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Viruses

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INTRODUCTION

Before the early 1970s the causes of human non-bacterial gastroenteritis were largely unknown. Extensive information has since been obtained about the aetiology and pathogenesis of viral gastroenteritis. There are four major subclassifications of gastroenteritis-causing viruses: rotavirus, enteric adenovirus, calicivirus (including Norwalk and Norwalk-like viruses) and astrovirus. Some characteristics of these viruses are compared in Table 1. The association of these viruses with gastroenteritis was based on their frequent detection by electron microscopy in stools and mucosal biopsies from diseased patients with diarrhoea compared to controls, absence of viruses after recovery from disease, and the subsequent development of virus-specific immunoglobulin responses in serum and intestinal secretions. In addition, rotavirus and Norwalk virus have been shown to cause gastroenteritis when inoculated into healthy volunteers (Christensen, 1989; Blacklow and Greenberg, 1991). This association has facilitated studies of epidemiology, classification, immunology, diagnostic methods, methods of treatment and, most importantly, strategies towards prevention through vaccine development.

Following the emergence of the acquired immunodeficiency syndrome (AIDS), other viral infections of the gastrointestinal tract (e.g. cytomegalovirus) have become increasingly common. Although it is relatively unusual for the common gastroenteritis viruses to be clinically important in patients with human immunodeficiency virus (HIV)-associated disease, adenovirus has recently been described (Janoff et al, 1991), as has the presence of HIV itself within the small bowel enterocytes (Ullrich et al, 1989; Heise et al, 1991).

This chapter highlights the major features of these agents, with special attention being given to molecular biology and prospects for vaccination. Prevention, treatment and vaccination will be discussed together, except for rotavirus, which will be considered separately.

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Table 1. Some clinical, epidemiological and diagnostic aspects of human gastroenteritis viruses.

Virus	Clinical features	Epidemiological aspects	Laboratory tests
Rotavirus			
Group A	Dehydrating diarrhoea for several days; vomiting and fever usual	Common cause of endemic diarrhoea in infants and young children (winter peak in temperate zone)	Immunoassay, electron microscopy, PAGE
Group B	Severe watery diarrhoea for 3–5 days	Outbreaks in adults and children reported from China	Electron microscopy, PAGE
Group C	Similar to those of group A rotavirus	Sporadic episodes in young children	Electron microscopy, PAGE
Enteric adenovirus	Diarrhoea for 5–12 days; vomiting and fever	Endemic diarrhoea of infants and young children	Immunoassay, electron microscopy with PAGE
Norwalk virus	Vomiting, diarrhoea, fever, myalgia, and headache for 24–48 h	Epidemics of vomiting and diarrhoea in older children and adults: occurs in families, communities and nursing homes: often associated with shellfish, other food or water	Immunoassay, immune electron microscopy
Norwalk-like viruses (small, round structured viruses)	Vomiting, diarrhoea, fever, myalgia, and heachache for 24–48 h	Similar to those of Norwalk virus	Immunoassay, immune electron microscopy
Calicivirus	Resembles rotavirus illness in children: Norwalk-like features in adults	Usually affects children but can be associated with shellfish and other food in adults	Immunoassay, electron microscopy
Astrovirus	Watery diarrhoea, usually for 2–3 days, sometimes for longer	Usually affects children but has been reported in nursing homes	Immunoassay, electron microscopy

From Blacklow and Greenberg (1991). Reprinted by permission of the New England Journal of Medicine 325(4): 252.
Laboratory diagnostic tests, other than those for rotavirus group A, are usually available only in research or diagnostic referral laboratories. Immunoassays are usually enzyme-linked immunosorbent assays or radio-immunoassays.
PAGE—polyacrylamide gel electrophoresis and silver staining of viral nucleic acid in stool.

