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Viral Gastroenteritis

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Infectious diarrhea is a universal health problem that is responsible for extensive morbidity in the United States and that accounts for tremendous mortality in underdeveloped areas of the world. It is estimated that acute diarrhea causes 3 to 5 billion episodes of illness annually, resulting in 5 to 10 million deaths in Asia, Africa, and Latin America.[72] Two classes of viruses have been clearly shown to be important intestinal pathogens with worldwide distribution. Acute viral gastroenteritis occurs in all age groups, although life-threatening illness generally occurs only in infants or in those who are debilitated. The economic consequences of gastroenteritis are enormous, both in lost work time and in expenditures for symptomatic treatment. The financial aspects of acute diarrheal illness are underscored by the enormous amount of work in veterinary science directed at understanding and preventing gastroenteritis in cattle and domestic animals.

During the past decade major advances have been made in our understanding of viruses that cause gastroenteritis. Previously, viral gastroenteritis was assumed when an acute, self-limited diarrheal illness occurred and no known bacterial or parasitic infection could be implicated. During the 1940s and 1950s Gordon and coworkers were able to induce gastroenteritis in volunteers by the oral administration of bacteria-free filtrates of diarrheal stool. Serial passage studies suggested replication of the infectious agents in the human intestinal tract.[51] Similar studies in Japan yielded other infectious agents that appeared to be related to the American viruses when studied by cross-challenge studies.[47] However, intensive study of these infectious inocula using the technique of cell culture and laboratory animal systems then available failed to yield any agent that could be identified, cultivated, or studied in any system other than the volunteer. Known enteric viruses were occasionally implicated in the production of gastrointestinal disease, but worldwide epidemiologic studies indicated that known enteric viruses were not a common cause of acute intestinal illness.[13]

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Interest in gastroenteritis viruses waned during the 1950s and 1960s but exploded again in the 1970s, encouraged by the development of new techniques and the reapplication of other established techniques to the study of these viruses. The replication of viruses in organ culture systems led to the adaptation of this technique for cultivation of enteric viruses. The examination of stool specimens using the electron microscope and immune electron microscopy has provided extensive information about a variety of agents of gastroenteritis. Two major classes of agents have proved to be frequent intestinal pathogens: the Norwalk-like viruses, which have been most thoroughly studied in controlled laboratory settings; and the rotaviruses, which are the major pathogens infecting infants of all mammalian species. These viruses are illustrated in Figure 1.

Renewed interest in nonbacterial gastroenteritis began slowly in 1968 with an epidemiologic study of an outbreak of gastroenteritis in Norwalk, Ohio. The fecal specimens obtained at this outbreak have been used to induce disease in volunteers. Subsequently, this virus has been visualized, partially purified, and analyzed. Exact description of the clinical syndrome induced by the Norwalk virus and studies of immunity to Norwalk and other viruses were made possible by carefully controlled volunteer studies, including rechallenge and cross-challenge studies.

Electron microscopy of stools was initially used to identify the Norwalk virus in the infectious fecal filtrates. This success led to examination by electron microscopy of stool specimens obtained from ill children. Rotavirus was rapidly identified as the major pathogen causing gastroenteritis in infants and young children. Rotavirus infection, which is relatively easy to detect, subsequently has been well characterized in extensive epidemiologic and clinical studies. The human pathophysiology of rotavirus infection has been less well studied than that induced by the Norwalk agent, but the presence of appropriate animal models has encouraged rapid advances in our understanding of rotavirus infections.

Careful examination of stools of patients ill with gastroenteritis has led to the identification of many virus-like particles in the stools of ill patients. These particles include astrovirus, minireovirus, coronavirus, adenovirus, and calicivirus; however, these viruses do not appear to be common widespread pathogens comparable to rotaviruses and Norwalk-like viruses.

NORWALK-LIKE VIRUSES

The Norwalk virus is the prototype virus that causes epidemic gastroenteritis infecting predominantly older children and adults.[120] Family, school, and community-wide outbreaks are common.[55] The original epidemic occurred in a public school in Norwalk, Ohio in 1968. Fifty per cent of the students developed illness, followed by a high secondary attack rate among family members. The incubation period approximated 48 hours. Symptoms of diarrhea, nausea, vomiting, fever, malaise, myalgias, and abdominal cramps lasted 24 to 48 hours. All patients recovered without complications or significant sequelae.[2]

