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PROGRESS IN GASTROENTEROLOGY

RECENT ADVANCES IN VIRAL GASTROENTERITIS

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Viral gastroenteritis is a common disease of short duration characterized by nausea, vomiting, abdominal cramps, diarrhea, and constitutional symptoms in varying combinations. It occurs in both epidemic and endemic forms and, although rarely fatal in developed countries, it is the second leading cause of illness in the United States.[1] Therefore, the economic consequences of lost work hours owing to gastroenteritis are considerable. In the chronically malnourished, the elderly, and in debilitated populations, gastroenteritis may also be a significant cause of death.[2]

Since the development of tissue culture methods for growth and detection of viruses, extensive efforts have been made to detect viral pathogens in stools of patients with gastroenteritis without evident bacterial pathogens. Until recently, potential viral pathogens have been documented in only a small minority of cases.[3-8] Within the last 5 years, two groups of viral agents, parvo-like viruses and reo-like viruses, have been implicated in the pathogenesis of acute infectious nonbacterial gastroenteritis. This has stimulated research related to the epidemiology, etiology, diagnosis, histopathology, and pathophysiology of this common illness. The purpose of this paper is to review recent progress in our understanding of viral gastroenteritis.

Historical Background

Early studies of viral gastroenteritis dealt primarily with epidemiology. In these, the natural occurrence of gastroenteritis was studied and stools from ill patients and controls were screened for possible causative agents. During the 1950's several surveys of infantile gastroenteritis in the United States resulted in isolation of known enteric viruses, such as echovirus, coxsackievirus and adenovirus in 20 to 50% of ill infants.[3-5, 8] However, up to 20% of controls shed these viruses and the multiplicity of viruses isolated made interpretation of the data difficult. In studies from underdeveloped nations, enteric viruses were identified in up to 80% of infants with diarrhea. However, 40 to 80% of controls excreted similar viruses in these populations.[7, 9, 10] In several surveys there was excretion of adenovirus[7, 11, 12] echovirus,[3, 8, 11, 13] or coxsackievirus[7, 8, 13, 14] in more stools of ill patients than controls, but these studies still left unexplained the majority of cases of gastroenteritis since specific viruses were isolated from only a minority of ill patients.

Another approach used in early studies was the attempted induction of disease in volunteers by administration of infectious, bacteria-free fecal filtrates under controlled conditions. In 1947, Gordon et al.[15] first demonstrated that epidemic gastroenteritis could be transmitted to volunteers by the oral ingestion of fecal filtrates derived from ill patients, but not by inhalation of nebulized throat washings from ill subjects. The infectious agent was designated the Marcy strain. Rechallenge studies suggested that immunity of reinfection with the Marcy strain persisted for at least 15 months,[16] but cross-immunity to a second agent, the FS strain, from a different epidemic was not found.[17] When frozen, the fecal filtrates remained infectious for at least 3 years.[16] Similar studies at that time in Japan yielded an inoculum (Niigata strain) which caused gastroenteritis and could be passaged serially through multiple generations of volunteers.[18] Six infectious agents, the Marcy and Niigata strains and four other inocula from other parts of Japan, were studied by cross-challenge tests. Volunteers were infected with the Marcy strain or one of the four other inocula and then rechallenged within 49 days with the Niigata strain. No illness resulted from the second challenge, suggesting a close immunological relationship or identity among these six agents.[18, 19] However, failure to identify or culture a specific etiological agent led to the abandonment of further research with these well studied inocula.

Recent developments in virological techniques have led to renewed efforts to study viral gastroenteritis. The cultivation of respiratory viruses in tracheal organ culture[20] led to successful attempts to establish human fetal intestinal organ cultures.[21, 22] The cultivation of some known enteric viruses has met with limited success.[23] The use of electron microscopy and immune electron microscopy to identify viral particles and their interaction with antisera[24, 25] have resulted in the identification and partial characterization of two types of viral gastroenteritis agents; parvovirus-like agents which appear primarily responsible for disease in adults

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and older children, and reovirus-like agents which appear to be a major cause of viral diarrhea in infants and young children.

Human Parvovirus-like Agents of Epidemic Gastroenteritis

Epidemiology. Three distinct, well circumscribed epidemics have been shown to be attributable to infection with parvovirus-like agents. The agents that caused them, the Norwalk, Hawaii, and Montgomery County (MC) agents, named after the sites of the epidemics, appear distinctive in that immunity to one of the agents does not appear to confer immunity to the other agents.[26] The Norwalk agent, the most extensively studied agent of this class of viruses, was derived from an outbreak of gastroenteritis originating in a public school in Norwalk, Ohio in 1968. Rapid spread throughout the community was observed with an attack rate of greater than 50% and an incubation period of approximately 48 hr.[27]

Clinical features. The clinical, pathophysiological, and histopathological features of parvovirus-like agent-induced gastroenteritis have been studied by inducing infection in healthy volunteers.