ROTAVIRUS

Introduction

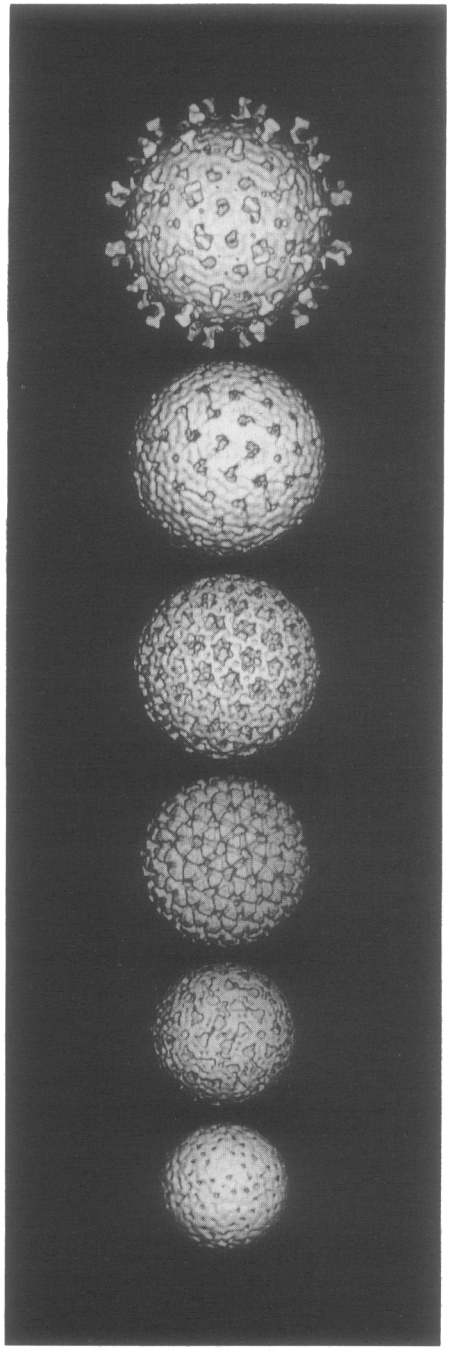
Rotavirus is the most common cause of severe gastroenteritis in infants and young children, accounting for approximately 3 500 000 episodes per year in the USA alone (Ho et al, 1988). This agent was initially discovered in the early 1970s when viral particles were seen in duodenal epithelial cells, followed by the detection of the virus in faecal extracts from children with acute gastroenteritis (Bishop et al, 1973, 1974). Rotavirus infects virtually every child in the USA by the age of 4 years and causes potentially lethal dehydration in 0.75% of children less than 2 years of age (LeBaron et al, 1990a).

Classification and molecular biology

These viruses are members of the *Reoviridae* family. The rotavirus genome contains 11 segments of double-stranded RNA, each of which encodes a single protein. The viral structural proteins assemble into a non-enveloped, multilayered icosahedral structure. The virions contain an RNA-dependent RNA polymerase as well as other enzymes which lead to the production of capped RNA transcripts (Estes and Cohen, 1989).

The viral genome itself is encapsulated by four major structural proteins (Figure 1). The two surface membrane proteins, VP4 and VP7, act as the major determinants of protective antibody-mediated immunity (Matsui et al, 1989). VP7 is the primary determinant of the viral serotype classification (see Epidemiology). Together with VP4, it makes up the outer coat of the virus and is perforated by 132 aqueous holes which provide channels from the viral surface to the viral genome in the centre of the virion. It is likely that these channels are the route through which viral mRNA leaves the transcription complex (Prasad et al, 1988). From this outer coat surface protrude the numerous spike-like structures of VP4, the viral haemagglutinin (Prasad et al, 1990; Yeager et al, 1990). Within the outer capsid lies the inner capsid, made up of VP6 trimers which constitute 51% of the virion protein by weight. VP6 is not only important for viral structure, but is also required for viral polymerase activity and may have a role in transcription (Estes and Cohen, 1989). There are holes in this inner capsid which are aligned with those in the outer capsid. This inner capsid surrounds a protein core which is thought to be made up of VP1, VP2 and VP3, which encapsulate the double-stranded RNA genome (Yeager et al, 1990).

The outer membrane protein, VP4, is encoded by RNA segment 4. This protein has several functions, including haemagglutination activity of certain rotaviruses, a role in virulence in mice, and induction of protective antibody-mediated immunity. VP4 is cleaved into VP5 and VP8 by proteolytic enzymes, such as trypsin, which leads to enhanced infectivity by permitting direct penetration of the virus through the cellular plasma membrane (Kalica et al, 1983; Suzuki et al, 1985, 1986; Offit et al, 1986; Hoshino et al, 1988; Kaljot et al, 1988; Matsui et al, 1989).



VP7 is encoded by RNA segment 9. It mediates viral neutralization and determines serotype specificity (see below). VP6 is encoded by RNA segment 6 and contains the subgroup-specific antigen and the antigen that determines group reactivity (Matsui et al, 1989). VP1, VP2 and VP3 are encoded by RNA segments 1, 2 and 3, respectively, while segments 5, 7, 8 and 10 are thought to encode non-structural proteins. Segment 11 encodes a protein that may be a minor protein of the outer capsid or a non-structural protein (Matsui et al, 1989; Kapikian and Chanock, 1990).

