CHAPTER 43

Viral Gastroenteritis

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The history of medicine, and medical virology in particular, is replete with impressive examples of the relatively rapid discovery of specific viruses as etiologic agents of frequently occurring illnesses such as smallpox, chicken pox, measles, poliomyelitis, mumps, rubella, the common cold, and influenza. Such discoveries led to the development of vaccines that prevented or modified certain of these diseases. Conspicuously absent from this list of medical conquests had been the etiologic agents of viral gastroenteritis, not only one of the most frequently occurring illnesses worldwide—second only to acute respiratory illnesses—but also one of the most important since it appears to be a major cause of mortality in infants and young children in developing countries [1]. However, with the discovery in 1972 of the 27-nanometer (nm) Norwalk virus, which is now known to be a major etiologic agent of a self-limited form of epidemic nonbacterial gastroenteritis of adults and children, and the discovery in 1973 of the 70-nm rotavirus, which is the single most important etiologic agent of severe diarrhea of infants and young children, a major segment of an important disease category was finally on the verge of exposure etiologically [1–5]. It is remarkable that in this era of tissue culture virology both of these agents were identified initially without the benefit of any *in vitro* tissue culture system since neither could be propagated directly in cell cultures from clinical specimens. Each of these viruses was detected initially by examination of clinical material by electron microscopy. This method has been termed “direct virology” since it entails the examination of material directly from clinical specimens without the conventional intermediate *in vitro* or animal *in vivo* systems characteristically required for virologic studies [5].

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Viral gastroenteritis consists of two distinct clinical entities with quite different epidemiological characteristics. One, which is associated with the 27-nm Norwalk group of agents, is designated epidemic viral gastroenteritis and occurs in family or community-wide outbreaks affecting adults, school age children, family contacts, and some young children as well. The clinical characteristics, which usually last only 24 to 48 hours, are self limited and mild and are comprised of a variety of signs and symptoms such as nausea, vomiting, diarrhea, abdominal pain, and low-grade fever or a combination of these [5-7]. Parenteral fluid replacement therapy is not usually required.

The 27-nm Norwalk virus was visualized by immune electron microscopy (IEM) in an infectious stool filtrate derived from a gastroenteritis outbreak in an elementary school in Norwalk, Ohio, which affected 50% of the students and teachers and had a secondary attack rate of 32% [2]. Attempts to recover an agent from this outbreak were unsuccessful. However, a rectal swab filtrate derived from a secondary case induced the disease in volunteers; a pathological lesion characterized by broadening and blunting of the villi of the jejunum was observed by light microscopy [6,6a]. Serial passages also induced illness [7]. In spite of the experimental transmission of the disease, attempts to cultivate an agent were once again unsuccessful [6,7]. However, with the IEM technique, antibody-coated 27-nm particles were visualized in an infectious stool filtrate following reaction with a convalescent serum [2,5]. Serologic evidence of infection with this particle was also demonstrated by IEM with paired sera from certain volunteers and individuals from the original outbreak [2]; in addition, a close temporal relationship between virus shedding and illness was observed by IEM [8]. From these and other studies, the 27-nm Norwalk virus was established as the etiologic agent of the Norwalk outbreak without the benefit of any *in vitro* system.

It soon became apparent that the role of the Norwalk agent in epidemic viral gastroenteritis was not limited to a localized area in the United States. The development of a radioimmunoassay blocking technique enabled the testing of paired serum specimens obtained from numerous outbreaks of nonbacterial gastroenteritis: 24 (34%) of 70 such outbreaks were associated serologically with the Norwalk agent [4,9]. Family and community outbreaks predominated, but several common source outbreaks were also observed including contaminated shell food, drinking water, and swimming water. Recently, a major outbreak of Norwalk gastroenteritis was associated with the ingestion of contaminated cake frosting [10]. Affected individuals in these outbreaks ranged from 4-year-old children to elderly adults [4,9].

Limited studies in infants and young children in both developed and developing countries show that the Norwalk virus is clearly not the long-sought agent of severe infantile gastroenteritis [5,11,12]. Further evidence of this was revealed from antibody prevalence studies in the United States, which demonstrated that Norwalk antibody was acquired gradually, beginning slowly in childhood and reaching 50% by the fifth decade. In contrast, rotavirus antibody was acquired early in life as over 90% of the same individ-

uals developed such antibody by the age of 36 months [5]. However, Norwalk antibody was acquired quite rapidly by infants and young children in several developing countries [9]; and in a study in Bangladesh, Norwalk seroresponses were observed in this age group and 1%–2% of the episodes of diarrhea were associated with Norwalk virus infection [13].

