outbreaks studied using the monoclonal antibody based EIA test (19). It was particularly noteworthy that the youngest children (less than 12 months of age) were most commonly infected and symptomatic.

C. Nosocomial Diarrhea

Astroviruses appear to be a common cause of nosocomial outbreaks of infant diarrhea. The limited number of reports of this entity all rely solely upon electron microscopy for diagnosis. One 16-month surveillance study at a Swedish Children's hospital uncovered 32 children with nosocomial gastroenteritis, of which 25 were part of two epidemic nosocomial outbreaks (20). Attack rates were highest in those under 12 months of age and in those with underlying gastrointestinal diseases. The 32 nosocomial astrovirus infections were similar in frequency to 33 nosocomial rotavirus cases detected and considerably more frequent than calicivirus or adenovirus infections. In this study, and in one other reported study using electron microscopy (21), 6 to 9 per cent of non-outbreak cases of nosocomial diarrhea in young children were due to astrovirus infection.

D. Epidemic Diarrhea of School Age Children and Adults

There are few reports of astroviruses causing epidemics of gastroenteritis. The best described outbreaks occurred in Japan. One large outbreak took place in 1991 among students and teachers at 14 primary and junior high schools in Osaka (22). Over 4700 persons were affected in a 5-day outbreak, believed to be caused by contaminated food prepared by a common supplier. Ten stool samples from the outbreak were positive for astrovirus when tested by a combination of assays including electron microscopy, monoclonal antibody EIA, and a newly developed polymerase chain reaction test. Illness was described as relatively mild since the school absenteeism rate increased only two to threefold. Another smaller disease outbreak affected children and staff at a kindergarten in Sendai (23). Based on the scanty number of reports available, it appears that astroviruses may be an uncommon cause of epidemic gastroenteritis in school aged children and their adult contacts.

E. Nursing Home Outbreaks

Several outbreaks of gastroenteritis due to astroviruses are reported in residential facilities for the elderly and on geriatric wards in the United States and United Kingdom (24–26). Limited numbers of patients with a definitive diagnosis are described, because only electron microscopy was used to study stool samples. A careful, prospective study of the role of astroviruses in diarrhea of the elderly is clearly needed. Although these viruses appear to infect primarily infants and young children, they could be a significant pathogen for elderly persons whose immunity to the virus has waned over time. It is known, in this regard, that IgM serum antibodies to astrovirus were detected in some elderly individuals as part of a seroepidemological study conducted in the United Kingdom (27), further suggesting circulation of astrovirus in this age group.

F. Diarrhea in HIV-Infected Patients

Several reports are published of the role of enteric viruses in the diarrhea of HIV-infected patients. Only one report conducted a comprehensive search specifically for astroviruses, using electron microscopy, monoclonal antibody EIA, and polymerase chain reaction (28). This study employed a broad repertoire of assays designed to detect all known human diarrhea viruses in an extensive virologic evaluation that had previously not been performed. A cohort of 65 patients in Atlanta with acute diarrhea (less than 28 days) and an equal number of controls without diarrhea were prospectively evaluated by CDC. An enteric virus was detected in 35% of fecal specimens from those with diarrhea and in 12% of samples from those without diarrhea ($p < 0.001$). Specimens from individuals with diarrhea were astrovirus-positive in 12% and from those without diarrhea in 2% ($p = 0.003$). Astrovirus was the most common enteric pathogen diagnosed in this study; it was recognized more frequently than any other enteric virus or any bacterial or parasitic enteropathogen. Most patients studied had severe HIV disease (CDC clinical stage IV-C) but detection of enteric viruses was not associated with levels of CD4 counts. It is clear that astroviruses play a role in AIDS-related diarrhea, and that additional studies need to be carried out to define further the extent of their involvement.

V. CLINICAL FINDINGS

The incubation period for astrovirus disease is 1 to 5 days, and illness lasts 1 to 5 days in the immunocompetent host (23, 26). Most

illnesses occur in infants and young children although there may be a resurgence of disease susceptibility in the elderly whose immunity may have declined over time.

The most comprehensive analysis of clinical findings was conducted in our study of endemic childhood diarrhea in Thailand (14). Clinical findings in astrovirus-infected children (from whom bacterial, parasitic, or other viral pathogens were excluded) were compared to clinical findings in similarly analyzed rotavirus-infected children (see Table 3). Watery or mucoid diarrhea, nausea, abdominal pain, vomiting and fever were noted in the majority of astrovirus- and rotavirus-infected children. Clinical differences between the two viral infections were not noted, except that dehydration was less common with astrovirus than rotavirus ($p < 0.08$). Only two percent of the astrovirus-infected children required hospitalization in this cohort.

Clinical features of infection were also evaluated in adult volunteers. In one study, one of 17 volunteers fed the virus developed overt illness with vomiting, profuse watery diarrhea and assorted constitutional symptoms, and five developed mild constitutional and abdominal symptoms without diarrhea or vomiting (29). Virus-specific antibody levels increased in most of the volunteers and fecal virus shedding in some. In a second study, one of 19 volunteers administered the virus developed nausea, vomiting and diarrhea, while serologic responses developed in himself and 8 asymptomatic persons (26). It, therefore, appears that astrovirus is of relatively low pathogenicity in immunocompetent, healthy young adults, in striking contrast to the effect of Norwalk virus in volunteers (1).

TABLE 3

*Clinical Findings Associated with Astrovirus and Rotavirus Gastroenteritis**

Findings	Astrovirus Infection (N = 44)	Rotavirus Infection (N = 175)
	Percent	
Watery	61	67
Loose stools	41	35
Mucoid stools	55	51
Bloody stools	7	6
Nausea+	71	88
Abdominal pain+	58	63
Vomiting	61	67
Fever	80	83
Dehydration ≥5%	5	15

* Only stool samples in which no bacterial, parasitic, or other viral pathogens were detected are included.