Epidemiology

Rotaviruses can be serologically classified into groups (currently A–G) which share cross-reacting antigens located on VP6. Groups A, B and C have been found in humans and animals, while the others have been found only in animals (Estes and Cohen, 1989). Rotaviruses can be subdivided within groups by serotypes that are based on neutralizing antibodies induced against VP7 during infection or immunization. This property is frequently established in vitro, when a virus is grown in tissue culture in the presence of a serum of known specificity. If viral neutralization is observed, the virus being tested is said to share a serotype with the virus used to produce the infection/vaccination serum. Recently, this cumbersome process has been obviated by the use of serotype-specific monoclonal antibodies that permit serotype classification in a simple enzyme-linked immunoassay. An important practical aspect of serotype classification is to enable investigators to enhance vaccine strategies through epidemiological studies that can define the serotypic diversity of the common human rotaviruses.

In both developed and developing countries, rotavirus is considered to be the single most common cause of dehydrating diarrhoeal illness, accounting for up to 71% of those episodes requiring hospitalization in children less than 2 years of age (Cook et al, 1990). The group A rotaviruses are the predominant cause of severe gastroenteritis leading to dehydration in children, although groups B and C are now being studied (Brandt et al, 1983; Koopman et al, 1984; Blacklow and Greenberg, 1991). During the first 3

Figure 1. Three-dimensional structure of rhesus rotavirus by cryoelectron microscopy and icosahedral image reconstruction. The shaded representations were obtained by truncating the three-dimensional maps with spherical envelopes to reveal the internal structure. The structures are as follows from top to bottom. The first is the surface view of rotavirus showing the outer capsid surface (dark) and the 60 spikes (light) attributed to the VP4 haemagglutinin diameter, 100 nm. In the second, the smoothly rippled outer capsid surface, attributed primarily to VP7, is perforated by 132 aqueous holes (diameter, 79 nm). The third shows the space between the outer and inner capsids, which forms an open aqueous network that may provide pathways for the diffusion of ions and small regulatory molecules as well as the extrusion of RNA (diameter, 72 nm). The fourth is the inner capsid, with a bristled outer surface composed of 260 trimeric columns, attributed to VP6 trimers (diameter, 66 nm). In the fifth, the VP6 trimers merge with a smooth inner capsid shell that is perforated by holes in register with those in the outer capsid (diameter, 58 nm. The sixth is a third protein shell (the core) thought to be formed by VP1, VP2 and VP3 and encapsulating the double-stranded, segmented RNA genome diameter, 53 nm. Adapted from Yeager et al (1990), with permission.

months of life, infants are relatively resistant to rotavirus infection because of passive immunity from transplacental transfer of immunoglobulin G (Tufvesson et al, 1986; Vial et al, 1988). The most severe episodes of rotavirus gastroenteritis occur between 6 months and 2 years of age.

The severe disease is primarily seen in children, but adults can be infected under a variety of conditions, most often after contact with an infected infant in the family or play group setting (Rodriguez et al, 1979). Other circumstances in which adult disease is common include water-borne outbreaks and travellers' diarrhoea (Hrdy, 1987). Nevertheless, due to the level of immunity acquired from previous infection as well as less well-defined maturational factors, subclinical infection tends to be the rule in adults. In one study, 55% of household contacts of children hospitalized for rotavirus gastroenteritis had serological evidence of recent or current rotavirus infection at the time of the child's hospital admission (Kim et al, 1977; Kapikian and Chanock, 1990). Other studies have also shown that rotavirus infection in children is much more likely to be symptomatic (Wenman et al, 1979).

The virus is transmitted by the faecal–oral route, although transmission by the respiratory route has also been postulated (Kapikian et al, 1983a; Kapikian and Chanock, 1990). The shedding of rotavirus has been documented both during and after the diarrhoeal stage (Pickering et al, 1988). In the Western hemisphere, rotavirus infection has a seasonal pattern, usually occurring in the winter months (LeBaron et al, 1990b). Utilizing the technique of electropherotyping (observing patterns of the 11 RNA gene segments separated on polyacrylamide gels), it has often been shown that several different strains of rotavirus may circulate and cause disease in a community at the same time (Konno et al, 1984; Ruggeri et al, 1989) although one strain usually predominates (Beards et al, 1989; Urasawa et al, 1989).