The nature of immunity to Norwalk virus remains an unusual and unresolved feature of the natural history of such infection. Adults are highly susceptible to both natural and experimental challenge. The attack rate reached over 80% in certain outbreaks and in addition, about 50% of unselected volunteers developed illness following oral administration [4,7,13a,b]. Volunteer studies reveal both short- and long-term immunity [14]. The long-term immunity is rather perplexing as volunteers challenged with Norwalk virus on two separate occasions 27 to 42 months apart either developed illness following both challenges or were resistant to both challenges [14]. The difference in susceptibility could not be explained on the basis of pre-challenge Norwalk virus antibody levels. In further studies, volunteers who developed illness tended to have higher serum antibody titers and significantly higher jejunal fluid antibody titers to the Norwalk virus than did volunteers who remained healthy following challenge [9].

Classification of the Norwalk agent has not been possible since it does not grow in cell cultures. However, study of the proteins of Norwalk virus by polyacrylamide gel electrophoresis following purification of antigens from stools revealed that the virus had a single structural protein with a molecular weight of 59,000 daltons and an additional soluble protein of 30,000-dalton molecular weight [15]. Such a pattern is consistent with that of the calicivirus. Although the Norwalk virus does not possess the distinctive cup-like surface morphologic features of the caliciviruses, it does have surface indentations in certain preparations and thus could be considered, at least, to be calicivirus-like. However, a final classification of this virus awaits further study including such properties as its nucleic acid content. It should be noted that other fastidious agents that are morphologically similar but antigenically distinct from the Norwalk virus have been detected by electron microscopy and associated with gastroenteritis outbreaks. These include the Hawaii, Ditchling or W, Cockle, Parramatta, Snow Mountain (Colorado), and Marin County agents [5].

Rotaviruses are the major known etiologic agents of severe diarrhea of infants and young children worldwide and are associated with the other form of viral gastroenteritis—sporadic infantile gastroenteritis, which differs from epidemic viral gastroenteritis in three major ways: (1) it is associated with a severe diarrheal illness in infants and young children, which may require hospitalization and fluid replacement therapy; (2) it does not usually occur in sharp outbreaks; and (3) the illness rate among family contacts of patients with this type of gastroenteritis is low (although the infection rate may be high) [5,16].

The human rotaviruses were discovered in 1973 in Australia by electron

microscopic examination of thin sections of duodenal biopsies obtained from infants and young children hospitalized with gastroenteritis [3]. Characteristically, pathological changes in the mucosal epithelial cells included shortening and blunting of the villi and the presence of virus-like particles within distended cisternae of the endoplasmic reticulum. Soon thereafter, rotaviruses were detected readily in stool suspensions by negative stain electron microscopy [17]. Initially, human rotaviruses could not be propagated in cell culture from clinical specimens and thus once again electron microscopy became the mainstay for the diagnosis of an enteric infection. However, second and third generation assays have been developed that also exploit the direct virologic approach. These include radioimmunoassay, ELISA, CIEOP, gel electrophoresis of RNA, dot hybridization, and many others [5,18]. The most widely used method worldwide is the ELISA.

The word, rotavirus, is derived from the Latin word *rota* meaning wheel, because the sharply defined circular outline of the outer capsid layer gives the appearance of the rim of a wheel placed on short spokes radiating from a wide hub [16]. Rotaviruses belong to the reoviridae family and possess a genome consisting of 11 segments of double-stranded RNA. Complete double-shelled particles have a density of 1.36 g/cm³ in cesium chloride [16]. In infected cells, rotaviruses code for 10 to 12 polypeptides of which 5 to 9 are considered structural; at least 3 are on the inner, and 2 on the outer capsid [16]. Rotaviruses also contain an RNA-dependent RNA polymerase, which is activated upon removal of the outer shell polypeptides [16].

In studies in Australia, United States, and Japan, rotaviruses have been associated with about 35% to 45% of acute diarrheal disease requiring hospitalization of children [11,12,19]. For example, in the United States study, from January 1974 to July 1982 rotaviruses were detected in the stool of 34.5% of 1537 pediatric in-patients with gastroenteritis [12]. In the temperate climates, rotaviruses display a unique temporal pattern of infection characterized by peaks, which occur almost exclusively in the cooler months of the year [11,12,19]. For example, in the United States study, between January 1974 and December 1981, rotavirus infections were not observed in the months of June, July, August, and September, whereas the major peaks of infection occurred in January and February [12]. The reason for the peaks during the cooler months is not known, although the association with cold weather periods has been shown; the influence of relative humidity has not been consistent [11,12]. In tropical climates, rotavirus infections are observed throughout most of the year, although less pronounced peaks can occur [1,11]. It should be noted that rotavirus can also cause mild gastroenteric illness not requiring hospitalization [1,5].