Oral administration of bacteria-free fecal filtrates derived from this outbreak induced illness in approximately two-thirds of volunteers not exposed during the epidemic.[28] Fecal filtrates of the Norwalk agent were subsequently serially passaged through several generations of volunteers via the oral route with no significant change in the rate of infection or resultant clinical symptoms.

The experimental incubation period is 18 to 48 hr and symptoms last for 24 to 48 hr.[28] Virus-like particles were detectable in stool filtrates during the first 72 hr after the onset of symptoms in approximately 50% of volunteers who developed illness.[29] Symptoms varied considerably in volunteers receiving identical inocula. Mild to marked vomiting without diarrhea was observed in some volunteers and mild to severe diarrhea without vomiting was seen in others; still others developed both vomiting and diarrhea, although all received identical inocula.[28] Low grade fever, abdominal cramps, malaise, anorexia, headache, and myalgias frequently accompanied the diarrhea and vomiting. Laboratory studies remained normal except for an occasional mild transient leukocytosis.[28] No prolonged illness or long term sequelae have been observed in volunteers ingesting inocula containing parvovirus-like agents.[30]

Physical characteristics. The Norwalk agent is relatively stable after exposure to ether, acid, or heat. Infectivity persists after heating to 60°C for 30 min, after exposure to 20% ether for 24 hr at 4°C, and after exposure to *p*H 2.7 for 3 hr at room temperature.[31] In 1972, Kapikian and co-workers[32] identified particles 27 nm in diameter in the infectious filtrate utilizing the technique of negative staining with immune electron microscopy, and then determined that the density of the particles in cesium chloride was 1.38 to 1.41 g per ml.[33] Subsequently infectious filtrates containing Hawaii and MC agents were found to contain morphologically indistinguishable particles 26 to 29 nm in diameter with a density in $CsCl_2$ of 1.37 to 1.39 g per ml.[34] These physical characteristics of gastroenteritis-causing agents are comparable to those of known DNA-containing parvoviruses,[33] although definitive classification awaits laboratory propagation of the agents.

Laboratory propagation. Attempts at in vitro propagation of the Norwalk agent have been unsuccessful. No experimental animals have been reported to be susceptible to Norwalk agent; rhesus monkeys, guinea pigs, rabbits, and mice have remained well after innoculation with the Norwalk agent.[30]

Human Reovirus-like Agent of Infantile Gastroenteritis (HRVL)

Epidemiology. A reovirus-like agent, which has been termed duovirus, rotavirus, orbivirus, and infantile gastroenteritis virus, by different investigators, appears to be responsible for both sporadic and epidemic outbreaks of gastroenteritis in infants and young children throughout the world.[35–42] During two separate year-long surveys, approximately 50% of all children hospitalized with gastroenteritis had evidence of infection with the reovirus-like agent.[43, 44] The peak prevalence of HRVL disease may approach 90% among infants and young children with acute gastroenteritis during the winter months and fall to 20% during the summer months.[35, 40, 43–45] Clinical infection is most common in the 6- to 24-month age group but also occurs in the neonatal period.[43–50] An unexplained male to female ratio of 3:2 has been observed in hospitalized patients.[44, 50] Transmission is presumed to be from person to person by the fecal-oral route.[51] Nosocomial spread has been observed.[52–54] There is evidence that subclinical infection with HRVL occurs quite often in adult relatives of infected infants.[41, 44] In one study 35% of the parents who were evaluated had evidence of HRVL infection by either serological tests or demonstration of HRVL in stool specimens.[44] HRVL infection in adults occasionally may be associated with diarrhea.[41, 44]

Clinical features. The clinical, pathophysiological, and histopathological features of reovirus-like agent-induced gastroenteritis have been determined in patients with naturally occurring infection. Diarrhea is the symptom by which infants with naturally occurring HRVL gastroenteritis are recognized, and by definition, has been present in 100% of patients. The duration of diarrhea varies but it may persist for as long as 5 to 8 days.[41, 50, 54, 55] Vomiting may precede or occur concomitantly with diarrhea. Fever and lymphocytosis occur in 50 to 60%.[44, 50, 54] Pharyngeal and tympanic membrane erythema accompanied gastrointestinal symptoms in 40 to 50% in one study.[44] The indication for hospitalization generally is dehydration requiring intravenous fluids. Death attributable to HRVL gastroenteritis, although rare, has been reported.[35]