Clinical features

The onset of symptoms occurs between 2 and 4 days after inoculation in experimental volunteers (Kapikian et al, 1983a,b). There is a broad spectrum of disease caused by rotavirus, ranging from subclinical infection through to fatal dehydration. Most paediatric patients with rotavirus infection are febrile to at least 37.9°C but a third to a half exceed 39°C, over 90% of patients have frequent vomiting and diarrhoea which may be severe; clinically significant dehydration (>5%) is observed in over 70% of those admitted to hospitals (Uhnoo et al, 1986). However, it is not possible to identify rotavirus infection (or any other type of viral gastroenteritis) based on clinical criteria alone (Blacklow and Greenberg, 1991).

Rotavirus infections are self-limited, with resolution of symptoms usually within a few days although at times symptoms may last up to 2 weeks. Chronic diarrhoea has been described in immunodeficient children (Saulsbury et al, 1980) and may lead to severe or fatal disease in bone-marrow transplant recipients (Yolken et al, 1982b). Haematochezia (bloody stools) and tenesmus are distinctly uncommon in rotavirus infection and often are due to

the presence of concurrent bacterial infection (Blacklow and Greenberg, 1991). Microscopy of faecal specimens may reveal occasional red blood cells or leukocytes.

Much has been learnt about the pathogenesis of rotavirus infection from studies in animals. The virus infects mature enterocytes on the tips of small intestine villi. The infection spreads in a proximal to distal pattern along the small intestine, lysing the intestinal enterocytes and leading to villous shortening and crypt hyperplasia (Blacklow and Greenberg, 1991). The pathophysiological mechanisms that cause diarrhoea are still unclear, with experimental evidence supporting a role for net intestinal secretion, and selective malabsorption of certain macromolecules. Secondary lactase deficiency and carbohydrate malabsorption also play a role. Recently, studies on cytokines, direct effects of viral infection on sodium transport prior to cell death, and villous ischaemia and subsequent oedema causing interference with countercurrent exchange mechanisms have all been reported (Yamashiro et al, 1989; Spencer et al, 1990; Starkey et al, 1990; Osborne et al, 1991). Establishing the relative importance of different mechanisms and their interactions may lead to improved treatments aimed at halting and replacing fluid losses.

Immunology

Antibodies directed at VP4 or VP7 can neutralize virus and protect the host from infection. This is not so with antibodies directed against the other viral proteins, including VP6, the most immunogenic of the rotavirus proteins (Offit et al, 1986; Hoshino et al, 1988; Matsui et al, 1989). Neutralizing epitopes conserved among several serotypes are located on the C-terminal trypsin-cleavage fragment of VP4, but high-titre antibodies directed at this region may not be induced during natural infection (Mackow et al, 1988a,b; Blacklow and Greenberg, 1991; Shaw et al, 1991). Most VP4-specific antibodies are directed at a hypervariable region (the region designated vp8* after trypsin cleavage of vp4) and are strain-specific. Although there exists at least one serotype cross-reactive epitope on VP7, most of the neutralizing antibodies to VP7 are serotype-specific (Taniguchi et al, 1988).

A role for cellular immunity (T-lymphocytes manifesting cytotoxicity or B-cell help) in response to rotavirus infection has been demonstrated (Offit and Dudzik, 1989; Offit et al, 1992). Precisely what part these responses play in the natural response to human infection remains unclear.

Diagnosis

There are a number of methods available to detect rotavirus in the stool. The virus is shed in very large amounts for the first 1–4 days of infection and for up to 21 days, depending on the length of symptoms. Usually the duration of diarrhoea corresponds to the shedding of the viral particles, although at times diarrhoea can last for up to 3 days beyond the termination of shedding (Kapikian and Chanock, 1990).

Although electron microscopy was the first method used to detect the

virion in stool, the most commonly used methods now are the enzyme-linked immunosorbent assay (ELISA) and latex agglutination (LA). In addition to detection of all group A viruses, monoclonal antibodies may be used in the ELISA to permit distinction of viral serotypes (Zissis and Lambert, 1980; Tufvesson et al, 1986). There are several studies comparing these antigen detection schemes with immune electron microscopy, the 'gold standard' of detection. In general, the predictive value of these assays has been greater than 90% (Christensen, 1989). Most commercially available diagnostic kits are based on variations of these assays.

Other methods of detection include dot hybridization using single-stranded RNA transcripts, which has been shown to have excellent sensitivity (Flores et al, 1983), although it is not commonly available as a rapid diagnostic tool. Viral RNA can be detected in faeces by polyacrylamide gel electrophoresis with silver staining as well as with the use of polymerase chain reaction (Gouvea et al, 1990).