+ Includes data from Study 1 only (33 children with astroviruses and 116 with rotaviruses).

Reprinted from reference (14).

VI. SEROLOGICAL FINDINGS AND IMMUNITY

Serum antibodies to astrovirus are common, as they are found in commercial preparations of human gamma globulin in the United States and Japan (23, 30). Antibodies to all viral serotypes are present in U.S. gamma globulin (30). Antibodies develop in over 70 percent of British children by 3 to 4 years of age and most adults possess serum antibodies (31). The protective role of serum antibodies against illness is uncertain. Volunteers who mount serum antibody responses after inoculation tend to be those who lack pre-existing serum antibodies and volunteers who are ill (26, 29). Since most astrovirus disease occurs in the very young, but disease outbreaks have been found in the elderly, it may be that most young adults may be protected by antibody levels that decline with age (30).

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DISCUSSION

Dupont, Houston: Neil, you have been instrumental to our understanding of enteric viruses and their importance as agents in clinical cases of diarrhea by taking laboratory identification to the field and showing the importance of various viral agents. It was in 1970 when you came to visit Dick Hornick and me to look at some stool samples that you had from an outbreak in Norwalk, Ohio. The Norwalk virus was soon thereafter identified and very important studies took place. I am glad to see you are now on track or on the trail of a new virus to see how important it is because we still have a majority of cases

of diarrhea in which we cannot ascribe an etiologic agent in most populations. So the quest for new agents is essential. The one group in which agents are almost never found is adults with diarrhea in any population. Even in tropical endemic countries, we cannot ascribe an agent to adult cases of diarrhea. Children quite often do have an etiologic agent identified, but I'd be very interested in the future in seeing if astrovirus could explain some of this illness. I'd also like for your comments about anything about the epidemology of astrovirus that might be different from Norwalk virus? Does it show the same seasonal pattern? What is the vehicle of transmission or the reservoir? Do we have any information about this? Is there a family of astroviruses like Norwalk or is it a single agent that we are talking about?

Blacklow: Doctor Dupont is a longtime colleague and former collaborator. Let me comment about astroviruses and other viral agents in adults. You are correct, that many of these agents produce most of their disease in young children, although that is not the case with Norwalk virus. The epidemiology of astrovirus is just now being unraveled and we are only a few years into these studies. However, as I mentioned, we know that astroviruses are responsible for a number of well described nursing home outbreaks. There was one outbreak that was well described and published caused by what was called the Marin County Agent. It was felt to be an agent related to the Norwalk virus. It turns out that particular agent is an astrovirus. We also know that if we look at large numbers of elderly patients, as the British have done, for IgM serum antibodies to astrovirus, (presumably indicating recent exposure to the virus in nursing home and geriatric wards) that a sizable percentage of these patients have astrovirus antibodies. So I suspect what probably happens with astrovirus is that immunity does develop in childhood, but probably wanes during adulthood. That is a hypothesis that needs to be tested.

Douglas, Whitehouse Station: Neil, it is nice to see how much progress we have made in the astrovirus field and in the field of viral etiology of gastroenteritis. I was thinking about methods of control. You started off your talk with a slide from the World Health Organization showing that there were something like 4.2 million diarrheal deaths in the world annually. It seems as if astroviruses probably are a small contributor, albeit important to that number. What are your thoughts on what should be done? The most successful methods of control in infectious diseases have been through either sanitation or immunization. Is either one of these a candidate here?

Blacklow: I think sanitation is critical for the prevention of diarrhea caused by all agents including bacteria, viruses and parasites. In terms of vaccination, I think it is far too premature to address the issue of astrovirus. I think we still have to know more about its overall medical importance in diarrheal diseases. Rotavirus is a much more important target for immunization and, as you know, a number of vaccines are under experimental study at this time.

Carey, Charlottesville: Worldwide, malnutrition has been an important risk factor for the development of diarrheal disease, especially chronic diarrhea. Could you discuss the possible role of malnutrition in the childhood diarrhea of viral etiology?

Blacklow: This is a good question, but one for which there are incomplete data. The mortality due to viral agents is increased in settings of malnutrition, but I am not sure that there is persuasive evidence that the incidence of infection with these agents changes markedly in conditions of malnutrition. I suspect it's the response of the host to the infection that changes with malnutrition.

Billings, Baton Rouge: With regard to the adult diarrheas, are there any therapeutic agents that are helpful? By that I mean the antimotility agents or any of the things that we commonly use. Are they in some cases harmful, like in the toxic diarrheas that we deal with in patients who are immunosuppressed?

Blacklow: A study was published by one of the earlier questioners, Dr. Douglas, that looked at the effect of bismuth subsalicylate in patients infected with Norwalk virus. A mild effect was noted on abdominal symptoms such as cramping, but not on viral shedding. No adverse effects were seen.

Billings: Are these diarrheal syndromes self limiting?

Blacklow: Yes, in the United States.

Billings: So none of this may be very serious.

Blacklow: There is the major exception of the third world where an overwhelming, dehydrating diarrhea can develop. Then mortality certainly is a major problem. In the case of rotavirus, there are about 875,000 deaths estimated per year by the World Health Organization.

Austrian, Philadelphia: Doctor Blacklow, have you observed dual infection with rotaviruses and astroviruses in any of your studies?

Blacklow: Yes, we have. Several of the children in the New England Journal study that I presented had dual infections, so in order to do some of the analyses, we had to exclude these children.