Rotaviruses have also been shown to be important etiologic agents of severe diarrheal illness in less-developed countries [1,20]. For example, in a study of the etiology of moderately severe or severe gastroenteritis in 6352 patients treated or hospitalized in a treatment center in Dhaka, Bangladesh, during a one-year period, rotaviruses were the most important pathogen in

patients two years of age or younger; 46% of such patients shed this agent [20]. It was of interest that toxigenic *E. coli* strains were next in importance in this age group, with 28% of a sample of patients shedding such strains. Bacterial agents were detected more frequently than rotaviruses among children 2 years of age and older.

Rotaviruses have also been implicated as important etiologic agents of diarrhea in numerous animals including calves, pigs, mice, foals, lambs, and many others [5]. Historically, it is of interest that the mouse, calf, and simian rotaviruses and the "0" agent (from intestinal washings of sheep and cattle) were described prior to the discovery of human rotaviruses [5,16]. The animal strains characteristically share common CF and immunofluorescent antigen(s) with each other and with human rotaviruses, as well, although recently a few human and animal strains have been described that do not share this common antigen(s) [5,16]. These have been designated tentatively as pararotaviruses. Rotaviruses also have a subgroup antigen that separates most strains into 2 subgroups [21]. The group and subgroup specificities are coded for by the sixth gene, the product of which is the major inner capsid polypeptide with a molecular weight of 42,000 daltons [16,21].

A major advance in the understanding of the natural history of rotavirus infection was made when Japanese investigators discovered that human rotaviruses could be grown efficiently in tissue culture following trypsin treatment of the virus prior to and during cultivation in roller tube cultures at 37°C [22,23]. The cell culture propagation of rotaviruses has allowed the conventional classification of rotaviruses according to neutralization specificity [24,25]. At present there is agreement on at least four serotypes, which are designated serotype 1 (Wa), serotype 2 (DS-1), serotype 3 (P or M), and serotype 4 (Saint Thomas No. 4) [24]. (A strain of each serotype is shown in parentheses.) It is striking that certain animal rotaviruses share neutralization specificity with human rotaviruses [24,25]. For example, rhesus (SA-11, MMU18006), canine (CU-1), feline (Taka), and equine (H-2) rotaviruses are indistinguishable from human rotavirus type 3 by neutralization [25]; however, except for the canine and feline strains, which have identical RNA gel electrophoretic patterns, each of these animal strains differs in its RNA pattern from the others and from the human strain, and in addition, differs in its subgroup specificity from the human strain [25]. The major protein responsible for neutralization specificity is coded for by the eighth or ninth gene, the product of which is an outer capsid protein that is glycosylated post-translationally and has a molecular weight of 34,000 daltons [16]. It is noteworthy that serotypes 1, 3, and 4 have subgroup 2 specificity whereas serotype 2 has subgroup 1 specificity [24,25].

Information on the mechanism of immunity to rotaviruses in humans is not clear. Volunteer studies reveal a correlation of serum antibodies with resistance to illness following challenge; in this same study the role of intestinal fluid neutralizing activity was not clearcut [26]. However, animal studies indicate that antibody in the lumen of the small intestine is the prime

determinant of protection against illness [1,5,16]. A paradoxical aspect of immunity to rotaviruses is the high rate of subclinical infection in neonates undergoing initial infection in newborn nurseries [16,27]. It is striking that such neonatal infections conferred significant protection against illness (but not infection) following documented second natural rotaviral infections for a period of about 3 years following the initial neonatal infection [27].