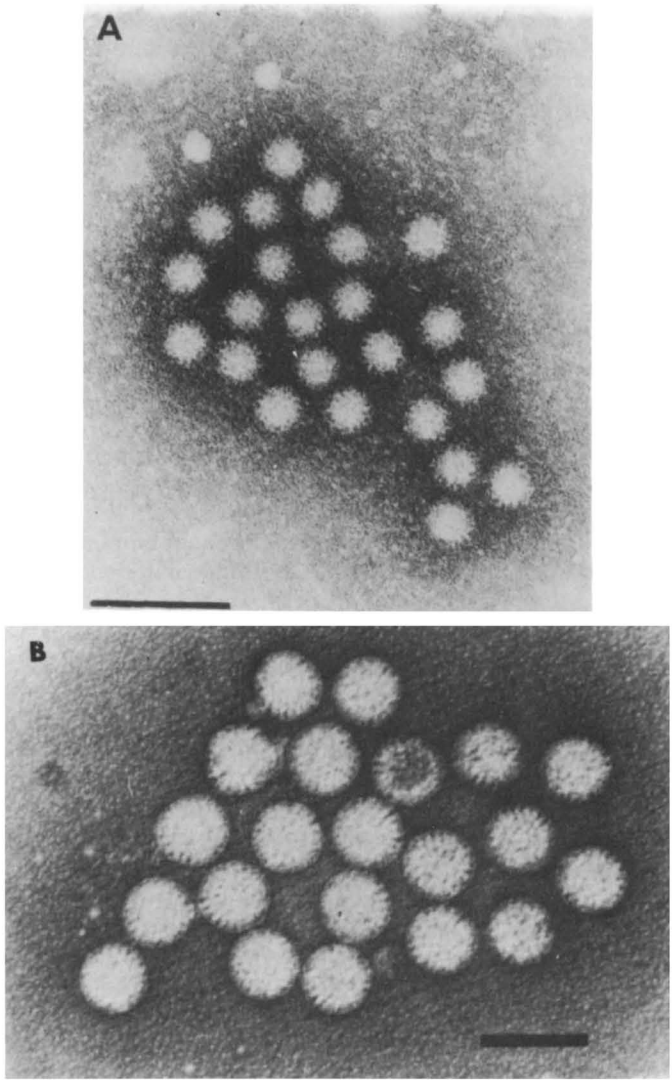

Figure 1. *A*, An aggregate of Norwalk virus particles visualized by immune electron microscopic examination of a human stool specimen. The bar = 100 nm. *B*, Human rotavirus observed by electron microscopic examination of a stool specimen from a case of infantile gastroenteritis. The bar = 100 nm. From Fenner, F.J., and White, D.O.: Medical Virology, Second Edition, New York, Academic Press, 1976.

Epidemiology

Studies of other discrete outbreaks of gastroenteritis have yielded viruses with properties similar to the Norwalk virus. Each agent is usually named by the location of the outbreak. Although indistinguishable from the Norwalk agent morphologically and physically, there are distinct antigenic strains. Outbreaks of gastroenteritis in places as diverse as Australia, England, and Japan were caused by Norwalk or antigenically related viruses.[157]

Other epidemics, such as those caused by the Hawaii agent or the Ditchling agent from England were due to viruses physically identical to but antigenically unrelated to the Norwalk agent.[55]

Approximately ⅓ of epidemics of gastroenteritis are produced by Norwalk virus or closely related viruses, as judged by serologic response to Norwalk virus. These epidemics have occurred in a variety of settings, including schools, nursing homes, recreational camps, and cruise ships.[157] Some outbreaks have been traced to contaminated foods, including an epidemic involving thousands of patients throughout Australia associated with the ingestion of oysters.[100] The worldwide prevalence of the Norwalk virus is further documented by population studies that show Norwalk antibody in 55 to 90 per cent of adults in diverse parts of the world. Only in one remote area of Equador has evidence of infection not been seen when sought.[54] In the United States, serum antibody is rarely present in infants. It begins to appear in late childhood and increases to the adult level of approximately 60 to 70 per cent.[71] The frequent occurrence of the Norwalk virus in outbreaks suggests that there may be a limited number of distinct agents that cause epidemic viral gastroenteritis.

Clinical Findings

Following the careful epidemiologic observations on the Norwalk outbreak, the virus was studied extensively in the laboratory under controlled conditions. A stool specimen obtained from an ill subject was filtered and found to be free of bacteria, toxins, or known viruses. Oral administration of the filtrate to volunteers resulted in illness in two thirds. Fecal filtrates of the Norwalk agent were serially passaged through several generations of volunteers without alteration in infectivity or in the clinical picture.[31]

The experimental incubation period is 18 to 48 hours, and illness usually lasts 24 to 48 hours. Diarrhea and vomiting are the hallmark symptoms. Either symptom could be minimal or severe; in some cases infected subjects have subclinical infection, although all receive identical inocula. Low grade fever, abdominal cramps, and myalgias frequently accompany vomiting or diarrhea. Infection has never been followed by prolonged illness or significant sequelae. As many as 10 per cent of volunteers develop infection documented by seroconversion or histologic lesions in the jejunal mucosa without significant symptoms.[13, 131] The Norwalk virus was visualized in the stool during the first 72 hours after onset of symptoms in 50 per cent of volunteers who developed illness.[137] Vomitus also occasionally contains the virus particles.[56] Treatment consists of supportive measures. Intravenous fluids are rarely needed, but they may be necessary in debilitated patients. In one study, bismuth subsalicylate decreased the mean duration of abdominal cramps without affecting diarrhea, vomiting, or virus excretion.[131]

Viral Properties

The agent is a nonenveloped, round virus with a diameter of 27 nm. The virus remains infectious after heating to 60° C for 30 minutes, after exposure to 20 per cent ether for 24 hours, and after exposure to pH 2.7 for 2 hours. The density in cesium chloride is 1.38 to 1.41 gm per ml. Based on these properties, the Norwalk virus was tentatively labled as parvovirus-

like.[120] However, a recent study has shown that the Norwalk virus appears to have a single primary structural protein with a molecular weight of 59,000.[58] Caliciviruses usually have a single protein with a molecular weight of 65,000. In contrast, parvoviruses generally contain three structural proteins. The size and physical characteristics of the Norwalk virus then are also compatible with inclusion in the calicivirus family. Definitive classification still awaits propagation, purification, and identification of the viral nucleic acid and proteins.