Physical characteristics. This virus was first identified in 1973 by Bishop et al.[56] in electron micrographs of biopsies of duodenal mucosa obtained during acute gastroenteritis in young children, and was subsequently recognized in negatively stained electron microscopic

preparations of stool suspensions from sick children.[46, 57] The HRVL particle is approximately 70 nm in diameter with a double-shelled capsid. Some particles are seen with the outer shell absent; these measure approximately 60 nm in diameter. Empty shells are also frequently seen. The virus appears to contain a 37-nm hexagonal core when viewed with the electron microscope using negative stains. The core seems to be surrounded by an electron-lucent layer from which capsomeres radiate. The surface is composed of 32 capsomeres.[58] Which of the structural components are necessary and sufficient for infectivity and antigenic recognition has not been determined as yet. The density in $CsCl_2$ has been shown to be 1.29 to 1.30 g per ml for empty shells and 1.36 to 1.37 g per ml for complete particles; double shell particles tend to aggregate at 1.37 g per ml and single shell particles aggregate at 1.36 g per ml.[59–61] The physical structure of this virus is extremely stable and it can be recognized easily by electron microscopy after storage at −20°C for up to 9 years.[49] Whether or not infectivity persists after prolonged storage of the virus is not known.

Laboratory propagation. Human fetal intestinal organ cultures, which support the replication of certain known enteric viruses,[21–23] will incorporate HRVL antigen into mucosal cells.[62] However, serial passage of HRVL in this culture system has not been uniformly successful.[35] It appears that only certain inocula of HRVL will infect the cultured fetal intestinal explants. Therefore, this system does not appear suitable for use as a diagnostic technique for detection of HRVL in stools. Replication of this virus in tissue culture systems has yet to be confirmed, although preliminary reports have suggested possible incorporation of HRVL in human embryonic gut monolayers,[63] pig kidney monolayers,[64] and human embryonic kidney tissue cultures.[65] The inability to obtain large quantities of this virus has limited its availability for diagnostic serological tests or characterization of physical and chemical properties beyond those mentioned above.

Infection of experimental animals with HRVL has recently been reported. The HRVL infects both gnotobiotic piglets,[53, 66, 67] and newborn, colostrum-deprived rhesus monkeys[68] under controlled laboratory conditions. These animals develop diarrhea and reovirus-like particles are found in the stool after oral inoculation with the HRVL.[66–68]

Other Viruses

In two circumscribed outbreaks occurring in hosptials, evidence implicating echovirus infection was presented. The first outbreak involved 3 laboratory workers; echovirus type 11 was recovered from serum and rectal swabs from the 2 laboratory workers examined. Seroconversion occurred in 2 of 3 of those ill.[69] In a larger outbreak, in a hospital nursery, echovirus type 18 was isolated from stools of 15 of 17 infants with gastroenteritis and all 17 infants developed serelogical responses to echovirus type 18. Nineteen hospitalized infants without gastroenteritis did not excrete the virus or develop antibodies to this virus.[70] However, echovirus has not been implicated as a common cause of gastroenteritis despite the ease of identification of this virus in the clinical laboratory.

The presence of coronavirus-like particles has been reported in the stools of several patients with gastroenteritis.[71, 72] Although coronavirus is a documented cause of gastroenteritis in animals such as pigs,[73] no definite pathogenetic relationship for this virus has yet been established in man.

Histopathology

Parvovirus-like agents. A characteristic though nonspecific histological lesion develops in the proximal small intestine during the course of gastroenteritis induced by the Norwalk and Hawaii agents. Mucosal architecture is altered; villi are shortened, and intestinal crypts are hyperplastic.

Infiltration of the lamina propria with polymorphonuclear leukocytes and increased numbers of mononuclear cells occurs early. The normally columnar surface cells commonly appear cuboidal and contain vacuoles.[74–77] Mitotic figures in the hyperplastic crypts are increased in number.[74, 75] Electron microscopic studies of involved villous epithelium show dilatation of the endoplasmic reticulum, widening of intercellular spaces, and increased numbers of lysosomes and multivesicular bodies.[76, 78] Dilated mitochondria and markedly shortened, irregular microvilli are also seen.[76–78] Viral particles have not been identified by electron microscopy in involved mucosa.[76, 77] The histological changes described may precede the onset of symptoms and may even develop in infected subjects who remain entirely asymptomatic.[74, 75]

Although it has been suggested that the lesion induced in the proximal small intestine by the Hawaii agent is not identical to that induced by the Norwalk agent,[77] we believe that these described differences are within the range attributable to variations in sampling and severity of infection.[74, 75] Because the histological changes are not specific but resemble other nonspecific jejunal mucosal lesions, such as those found in tropical sprue and intraluminal bacterial overgrowth,[79] a diagnosis of parvovirus-like agent gastroenteritis cannot be made by proximal small intestinal biopsy alone. Because only the proximal small intestinal mucosa has been studied to date, the extent of small intestinal involvement is not known. Whether or not the severity of symptoms correlates with the extent of intestinal involvement by the lesion remains to be determined.