Since it is clear that a rotavirus vaccine is needed in the developing as well as the developed countries, various approaches are being pursued in producing such a vaccine. (1) *A live attenuated human rotavirus vaccine*. The Wa strain, which was successfully cultivated in primary African green monkey kidney cell cultures following prior serial passage in piglets, was studied as a vaccine candidate initially in animals, then in volunteers [26,28]. This vaccine appeared promising as none of the volunteers developed illness and 6 of 10 with low levels of prechallenge antibody developed a seroresponse [26]. However, studies with this vaccine lot were suspended when unexpected serum transaminase elevations were observed in three volunteers, only one of whom had developed a rotavirus seroresponse [26]. The etiology of these elevations is not yet known, although various possibilities are described [26]. (2) *A live attenuated vaccine derived from a heterologous host*. A bovine rotavirus (Lincoln strain of NCDV), which had been attenuated by multiple passages in calf kidney cells at low temperature (this strain is used as a vaccine in newborn calves and in cows) was administered orally to adult volunteers initially and later to children [29]. It did not cause illness and in children induced frequent homotypic and infrequent heterotypic rotavirus serum antibodies. This approach was based on previous animal studies that demonstrated that prior administration of bovine rotavirus to calves while *in utero* protected against challenge with human rotavirus (serotype 1) at birth [30]; in addition, prior administration of this candidate vaccine to piglets significantly decreased the shedding of human rotaviruses following such challenge [29]. Recent studies indicates that the calves developed not only homotypic neutralizing antibody, but also heterotypic antibody to human rotaviruses following the *in utero* administration of bovine rotavirus [30]. A rhesus rotavirus is also a possible vaccine candidate since it not only represents a strain from a heterologous host but is serotypically identical to human rotavirus type 3 [24,25]. (3) *An attenuated human-animal rotavirus reassortant vaccine*. This approach was used successfully for the propagation of noncultivable human rotavirus strains [31]. It took advantage of the well-known characteristic of the reoviridae to undergo genetic reassortment with efficiency during mixed infection in cell culture [31]. Noncultivable human rotaviruses were cocultivated with a *ts* bovine rotavirus mutant at permissive temperature and harvests were subjected to selection by growth at a restrictive temperature in the presence of bovine rotavirus antiserum. The resulting progeny virus not only grew efficiently in cell culture but also had the neutralization specificity of the human rotavirus parent [31]. Thus,

noncultivable human rotavirus strains were "rescued" for study of the serotypic diversity of naturally occurring strains. This basic approach is now being used for development of reassortant rotavirus vaccines that possess ten animal rotavirus genes and the major neutralizing gene from a human rotavirus parent except that wild type bovine rotavirus or rhesus rotavirus (i.e., *ts* +) is used as the donor of the heterologous genes [32]. (4) *Cloning the rotavirus genome by DNA technology*. Major advances have been made in sequencing various genes of several rotaviruses. Of special note has been the sequencing of the genes that code for the major SA-11 and UK rotavirus, neutralization protein; in both instances the gene was 1062 bases long [33,34]. Insertion of a complete DNA copy of such a gene, or that portion responsible for its major antigenic sites in an acceptable vector and its expression in a suitable vehicle might provide an efficient method for immunization [33,34a]. (5) *Production of synthetic vaccines*. Since the major neutralizing gene of at least two rotaviruses has been sequenced, it should be possible to define the region that codes for type-specific antibody. It may then be feasible to produce a synthetic vaccine containing the peptide(s) responsible for inducing protective antibodies.

It should be noted that administration of rotavirus antigens to expectant mothers as a means of boosting breast milk antibody is being considered as a means of imparting protective antibodies passively to the infant. In addition, significant passive protection of neonates against rotavirus-induced gastroenteric symptoms has been demonstrated following oral administration of human gamma globulin containing rotavirus antibody [35]. Such approaches have been shown to be effective in animal studies [1,5]. Also, rotavirus antibody-containing milk has been administered as a therapeutic measure to immunodeficient patients with chronic rotavirus infection [36].

Remarkable progress has been made in elucidating the major etiologic agents of viral gastroenteritis. Not considered in this discussion has been the role of other enteric viral agents associated with gastroenteric illnesses, such as enteric adenoviruses, calciviruses, astroviruses, small round viruses other than Norwalk, putative coronaviruses, and the Otofuke agent [5]. With the exception of the enteric adenoviruses, which are associated with approximately 5% of diarrheal illnesses of infants and young children requiring hospitalization, the contribution of these other agents in the etiology of severe infantile diarrhea appears minimal. Although numerous agents—viral, bacterial, and parasitic—are detectable in stools, it should be emphasized that even with the most advanced techniques, an etiologic agent cannot be found in approximately 40% of all pediatric patients hospitalized with diarrhea. Thus, elucidation of the etiology of these undiagnosed diarrheal illnesses remains an important area of research that must be pursued vigorously if the morbidity and mortality from diarrheal diseases is to be controlled effectively and comprehensively. Although much progress has been made in the field of viral gastroenteritis, it is clear that major efforts are still needed to conquer this very common and important affliction.

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