Pathophysiology

The Norwalk virus induces a characteristic, albeit nonspecific, histologic lesion in the proximal small intestine in all volunteers who develop illness.[3, 118] The Hawaii virus, a virus with physical properties placing it in the Norwalk group, but antigenically distinct, produces an intestinal lesion indistinguishable from that produced by the Norwalk virus.[33, 118] Jejunal villi are shortened and the crypts are hyperplastic and contain increased numbers of mitoses. The normally tall columnar epithelial cells are cuboidal and often are filled with vacuoles. The lamina propria contains polymorphonuclear leukocytes and increased numbers of mononuclear cells. Viral particles have not yet been detected in the involved intestinal epithelium. Histologic abnormalities often precede clinical illness and may be seen in asymptomatic subjects who seroconvert, presumably reflecting subclinical infection.[118, 119] Histologic abnormalities often persist for one week but return to normal by two weeks. The intestinal lesion results in malabsorption of fat, lactose, and xylose, indicating intestinal absorptive cell dysfunction.[120] Intestinal brush border enzymes are depressed in conjunction with the histologic lesion.[3] Adenylate cyclase levels remain unchanged, suggesting that cyclic adenosine monophosphate–mediated intestinal secretion is not part of this pathophysiologic process.[80] The malabsorption and histologic abnormalities may persist for one week, although symptoms are present for less than two days.

Despite the symptoms of nausea and vomiting, gastric fundic and antral mucosae remain histologically normal.[150] The secretion of acid, pepsin, and intrinsic factor are not altered during illness. However, gastric motor function appears impaired, resulting in markedly delayed gastric emptying of liquids in all infected subjects.[91] The rectal mucosa has been normal in the few subjects studied and fecal leukocytes are absent, suggesting that these viruses do not cause colitis.[120]

Diagnostic Tests

Until recently, Norwalk-like viruses could be detected only by immune electron microscopy (IEM) of fecal filtrates, which consists of mixing a stool filtrate containing virus with serum. The virus and serum antibody form aggregates that can be visualized using the electron microscope following appropriate centrifugation and staining. Semiquantitative assessment of serum antibody to Norwalk-like viruses is also possible by IEM by visually estimating the amount of antibody coating viral particles. Although this procedure has been invaluable in accumulating experimental information, technical limitations have made it useful as a research tool only.

Recently, a radioimmunoassay has been developed for the Norwalk agent. This radioimmunoassay has been used to identify viral antigen in stools and to quantitate antibody in serum and secretions.[12, 53] However, the class of antibody present in serum and intestinal secretions has not been defined. Radioimmunoassay allows rapid screening of large numbers of samples. However, the reagents used require fecal filtrates and sera from experimentally infected volunteers. Therefore, the procedure has been limited to the research laboratories actively investigating Norwalk virus infection. Radioimmunoassay has not yet been developed for the other Norwalk-like viruses.

Immunity

Illness developed in approximately two thirds of normal volunteers after ingestion of Norwalk virus.[31] The one third who remained well had low or absent levels of pre-existing antibody to Norwalk virus in serum and intestinal secretions.[12, 103] In this latter group ingestion of the Norwalk virus resulted in neither illness nor subsequent antibody response.[103]

The virus seems to pass through the gastrointestinal tract without ever gaining access to the intestinal mucosa or the immune system. If this group is re-exposed to Norwalk virus after extended delay, it will still remain uninfected.

Those volunteers in whom illness developed after ingesting Norwalk virus frequently had pre-existing antibody, suggesting prior experience with this agent.[103] Serologic response follows Norwalk illness. When rechallenged with the same virus within 14 weeks, these volunteers were immune to reinfection.[103, 120] However, this immunity is relatively short lived. If rechallenged after 27 to 42 months, all volunteers previously sick will develop illness again.[103]

Interferon does not appear to play a significant role in recovery or immunity to infection.[32] Cross-challenge studies show that infection with the Norwalk virus does not protect against the unrelated Hawaii agent, but does protect against the serologically related Montgomery County agent.[154] The differences between subjects who resist infection and those who are susceptible have not been explained. Nonimmune intestinal factors may be responsible for these differences. Further work on the basic mechanism of infection of the gastroenteritis viruses is necessary to explain these paradoxical findings.

ROTAVIRUS

Rotavirus infection is a major cause of morbidity in man and animals, causing more severe and frequent disease in the young. Bishop et al. first implicated rotavirus as a cause of infantile gastroenteritis in 1973 when virus particles with a diameter of 65 nm were found within intestinal epithelial cells in 6 of 9 duodenal biopsies from children with acute gastroenteritis.[8] Subsequently, viral particles with a diameter of 65 to 75 nm were recognized by electron microscopy in negatively stained stool of many children with acute gastroenteritis.[9, 42, 67, 92] The discovery of rotavirus infection in

children stimulated research on the epidemiology, clinical course, diagnosis, histology, pathophysiology, physical characteristics, and molecular biology of rotavirus infection in man and animals.