A histological lesion of the gastic mucosa does not develop during gastroenteritis induced by the Norwalk agent. Biopsies of antral and fundic mucosa in 9 volunteers who developed symptoms after ingesting an inoculum containing the Norwalk agent were unchanged compared to control biopsies.[80] The colonic mucosa has also been reported to be normal during Norwalk agent-induced gastroenteritis in the few cases examined.[30, 76]

Reovirus-like agents. The histological changes reported in association with gastroenteritis caused by reovirus-like agents have varied. In one report of biopsies

from 31 infants hospitalized with acute nonbacterial gastroenteritis, there were 5 normal specimens, 21 with mild to moderate abnormalities similar to those seen with parvovirus-like agent gastroenteritis, and 5 flat mucosal biopsies.[81] Infiltration of the lamina propria with chronic inflammatory cells and a cuboidal appearance to the normally tall columnar surface absorptive cells were noted.[81] However, in this study, stools were not examined for reovirus-like agents; thus, the etiology of gastroenteritis in these infants was not fully established. All 9 children studied by Bishop et al.[56] with duodenal biopsies 1 to 5 days after onset of symptoms of gastroenteritis, showed histological abnormalities ranging from mild to severe. In 6 of the patients HRVL particles were seen by electron microscopy in intestinal epithelial cells. These particles were identified in distended cisternae of the endoplasmic reticulum and in the cisternae between inner and outer nuclear membranes. Enveloped particles had a diameter of 87 to 90 nm; others not enveloped were about 67 nm in diameter.[56] Microvilli of absorptive cells containing particles were often irregular and distorted.[82] The abnormal infected cells were seen scattered among absorptive cells with normal fine structure. Cells with degenerative changes were observed which appeared to be discharging virus into the intestinal lumen.[82] Swollen mitochondria, increased numbers of lysosomes, and multivesicular bodies were also noted in involved absorptive cells.[83] Particles were noted in goblet cells and in cells within the lamina propria in one report,[83] but virus particles were not found in cells of the lamina propria in other studies.[56, 84] Histology reverted to normal and HRVL particles were absent in 3 patients 4 to 8 weeks after the HRVL particle-containing lesions were first noted.[56] Unlike gastroenteritis induced by parvovirus-like agents, a definitive diagnosis of HRVL gastroenteritis can be made by small intestinal biopsy when the typical particles are seen by electron microscopy.[35, 56, 82, 83] However, intestinal biopsy for diagnosis is not generally indicated since simpler diagnostic tests are now available (see Diagnostic Tests).

The gastric and colonic mucosae have been reported to be normal in the few infants with HRVL gastroenteritis in whom they have been examined.[35]

Pathophysiology

Because diarrhea is a major symptom of acute viral gastroenteritis, and histological abnormalities appear confined to the small intestinal mucosa, studies have been performed to characterize impaired intestinal absorption which may accompany this disease.

Parvovirus-like agents. Steatorrhea was demonstrated in early studies of volunteers who became ill after ingesting the Norwalk agent.[30] Malabsorption of fat was also noted in several volunteers who did not develop overt clinical illness after ingesting that agent.[30] However, the latter group may well have had subclinical infection capable of causing significant malabsorption in the absence of overt symptoms. Abnormal xylose absorption during illness suggests that mucosal pathology contributes to the observed malabsorption.[30, 74] The malabsorption of fat and xylose may persist for at least 1 week after infection, although clinical symptoms last only 24 to 48 hr.[30, 74]

Decreased levels of the brush border enzymes trehalase and alkaline phosphatase were regularly observed during acute Norwalk illness; decreases in the levels of sucrase and lactase were less consistent. However, oral lactose tolerance tests show decreased absorption during acute Norwalk illness and may remain abnormal for at least 1 week.[30] Twenty-three volunteers who developed illness after ingesting either the Norwalk or the Hawaii agents had assays of adenylate cyclase in the proximal jejunal mucosa before and after the ingestion of inocula. No significant changes in intestinal mucosal adenylate cyclase levels were detected during clinical illness or during the recovery period up to 3 weeks after illness.[85]

Serial bacteriological analysis of jejunal contents showed a "modest" increase in the number of bacteria present during the Norwalk illness. However, recovery of increased bacteria from the proximal intestine did not correlate with the presence or absence of D-xylose malabsorption.[30]

It is evident that available information regarding gastrointestinal function during parvovirus-like agent disease is limited. Studies which characterize gastric and intestinal motor function, fluid, electrolyte, sugar, and peptide absorption are needed. Further study of the alteration of the intraluminal bacterial flora during acute disease and convalescence also seem indicated.

Reovirus-like agents. The pathophysiology of infantile gastroenteritis attributable to HRVL has been studied even less extensively than that caused by Norwalk agent, because studies are limited to uncontrolled evaluation of sick infants. These studies of the pathophysiology of acute infantile gastroenteritis were carried out on infants without a definitive diagnosis, although it seems likely that many of these subjects were infected with HRVL.

The disaccharidases maltase, sucrase, isomaltase, and lactase are usually depressed in infantile gastroenteritis,[81, 86] and the severity of the histological lesion correlates with the degree of disaccharidase depression.[81] *Candida albicans* was cultured from the proximal small intestine in 30% of infants with gastroenteritis and 10% of controls. However, this was not statistically significant.[87] No significant change in the bacterial flora of the proximal small bowel was noted.[86, 87] The significance, if any, of Candida colonization or alterations in bacterial flora is not known.

Diagnostic Tests

Parvovirus-like agents—electron microscopy. The technique of immune electron microscopy (IEM), has proved useful in the study of volunteers with experimental gastroenteritis. IEM involves mixing a virus particle-containing filtrate with convalescent serum from a person known to have had recent infection with the appropriate viral agent. After suitable incubation, antibody in the convalescent serum and the viral particles form aggregates which can be recovered by centrifu-

gation and visualized with the electron microscope after negative staining with phosphotungstic acid.[24] The 27-nm Norwalk article was first detected in infectious fecal filtrates using this technique.[32] Subsequently, similar appearing 27-nm particles were detected in the stools of volunteers with experimentally induced gastroenteritis caused by the Hawaii and MC agents.[34] Approximately 50% of volunteers experimentally infected with the Norwalk agent will excrete 27-nm particles in sufficient quantity to be detected by IEM of stools during the first 72 hr after the start of symptoms. Viral particles are not detected before the onset of symptoms and can be detected in less than 20% of stool specimens 72 hr or more after onset of symptoms.[29] Various technical manipulations such as concentration of fecal filtrates and alterations of antigen-antibody ratios are often required to achieve optimal visualization of particles by IEM.[34] To date, the technique has been used primarily in the study of experimentally induced infection; therefore, the usefulness of IEM in the diagnosis of naturally occurring parvovirus-like agent gastroenteritis remains to be established. Serum antibody levels to parvovirus-like agents can be partially quantitated by determining, with IEM, the degree of coating by antibody of viral particles when serum is mixed with fecal filtrates known to contain appropriate viral particles.[26, 32, 34, 77] However, there is as yet no convenient and accurate serological test for the diagnosis of parvovirus-like agent-induced gastroenteritis.

HRVL-electron microscopy. Shortly after the HRVL agent was detected in tissue sections of the duodenal mucosa of ill infants,[56] it was reported that HRVL can be detected in stools from infected infants and young children by electron microscopy using negative staining with phosphotungstic acid.[46, 57] This observation has now been confirmed in many centers.[35, 38, 41, 54] These HRVL particles are rarely seen in the stools of healthy infants and children.[41, 45–57] Indeed, examination of feces passed during the first few days of illness was soon demonstrated to be more sensitive than duodenal biopsy for detecting HRVL particles.[57] Few technical problems are associated with this method and relatively little processing of stool samples is required for specimens to be rendered suitable for electron microscopy. Thus, in contrast to the vagaries associated with the detection of parvovirus-like viruses of gastroenteritis in the stool, which requires antibody coating, centrifugation, and filtration, the diagnosis of HRVL-induced gastroenteritis can be made easily in infants and young children by finding the 70-nm HRVL particles by electron microscopy in stool specimens passed during acute illness.

HRVL-immunoelectrophoresis. Although examination of stools for HRVL particles by electron microscopy is quite simple, the technique is time consuming and requires trained personnel and equipment not universally available. The detection of viral antigen in the stools of children with HRVL gastroenteritis has recently been reported using the technique of counter-immunoelectroosmophoresis.[88, 89] For this, stool suspensions are placed in one well of an agarose slide. Antiserum produced in guinea pigs after immunization with HRVL is placed in an adjacent well.[89] After appropriate incubation and application of an electrical current, a characteristic precipitin line develops between the wells of stool suspensions which contain HRVL particles and the wells of antisera, but not between control stool specimens and antisera. This precipitin band has been shown by electron microscopy to contain viral particles.[89] The technique appears to have approximately the same sensitivity and specificity as does electron microscopy of stool suspensions, but can be used to screen large numbers of specimens rapidly and does not require an electron microscope.[89]

HRVL-complement fixation. Serum serological tests have been developed, extensively tested, and compared to the efficacy of stool examination by electron microscopy. Kapikian and co-workers[38] demonstrated a rise in serum antibody titer to HRVL stool antigen with a complement fixation (CF) test using acute and convalescent sera of infants who excreted the HRVL in their stools. The concordance between IEM and CF was 85% in their large series.[44]