Rotaviruses have been found in almost every species examined.[1, 8, 16, 43, 79, 84, 89, 124, 142] Although cross-species infection is not common, it does occur.[61, 90, 93, 143, 156] The first transmission of rotaviral disease to another species was accomplished by Light and Hodes in 1943 when calves inoculated with diarrheal stools from children with acute gastroenteritis developed diarrhea. When examined recently by electron microscopy, the calf stool was shown to contain rotaviruses.[61] Human rotavirus has successfully caused symptomatic and asymptomatic infection in gnotobiotic calves,[61, 90] lambs, [143] piglets, [93, 143] and monkeys.[156]

Biophysical Characteristics

Rotaviruses are particles with a diameter of 65 to 75 nm when examined by negative staining under the transmission electron microscope.[42, 67, 92] They are members of the Reoviridae family.[26, 108] Rotaviruses were named for their wheel-like (rota = wheel in Latin) appearance. In early reports they were also called orbiviruses,[92] reovirus-like agent (HRVL),[42, 67] infantile gastroenteritis viruses,[62] or duoviruses.[26] Rotaviruses consist of a dense core of 33 to 40 nm surrounded by a rough-appearing inner capsid and a smooth outer capsid.[35, 41, 62, 102] The rough particle, which lacks the outer capsid, is 60 to 65 nm in diameter[9, 35, 41, 42, 62, 92, 102] and is probably not infectious.[15, 34] The smooth particle with a diameter of 70 to 75 nm[8, 9, 35, 41, 42, 62, 92, 102] represents the complete virus and is thought to be the infectious form of the virus.[15, 34] In natural infection rough particles, smooth particles, empty capsids, and tubular forms are found in the feces.[9, 42, 62, 67, 92] The rotaviruses of different species are morphologically identical and appear to share common complement fixation and immunofluorescent antigens on the inner capsid,[69, 153] whereas species-specific neutralization antigens are found on the outer capsid.[153] They are stable after prolonged storage at −70°C,[97] and treatment with acid, ether, chloroform, or genetron.[38, 102] Rotaviruses are unstable at extremes of pH, and the monkey rotavirus, SA_{11}, is inactivated by 95 per cent ethyl alcohol within 15 seconds and by betadine within one hour.[38, 133]

The rotavirus inner capsid contains four or five polypeptides with molecular weights of approximately 41,000 to 128,000. The outer capsid of the rotavirus has been thought to contain three to five polypeptides with glycosylation of the major outer proteins.[108, 109, 138] However, recent evidence suggests that there may only be two outer capsid proteins.[37] The final resolution of the structural polypeptides in the rotaviruses will have to await further experimentation.

The rotaviruses also contain an RNA polymerase[22] and 11 segments of double-stranded RNA.[65, 85, 123] Using the techniques of in vitro translation or transcription and translation, five proteins designated as structural proteins and two to six nonstructural proteins have been found.[85, 123] In the future, experiments using in vitro transcription and translation methods should allow isolation of the polypeptides and understanding of their function.

Tissue culture adaptation of calf (NCDV), [40] porcine (OSU, EE), [136] monkey (SA_{11}),[84] and sheep (O)[84] agents has been possible for many years, allow-

ing high titer production of these viruses. Trypsin enhances productivity and infectivity of the cultivatable rotaviruses in tissue culture.[5] Until recently, human rotavirus could be passaged only in human fetal intestinal organ culture in low titer.[155] In 1980, Wyatt et al. were able to adapt a strain of human rotavirus to high titer propagation in tissue culture. The strain of human rotavirus first had to be serially passaged 11 times in gnotobiotic pigs, probably resulting in a mutation in the virus allowing it to replicate in tissue culture.[158] A new approach to obtain a cultivatable human rotavirus by gene reassortment during mixed infection with bovine rotavirus recently has been described by Greenberg et al. This technique involves co-culturing an animal rotavirus that grows well in tissue culture at certain temperatures with a human rotavirus that does not grow in culture. By selecting the appropriate conditions, only mutants of the animal rotavirus or recombinants of the animal and human rotavirus will grow.[57] These recombinants may be helpful in developing viral vaccines as well as assigning the function to the various RNA gene products.

Epidemiology

Rotavirus infection is found throughout the world with a peak incidence in six-month-old to 2-year-old children,[26, 36, 75, 112, 135] although infection has been reported in all age groups. It has been associated with sporadic cases, epidemic outbreaks,[45, 74, 117] nosocomial infections,[26, 44, 112] and traveler's diarrhea.[122, 144] In some studies of hospitalized children there is a 6:4 male to female ratio,[18, 39] although others report an equal incidence in males and females.[112]

In temperate climates throughout the world there is a marked predominance of infection in the fall and winter months with a low incidence of infection in the summer.[26, 60, 70, 75, 92, 98, 135] The incidence of rotavirus infection in the winter months in children hospitalized with gastroenteritis is approximately 50 per cent but can reach a peak of 80 per cent. In the summer, rotavirus infection may not be seen. In tropical countries where temperature variation is only a few degrees, the incidence of infection by rotavirus has been reported to increase with either extreme of humidity. In Darwin, Australia, 50 per cent of children with gastroenteritis admitted during the rainy season had serologic evidence for rotavirus infection, whereas only 13 per cent of those admitted with gastroenteritis during the dry season had rotavirus infection.[146] In contrast to these findings, the peak incidence of infection of 30 to 40 per cent in San Jose, Costa Rica was at the beginning of the dry season with rotavirus infection present throughout the year.[60] In other studies there was no relation between rainfall and rotavirus infectivity, with infection present throughout the year.[46, 139] It is therefore evident that seasonal variation may have a profound effect, but variation of the humidity is not a major determinant of rotavirus infection.