Because of the limited quantity of HRVL stock antigen available, a related reovirus-like agent called the Nebraska calf diarrhea virus (NCDV) is often used as a substitute antigen in place of the HRVL.[38, 90] The NCDV, which causes diarrhea in infant calves,[91] is morphologically identical to and antigentically related to the HRVL.[38, 90] (See relevant animal models below.) When tissue culture-grown NCDV is used as antigen for the CF tests and is compared with the CF test which uses crude stools containing HRVL as antigen, the concordance is 80% at best. In such comparative studies, HRVL is clearly more efficient in detecting human antibody response.[44, 55, 92, 93] Nonetheless, the NCDV has been substituted for HRVL in some laboratories because it can be readily grown in tissue culture and therefore is available in the quantities required.

HRVL-fluorescent antibody. Detection of antibody by indirect immunofluorescent microscopy has also been reported. In the original report of indirect immunofluorescence, Davidson et al.[84] used as antigen human duodenal mucosal absorptive cells from infected infants proved by electron microscopy to contain HRVL. Immunofluorescence was patchy and the fluorescence was confined to the supranuclear cytoplasm which correlates with the distribution of viral particles as observed by electron microscopy. However, acute as well as convalescent sera reacted with the antigen. Wyatt and coworkers[62] used human fetal intestinal organ cultures infected with the HRVL as the antigen to examine the sera of infants with gastroenteritis for antibody by indirect immunofluorescence. Whereas acute sera did not contain antibody detectable by immunofluorescence, convalescent sera from 8 of 9 gastroenteritis patients with HRVL in stools, as well as 2 of 4 with negative stools, contained antibody detectable by this method.[62] Infant rhesus monkey intestine infected with the HRVL can also be used as antigen for fluorescent antibody (FA) tests and appears slightly more sensitive than the complement fixation test which utilizes HRVL for the detection of HRVL seroconversion.[68] However, these FA

tests are impractical as they require either human fetal intestinal explants or HRVL-infected intestine from newborn monkeys. In the most convenient FA tests available, NCDV grown in tissue culture is used as the antigen.[90, 93, 94]

Gnotobiotic piglets infected with the HRVL develop serological conversion by the FA test[66, 67] but not by CF tests,[66] indicating that these tests recognize different antigen-antibody reactions.

Immunity

Parvovirus-like agents. The development of immunity to gastroenteritis is poorly understood. Experimental infection with the Norwalk agent results in resistance to reinfection with the same agent for at least 14 weeks.[31] However, within 3 years after infection, immunity to reinfection with the Norwalk agent is lost (Parrino et al., *unpublished results).* In the original outbreak of Norwalk gastroenteritis, 2 ill patients who did not develop a measurable rise in serum antibody titer by IEM during convalescence had high levels of antibody in their sera obtained during acute illness.[32] This would suggest that the presence of serum antibody to Norwalk agent as measured by IEM is not sufficient for immunity. Whether immunity to one parvovirus-like agent provides at least temporary protection against infection with other agents is not fully resolved. The Norwalk and Hawaii agents appear to be antigenically distinct, and infection with either agent provides no protection against infection with the other agent.[26] An immunological relationshp between the Norwalk and MC agents has been suggested based on (1) a slight rise in serum IEM antibody titer to the Norwalk agent after infection with the MC agent in a single patient, and (2) the inability to induce overt clinical disease with MC agent after recovery from Norwalk enteritis in 8 volunteers.[26] However, experimental illness with the MC agent often resulted in mild symptoms which were difficult to evaluate.

Inasmuch as these data derived from small numbers of volunteers and relatively crude criteria for illness were used, we believe that cross-immunity between distinct parvovirus-like agents which induce gastroenteritis has not been documented. Although only three distinct disease-producing parvovirus-like agents are recognized at this time, the number of agents in this group is unknown and may be substantial.

The local mechanisms which may be involved in recovery from viral gastroenteritis have not yet been elucidated. The short duration of clinical illness (12 to 48 hr) has led to speculation that interferon may play a role in controlling intestinal infection. However, no interferon was detected in the jejunal secretions, jejunal homogenates, or sera of volunteers with gastroenteritis induced by Norwalk agent.[95]

Studies of the local immunological response to parvovirus-like agents have been limited. The synthesis of crude IgA by intestinal biopsies in vitro obtained from volunteers before and after Norwalk enteritis has been measured. Two weeks after ingestion of the Norwalk agent, there was a doubling of radioactive leucine incorporation into tissue IgA both in the volunteers who had been ill as well as those who remained asymptomatic.[96] However, variation was extreme and the significance of these studies is difficult to interpret since specific antibody to the viral antigen was not measured. The measurement of total IgA synthesis does not necessarily reflect changes in a single specific antibody to a virus. More accurate evaluation of the immunological response of these agents will probably require the production of purified viral antigen.