Acute rotavirus infection in children is usually a self-limited disease, although a few deaths have been reported.[17, 74, 92] The average incubation period is two to three days.[26, 92] Vomiting, diarrhea, and fever are the most frequent initial symptoms, with vomiting occurring in 58 to 100 per cent and diarrhea in 36 to 100 per cent of reported symptomatic cases. The vomiting

usually lasts one to five days, with a mean duration of four to seven days.[18, 29, 50, 60, 74, 112, 121, 132] Symptoms of upper respiratory infection are frequently (26 to 75 per cent) associated with rotavirus infection and occur more frequently in patients with gastroenteritis due to rotavirus rather than gastroenteritis due to other causes.[49, 60, 81, 112, 135] The most common respiratory tract symptoms are cough, nasal discharge, otitis media, and erythematous throat. Fever has been reported in 63 to 100 per cent of patients. Most commonly the fever is low grade, with a fever of greater than 39° C being uncommon, although temperatures of greater than 40° C have been reported.[18, 60, 112] On occasion, pyrexia rather than nausea or vomiting is the initial symptom.[18] Dehydration occurs in 40 to 83 per cent of cases. The level of dehydration is usually less than 5 per cent, although it has been reported to be greater than 10 per cent in up to two fifths of cases.[29, 60] The serum electrolytes are usually normal, although serum hypertonicity and hypotonicity have been reported. The mean duration of illness is 7 days, although symptoms can persist for up to 26 days.[60, 92, 112, 121] Virus is usually excreted for less than 8 days, [26, 74] although prolonged excretion has been reported.[44] Intussusception, Reye's syndrome, and encephalitis have all occurred in association with rotavirus disease.[76, 115] An analysis of 21 deaths associated with rotavirus showed that the children who died were 4 to 30 months of age with a mean age of 11.4 months. Many of the children were 10 to 20 per cent dehydrated. Fatty liver, bowel dilation, and splenic congestion were prominent at death. Sixteen of the 21 had made contact with a physician during the acute illness.[17]

Laboratory evaluation of patients with rotavirus infection is nonspecific. The stools are usually watery and without blood, containing fecal leukocytes in 16 to 31 per cent of cases and mucus in up to 50 per cent of infections.[60, 112] Rotavirus infection is often associated with white stools in Japan.[74] The white cell count is usually between 7000 to 12,000 per cu mm.[60, 112] Elevation of serum glutamic axalic transaminase has been reported.[132] The disease is usually self-limited, but symptomatic relapses can occur.[29, 132]

Rotavirus disease in adults is less likely to occur than in children, and when it does it is less likely to be symptomatic. In a prospective study of 98 families, Wenman found an attack rate in children of 32 per cent per year and in adults of 17 per cent per year. Of those who were infected, 70 per cent of children and 40 per cent of adults developed symptoms.[148] Adults have developed symptomatic infection as a result of secondary spread from infected children, as a result of primary infection, and as a type of traveler's diarrhea. Adults can experience loose stools, malaise, abdominal pain, fever, and vomiting. In two studies of traveler's diarrhea, rotavirus was responsible for one third of the cases in both Peace Corps volunteers traveling to Honduras and in Panamanians traveling to Mexico.[122, 144]

Rotavirus infection in the newborn infant is less common than in the six month to two year old group and is often manifested by asymptomatic excretion of virus. Excretion of rotavirus by newborn infants has been found in up to one-half of babies surveyed. Of newborn infants who excrete rotavirus, only 8 to 24 per cent have diarrhea or vomiting.[10, 20, 99] In one study the most important factor responsible for the occurrence of diarrhea in rotavirus in-

fection was proximity to other newborn infants and frequency of handling by adults.[10] Although breast-feeding is not always protective, many investigators have reported significantly less frequent rotavirus infection in babies who were breast-fed (22 per cent) compared with babies who were bottle-fed (58 per cent).[10, 87] Bottle-fed babies who were rotavirus positive excreted greater amounts of rotavirus than breast-fed babies.[20] Others have found an inverse correlation between the level of secretory IgA in colostrum of mothers and rotavirus infection in breast-fed infants.[88] Analysis of rotavirus RNA patterns has documented two principal varieties of rotavirus isolated from infants that are distinct from those isolated from young children, implying that there may be a difference in the virus as well as in host factors involved in neonatal infection compared with that in young children.[111]

There are at least two functional serotypes of human rotavirus as detected by enzyme-linked immunosorbent assay (ELISA). These two serotypes have shown a shift in their prevalence over the years. In Washington, D.C., the prevalence of Type 2 rotavirus in 1973 to 1977 was 100 per cent, whereas in 1977 to 1978 it was only 57 per cent. In addition, Type 2 may cause more severe disease than Type 1. Where recurrent rotavirus infection has been evaluated, it appears that reinfection occurs with a different serotype of rotavirus.[163] By using serum neutralization techniques, four and possibly five serotypes have been defined. However, because of extensive cross-reactivity it has been difficult to determine the exact number of serotypes.[7]

Immunity

Following infection with human rotavirus, IgM serum antibody appears after about five days, persists for at least three weeks, and eventually disappears. IgG serum response is present by two to four weeks postinfection. An IgA response has also been shown.[161] Stool antirotavirus antibody titers of IgG, IgA, and IgM all reach peak levels between two and four weeks after infection, then drop to undetectable levels after two months.[129]

By routine screening of sera for antibodies to rotavirus, it has been determined that with the first three months, 60 to 75 per cent of infants have serum antibody to rotavirus that is probably maternally acquired. The incidence falls at three to six months to approximately 10 to 25 per cent and then rises again. By one year of age 50 to 70 per cent of children have antibody to rotavirus, and by two years 80 to 95 per cent of children have antibody to rotavirus.[11, 163] The incidence of acquisition of antibody to Type 1 or Type 2 rotavirus is the same. In the postinfection period a serotype-specific antibody response occurs.[163] It is not clear whether type-specific IgG serum antibody protects against recurrent infection with the same serotype of rotavirus, but it is unlikely that the serum IgG antibody does protect, as rotavirus is common even though 50 to 70 per cent of one-year-old children have Type 1 and Type 2 antibodies.

The importance of local intestinal IgA antibody levels has recently been investigated. A small number of adult volunteers were orally inoculated with Type 2 rotavirus. Resistance of clinical disease appeared to correlate better with the presence of jejunal fluid Type 2–specific IgA antibody levels

than with serum IgG. The presence of Type 1–specific IgA antibody did not correlate with resistance to Type 2 rotavirus infection.[72]

Local immunity is also believed to play a role in protection of suckling neonates from rotavirus infection. High titers of IgA antibody to both Type 1 and Type 2 rotaviruses are present in 88 per cent of colostral and milk samples, although the levels fall shortly after birth.[25, 140, 162] In one study in Australia, 19 of 42 neonates who were breast-fed developed rotavirus infection. However, no neonate who received breast milk with an antirotavirus secretory IgA of 280 μg per ml or more became infected, suggesting a role for specific IgA in protection of the neonate from rotavirus infection.[88] However, in another study the presence of IgA antibody in breast milk did not correlate with protection from rotavirus infection.[140]

Further information suggesting a role for partial protection of neonates by colostral and milk antibodies comes from animal studies. One calf fed colostrum with a neutralizing antibody titer of 1:320 to bovine rotavirus 4 and 24 hours after birth was protected from disease when inoculated with rotavirus at 12 hours after birth.[14] Gnotobiotic lambs fed 450 ml (but not those fed only 100 ml) of colostrum on the first or second day of life and challenged two days later with rotavirus were protected from disease.[126] Also protected from disease were lambs fed 40 ml per kg of colostrum for four consecutive days after birth and challenged with rotavirus on the second day.[125] In addition, two infant lambs fed high titer antirotavirus hyperimmune serum on days two to four did not excrete rotavirus or develop diarrhea when challenged on day two with rotavirus.[126] Thus, oral ingestion of substantial amounts of antibody directed against rotavirus seems to be protective in lambs and cows.

Pathophysiology

Rotavirus directly infects the intestinal epithelial cells on the villi rather than in the crypts.[8] The light microscopic findings are nonspecific. Of 17 patients studied by Davidson and Barnes with rotavirus gastroenteritis, two had severe abnormalities, including loss of villi, crypt hypertrophy, cubiodal epithelium and marked inflammatory infiltrate in the lamina propria. Eight more showed moderate damage with blunting of villi, increased crypt depth, flattening of epithelial cells, and an increase in inflammatory cells in the lamina propria. The remaining seven had mild, nonspecific abnormalities. By three to eight weeks postinfection, all of the patients who were rebiopsied had normal intestinal morphology.[28]

Electron microscopy of the small bowel is pathognomonic for rotavirus infection. Virus particles are found within distended cisternae of endoplasmic reticulum. The particles have a central dense core (33 nm in diameter) surrounded by a moderately dense capsid zone about 67 to 70 nm in diameter. About 10 per cent of particles within vesicles are enveloped (87 to 90 nm in diameter). Some enveloped particles appear to acquire an envelope from the endoplasmic reticulum. Areas of "viroplasm" or "virus factories" may be found in the cytoplasm, not membrane bound, containing moderately electron-dense, granular, or finely fibrillar material. Microvilli of infected cells are often irregular and distorted. Degenerating cells appearing

to discharge virus into the lumen have been observed.[8] In addition to the above findings, rotavirus infection in some animals is associated with nuclear and cytoplasmic tubular inclusions.[6]

The gastric and colonic mucosae have been reported to be normal in the few rotavirus-infected infants in which they have been examined.[92]

Depression of disaccharidase (maltase, sucrase, or lactase) levels was found in 14 out of 16 children. Although most of the disaccharidase activities return to normal by three to eight weeks, one or more may remain abnormal longer, thereby making the patient relatively intolerant to disaccharide.[28] In piglets infected with human rotavirus, net Na^+ and Cl^- fluxes are normal under basal conditions, but glucose-coupled Na^+ transport was blunted in the jejunum. Cyclic adenosine monophosphate concentration in isolated jejunal villus enterocytes did not differ significantly in control and infected animals. Davidson concludes that the diarrhea in the rotavirus-infected animals was caused by repopulation of the damaged mucosa with immature cells that cannot absorb as well as mature cells.[27]