Reovirus-like agents. The immunological response to HRVL gastroenteritis and its significance is even less clear than that to parvovirus-like agents because data are derived from naturally occurring disease rather than from experimentally induced illness. Moreover, there is little information regarding antigenic variation among HRVL agents which induce gastroenteritis. As expected, there is no evidence for antigenic cross-reactions between the HRVL agents and the parvovirus-like agents.[38] There probably are antigenic sites in both the inner and outer shell of HRVL agents as human convalescent sera will agglutinate both single and double-shelled HRVL particles.[67, 90] In the only report in which human agents from two areas of the world were compared, coating of particles from stools of children from India by convalescent sera of Australian children occurred only when the outer shell was absent.[42] These results remain to be confirmed but raise the possibility that there may be more than one antigenic variety of HRVL. Although reinfection of patients with gastroenteritis-inducing HRVL's has not been reported, children with preexisting serum antibody have developed illness attributable to HRVL.[44] This would suggest infection with a second antigenically related HRVL or reinfection with the same HRVL.

A serum antibody detected by immunofluorescense in human infants with gastroenteritis is IgM, not IgA.[84] Its significance remains to be determined.

The prevelance of serum antibody to HRVL as measured by CF tests increases steadily from 10 to 28% at 6 months of age to 50 to 90% by age 2 years.[41, 44, 92, 93] Although the reported prelavence of circulating antibody to HRVL varies with slightly different laboratory techniques and differing patient populations, the prevalence of antibody at 2 years of age is comparable to the prelavence of antibody in adults in each individual study.[41, 44, 92, 93] One survey showed a prevalence of serum CF and FA antibody of 75 to 80% in neonates, which then decreased, reaching a nadir of 10 to 15% at approximately 6 months of age.[93] This antibody, found in neonates, is probably maternal in origin.

Many patients who demonstrate a rise in serum antibody titer by either FA or CF techniques after gastroenteritis have significant levels of antibody before infection.[44] It is clear therefore, that the presence of these serum anti-HRVL antibodies alone is not sufficient for immunity. Infants aged 6 to 24 months appear more susceptible to infection regardless of the presence or absence of serum antibody. The low incidence of infection in infants less than 6 months of age is most likely attributable to immunity acquired from the mother.

The greater resistance to infection seen in children 2 years and older may reflect functional maturation of the intestine and its immune mechanisms as well as the increased likelihood of active immunity from prior exposure to HRVL.

The intestinal secretions from infants with gastroenteritis caused by the HRVL contained normal amounts of IgA both during acute illness and convalescence.[97] However, in this study no information was obtained regarding specific antibody to HRVL antigen, so its significance is difficult to interpret.

Relevant Animal Models

Parvoviruses. Infectious enteritis in animals caused by parvoviruses has been reported in cats and mink.[98, 99] All members of the cat family appear to be susceptible to a feline enteritis virus which is commonly termed feline panleukopenia (FPL) virus. Mink enteritis virus appears to be closely related to or identical to FPL virus based on physical properties, serological tests, and cross-challenge studies of immunity.[99, 100] Clinically, FPL is characterized by a 3- to 7-day incubation period followed by high fever, lethargy, loss of appetite, vomiting, dehydration, and abdominal tenderness. Diarrhea, which may be bloody, usually begins 2 to 3 days after the onset of symptoms and persists for 5 to 7 days. The fatality rate is 50 to 90%.[101] A characteristic finding is marked leukopenia with WBC generally less than 3000 and often less than 500.[101]

Histopathological findings include necrosis of the epithelium of villus tips in the small bowel, ballooning of surface cells, and loss of the basal nuclear polarity of epithelial cells. Eosinophilic intranuclear inclusions are seen in the crypt epithelial cells. Mesenteric lymph nodes are characterized by lymphocyte depletion and reticuloendothelial cell hyperplasia, whereas the bone marrow shows a marked decrease in myeloid precursors.[102] The FPL virus replicates in tissue culture monolayers of kitten kidney cells, providing the cells are dividing rapidly.[103] Serological methods such as serum neutralization and hemagglutination inhibition tests are available for the detection of infection with FPL or mink enteritis virus.[104]

Reovirus-like agents. Enteric infection of young animals with reovirus-like agents appears to be widespread throughout the animal kingdom. The viruses about which the most is known are those which infect the calf (NCDV)[91] and mouse (epizootic diarrhea of infant mice—EDIM).[105] Other species which develop enteritis caused by reovirus-like agents include the pig[106] and the lamb.[107] Infection is most common during the neonatal period.[105, 108]

Infection of the small intestine in these animals, with the appropriate species-specific agent, produces a histological lesion which is similar to that seen in human infants with viral gastroenteritis.[109, 110] These viruses are morphologically indistinguishable from each other and from the HRVL.[59, 91, 111] These agents are all immunologically distinctive and, in terms of infectivity, appear to have clear-cut species specificity based on factors which are not yet understood.