Lactase has been postulated to serve as the intestinal receptor and uncoater of rotavirus because decreases in lactase activity correlate with decreased susceptibility of animals to rotavirus infection.[63] Furthermore, lactase partially uncoats bovine rotavirus *in vitro*. Recent data in mice, however, suggest that lactase is not the receptor.[152]

Diagnostic Methods

Many methods are available for making a rapid and accurate diagnosis of rotavirus infection. One of the methods most commonly used for detecting rotaviruses is examination of negatively stained stool by transmission electron microscopy.[9, 42, 67, 92] This method is limited by the necessity of having a large number of particles ($> 10^6$ particles per gm feces), and the inability to determine the serotype of the rotavirus. Immune electron microscopy can be used to determine serotype and presence of antibody in sera but otherwise has many of the same disadvantages.[67] Both techniques are too inconvenient and time consuming to screen large numbers of specimens and require ready access to an electron microscope. One of the best methods for rapid screening of a large number of stool samples for virus and sera for antibody is the enzyme-linked immunosorbent assay (ELISA).[159, 161, 193] The ELISA has been adapted to measure serotypes of rotavirus and type-specific serum antibody responses, and to determine the class of antibody response. Both radiommunoassay (RIA) and the ELISA are fast and efficient and at least as sensitive as electron microscopy in detecting rotavirus infection and serum antibody.[24, 66]

Other techniques that have been used to detect rotavirus antigen and/or antibody include immunofluorescence,[11, 150] counterimmunoelectrophoresis,[94, 141] immune adherence hemagglutination,[86] and complement fixation.[11, 68]

Treatment and Prophylaxis

Children who have been admitted to hospitals for rotavirus enteritis may be treated with intravenous solutions depending on the degree of dehydration. Intravenous therapy is usually not necessary. There have been

two double-blind studies, one in Bangladesh[114] and the other in Costa Rica,[101] comparing oral sucrose with glucose electrolyte fluids for treatment of children with diarrhea of whom 64 per cent and 30 percent, respectively, had rotavirus. All children were successfully treated with the oral glucose-electrolyte solutions, and almost all children were successfully treated with the oral sucrose-electrolyte solutions. Therefore, it appears that in most cases oral therapy of rotavirus diarrhea is successful and that glucose-containing fluids may be slightly better than sucrose-containing fluids. The recommendation for oral fluid management would be solutions containing 20 gm per L glucose or 40 gm per L sucrose with Na^+ (90 mmol per L), K^+ (20 mmol per L), Cl^- (80 mmol per L), and HCO_3^- (30 mmol per L).

Although the mortality is low, the morbidity caused by rotavirus infection throughout the world is high and immunization to prevent disease is important. Currently, development of a rotavirus vaccine is a major thrust of much research.[72] Prospective vaccines can be divided into two groups. The first is an oral, live, attenuated vaccine that could provide protection by stimulation of local intestinal immunity. The second approach is to vaccinate adults in order (1) to reduce the reinfection rate among adults and thereby decrease a potential reservoir of infection, and (2) to stimulate increased milk and colostral antibody production. The ability to produce recombinant human-bovine rotaviruses that grow well in tissue culture[57] and tissue culture growth of a Type 2 rotavirus strain passaged in pigs[158] should make development of a vaccine more feasible.

Oral attenuated vaccines have been tested in newborn calves with variable results. When evaluated in two dairy herds in the 1977 calf season in the Netherlands, no significant differences were observed in the incidence rates or severity of undifferentiated neonatal calf diarrhea or rotavirus-associated late diarrhea between calves given a placebo and vaccinated calves in the herds. Possible neutralization of the vaccination virus by the ingested antibody-containing colostrum was considered to be the reason for vaccine failure.[30] As discussed earlier, in calves and lambs colostrum can prevent or delay onset of rotavirus disease. Vaccination of adult ewes and cows with inactivated rotavirus results in a marked increase in colostral antibody.[128, 147] Calves of vaccinated cows, when challenged with rotavirus on day seven had lengthening of the incubation period but diarrhea of equal severity when compared with calves of unvaccinated cows. The experience in animal vaccines will be helpful in developing a human vaccine. Based on experience with animals, the oral, attenuated vaccine is the most promising at this time.

OTHER ENTERIC VIRUSES

In addition to the Norwalk-like viruses and rotaviruses, there are several other candidates as etiologic agents in gastroenteritis. However, they appear to be much less important epidemiologically than the rotaviruses and Norwalk-like viruses. Of the agents identified, astroviruses, caliciviruses (unrelated antigenically to the Norwalk-like viruses), and adenoviruses are likely etiologic organisms. The Otofuke agent and minireovirus (minirota-

virus) are less well established but are probable causes of gastroenteritis. Coronaviruses have not been proved to cause diarrhea in humans.

Astroviruses are particles with a diameter of 27 to 29 nm and a smooth, circular outer edge. The surface structure is a five- or six-pointed star with no central hollow.[83] Astrovirus has been associated with two outbreaks in children's wards in England. The patients presented typically with a watery diarrhea, with 30 to 60 per cent having an episode of vomiting. Vomiting could occur without diarrhea. The disease occurred in adults and children. The diagnosis was made by identification of astrovirus in the stools and/or demonstration of serum antibody rise or antibody presence by immunoelectron microscopy.[4, 77] Of eight adult volunteers given astrovirus, one developed vomiting 74 hours postinfection, followed by nausea, anorexia, headache, malaise, abdominal discomfort, slight pyrexia, and diarrhea with profuse shedding of astrovirus in the feces. Only one other adult had mild symptoms. Nine other volunteers given the ill patient's stool remained healthy, although two shed astrovirus. Thirteen of the 17 patients seroconverted.[78] Electron microscopic examination of lambs inoculated with astrovirus reveals infection of mature columnar epithelial cells covering the apical two thirds of villi from 14 to 30 hours postinoculation. Crystalline arrays are found within the cytoplasm and virus particles are found within apical pits and tubules. Virus particles are released by desquamated cells disintegrating in the lumen.[52]

Caliciviruses, RNA-containing viruses with a diameter of 31 nm, have also been associated with a few outbreaks in Japan, London, and Montreal.[19, 23, 130] The virus has a feathery, scalloped edge and looks like a six-pointed star with a central hollow.[82]

Disease may be manifested by vomiting diarrhea, abdominal colic, or fever. Illness has been reported in school children and adults.

Enteric adenoviruses are found commonly in stools of patients with and without gastroenteritis. They have been found in up to 13 per cent of stools of children with gastroenteritis and six per cent of control patients. In some of these cases a serologic response has been noted.[75, 95, 139] Adenovirus has been found in the duodenal aspirate of a few children and has been associated with a decreased absorption of D-xylose.[87] Adenovirus was probably the etiologic factor in an outbreak of gastroenteritis in children at a Royal Air Force station in the United Kingdom. The incubation period was eight to ten days, and the duration of disease was four to nine days. Diarrhea was the predominant symptom, with a frequent association of nausea and anorexia.[107] Adenovirus was thought to be responsible in the deaths of two children 16 months old who had gastroenteritis.[149] In 45 per cent or more cases, adenovirus seen by electron microscopy in stool samples could not be grown in cell culture. The enteric adenoviruses are probably one or more serotypes distinct from established adenovirus serotypes.[48, 64, 106]

Other viruses have been associated with gastroenteritis in humans. Minirotavirus (minireovirus), a particle with a diameter of 30 to 32 nm has been found in association with outbreaks of gastroenteritis in Glasgow, Montreal, and Toronto. Diarrhea and vomiting were common symptoms.[82, 95, 130] A particle with a diameter of 35 to 40 nm in stool specimens was associ-

ated with an outbreak of gastroenteritis in Otofuke, Japan in patients 22 to 51 years of age. The disease was characterized by vomiting (73.5 per cent) and less frequently with nausea (32.4 per cent), diarrhea (38.2 per cent), abdominal pain (11.8 per cent), and fever (5.9 per cent).[134] Coronaviruses are well established as causes of gastroenteritis in pigs (transmissible gastroenteritis virus) and cows.[96, 104] Coronavirus has not been established as a pathologic agent in humans, being found only occasionally in stools of patients with gastroenteritis, and when it is found it is as frequent in asymptomatic as in symptomatic people.[21] Parvoviruses are also well-established causative agents in severe enteritis in dogs, cats, and mink.[105, 151]

CONCLUSION

The last decade of research has resulted in an explosion of knowledge about viral gastroenteritis. Great advances have been made in delineating the causative agents, epidemiology, pathophysiology, and immunity of viral gastroenteritis. Despite the major advances reviewed here, there has been no significant change in practical diagnosis, therapy, or prevention of gastroenteritis.

The diagnosis of rotavirus gastroenteritis is now possible using technology readily available in clinical laboratories and undoubtedly will be the first major advance gaining widespread use. However, at this time the diagnosis of viral gastroenteritis continues to rest on the typical clinical picture and exclusion of common bacterial pathogens. The diagnosis of all other gastroenteritis agents remains the province of the highly sophisticated research laboratory that uses electron microscopy or experimental radioimmunoassay requiring carefully defined and difficult to acquire fecal and serum samples.

Therapy is still limited to nonspecific supportive measures. No intervention has significantly altered the course of illness or prevented infection. The selection of oral or intravenous replacement fluids remains a function of severity of illness and risk of complications. The high-risk patients include the elderly, debilitated, or very young, and these may require hospitalization or parenteral fluids.

Development of a vaccine for rotavirus appears feasible.[72] The limited antigenic varieties, laboratory growth *in vitro*, related animal viruses, and the potential for production of hybrid strains make this a fertile area of exploration. The need to protect for a relatively limited period of time (approximately six months to two years of age) enhances the likelihood of success. However, the mixed results of vaccination of calves to prevent rotavirus gastroenteritis warns of potential difficulties ahead.

Many basic research advances are required before vaccines for Norwalk-like viruses can be developed. The viruses must be purified, cultivated, and the antigenic varieties delineated. Even if these formidable problems are overcome, vaccination may not be practical owing to the variety of antigenic strains and the relatively short immunity in naturally acquired infection.

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