Animal reovirus-like agents are antigenically related to the HRVL.[38] Human convalescent sera of infants who recovered from HRVL infection agglutinate the NCVD[67, 90] and the reovirus-like agent which produces diarrhea in piglets.[67] However, convalescent sera from piglets or calves infected with their specific reovirus-like agent agglutinate only HRVL particles devoid of the outer shell.[67, 90] This implies that common antigens are present in the inner capsomere whereas species-specific antigens may be carried in the outer shell. These findings were confirmed and expanded by Kapikian and co-workers,[38, 92] who showed an antigenic relationship between the human agent (HRVL), NCDV, and the virus of EDIM by using CF tests.[38, 92] Additional CF tests failed to demonstrate any immunological relationship between the HRVL and known reoviruses or 20 standard orbiviruses, groups of viruses with a similar morphological appearance.[38, 92] Antisera to the three known types of reovirus also do not bind to HRVL in intestinal tissue using immunofluorescent antibody techniques.[84]

The NCDV has recently been propagated successfully in tissue culture.[108, 112] As a result, a vaccine has been developed to combat this disease. Immunity to NCDV develops in newborn calves in 2 days after oral inoculation with the vaccine, before the development of significant serum antibody levels, which suggests an immune protection at the intestinal mucosal level.[113] Field studies in newborn calves have also shown that the presence of passively acquired antibody of maternal origin is not sufficient to protect calves from clinical NCDV illness.[113]

Coronaviruses. In addition to the reovirus-like agent of piglets there is a coronavirus which causes transmissible gastroenteritis of swine by infecting the small intestine[114] of neonatal piglets.[115] Protection is afforded by the presence of a specific anticoronavirus IgA antibody in the milk of the mother.[116, 117] In infected piglets with diarrhea, there is net intraluminal secretion of water and electrolytes in the proximal jejunum which is associated with increased active and passive sodium efflux. Intestinal mucosal adenyl cyclase levels are normal, but sodium-potassium adenosinetriphosphatase levels are decreased in infected animals.[118, 119] The rate of cell migration from crypts to villus tips is increased and the crypts are hyperplastic.[118, 119] It has been suggested that impaired intestinal epithelial maturation may contribute to the observed secretory diarrhea.[119]

Future Investigations

Although progress has been made recently in defining the clinical features, the histopathology and some aspects of the pathophysiology of viral gastroenteritis, much remains to be learned.

The mode by which the viruses which cause gastroenteritis gain access to intestinal mucosal cells is unknown. Whether there are specific receptors to which viral particles attach or whether virus is taken up by nonspecific transport mechanisms has not been determined. However, the finding that the age of the host is an important determinant of susceptibility to infection

with HRVL implies that structural or functional changes associated with maturation may significantly impair the ability of the HRVL to infect the intestinal mucosa.

Development of practical methods for cultivation of the viruses which cause gastroenteritis are needed. Large quantities of pure virus would greatly facilitate clarification of the physical and biochemical characteristics of the agents and could be used to study the mechanisms of infection. To date, attempts to grow parvovirus-like agents in tissue and organ culture or to induce replication of the virus in laboratory animals have failed. The HRVL agent appears to replicate in organ cultures of human fetal intestine,[62] but large amounts of purified virus have not been produced by this method nor has it proved practical for diagnostic purposes.

The factors that determine host resistance to viral enteritis remain unknown. The potential roles of serum antibody, local intestinal antibody, interferon, specific viral receptors, and local mechanical factors in the intestine remain to be determined.

The interrelationship of viral gastroenteritis to chronic intestinal diseases requires extensive study. The intestinal lesion which develops in viral gastroenteritis is histologically indistinguishable from that seen in tropical sprue, a disease of unknown etiology. A single report of a number of patients who developed clinical features of tropical sprue after an outbreak of apparent gastroenteritis is provocative.[120] The interrelationship between chronic malnutrition and gastroenteritis has immense world-wide public health implications and requires further study. Acute gastroenteritis with significant mortality has been reported in a chronically malnourished population.[2] This might reflect a variety of host factors which could increase the susceptibility or inhibit the recovery of such individuals from viral infection of the intestine.

The feasibility of immunization against gastroenteritis has not yet been explored. Reovirus-like agents which appear to produce disease only in specific animal species (NCDV, EDIM, or other reovirus-like agents) share antigens with HRVL. By administering these agents to human beings, it may be possible to induce resistance to HRVL infection without production of significant illness, much as has been done with smallpox vaccination.

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