There are various techniques for measuring serological response to rotavirus infection, but ELISA using intact rotavirus particles as the antigen has been most widely used. Evaluation of rotavirus serology is used primarily in epidemiological and vaccine studies rather than for diagnosis. However, the evaluation of anti-rotavirus serum immunoglobulins has some limitations. Serum IgG, the most frequently measured immunoglobulin, does not necessarily reflect acute or recent infection. In newborns, serum IgA has been useful as IgG levels can be affected by maternal transfer of immunoglobulin. However, it has recently been proposed that helper T-lymphocyte activity may be a more useful measure of the exposure of infants to rotavirus (Offit et al, 1992).

Considerable uncertainty exists regarding the relation of serum immunoglobulins to intestinal immunity. Circulating IgA levels have been suggested as a reflection of the immune status of the intestine (Davidson et al, 1983). The serum IgM response can be detected as early as 5 or 6 days after the onset of illness (Kapikian and Chanock, 1990). Copro-IgA has been proposed as an indicator of infection (Coulson et al, 1992), but copro-antibodies are not easily measured. Indeed, the correlation of antibodies in serum or intestinal fluids to disease protection is not easily established. Efforts have been made to refine the antigenic specificities of the antigens used in assays to help correlate responses to protective states. For instance, an individual rotavirus neutralizing protein (VP4) has been expressed in baculovirus expression systems and the specific responses to this potentially 'protective' antigen have been evaluated in mice (mucosal immunity) and vaccinated children (serum) (Shaw et al, 1991; Padilla-Noriega et al, 1992). Considerably more information is required to fully understand the relationship of antibody responses and disease protection.

Treatment

Treatment of rotavirus infection is supportive with oral rehydration with glucose–electrolyte solutions being the mainstay. If severe dehydration or electrolyte abnormalities exist or if the patient is unable to tolerate liquids by

mouth, intravenous supplementation is required (see Chapter 10). After several days of rehydration and maintenance, gradual return to normal diet is advocated (Tolia and Dubois, 1985; Christensen, 1989). Modification of the carbohydrate content of the diet may be required temporarily, depending on the presence and severity of intolerance to dietary disaccharides and/or monosaccharides (Gracey and Anderson, 1993). Better recipes for rehydration solutions to enhance efficacy and promote mucosal regeneration are always being sought, with recent attention being given to the substitution of glutamine for glucose (Rhoads et al, 1991; see also Chapter 10).

Prevention and vaccination

Given the worldwide morbidity, mortality and economic impact of this disease, prevention is of great importance. The virus is resistant to a wide range of commonly used disinfectants, but there are several which are commonly available that may be effective in preventing and controlling outbreaks (Table 2).

Several issues have been important in the shaping of current vaccine strategies. These include host characteristics such as the perceived importance of local immunity and the benefits of live attenuated rotaviruses as vaccines; the completeness and breadth of protection arising from a single infection or vaccination; and the ability of very young infants to mount immune responses to vaccines. The development of a better epidemiological database and improved (but not yet completely satisfactory) means of evaluating host responses to infection and vaccination and the implications of these findings regarding disease protection have also been important in devising strategies. Progress in these areas has been comprehensively reviewed by Kapikian et al (1989).

Extensive experience with vaccine field trials has been accumulated in developed country and underdeveloped country settings. The principal difficulty with live virus vaccines has been the serotype specificity of the responses (Flores et al, 1987; Bernstein et al, 1990; Rennels et al, 1990). Initial studies with bovine rotaviruses which were serologically distinct from human strains (but shared non-protective group antigens) were promising

Table 2. Some commonly available disinfectants that help prevent the spread of rotavirus (Springthorpe et al, 1986).

Hydrochloric acid
Peracetic acid
Isopropyl alcohol
Chlorhexidine gluconate
Glutaraldehyde
Chloramine-T
Povidone–iodine complex
Sodium-*O*-benzyl-*p*-chlorophonate
Quaternary ammonium compounds

(Vesikari et al, 1984) but subsequently numerous studies have shown very limited protective capability (Kapikian and Chanock, 1990). An attempt to make the live attenuated vaccine more effective used a simian rotavirus (rhesus rotavirus MMU 18006) sharing serotype 3 characteristics with human rotaviruses. This vaccine was immunogenic and sometimes provided substantial protection against serotype 3 infection (Perez et al, 1990).

Unfortunately, with four major human rotavirus serotypes, protection against only one is not sufficient. Rotaviruses naturally reassort genes during mixed infection, a property which has been exploited to prepare a quadrivalent vaccine made up of four reassortant viruses, each with a VP7 antigen representing a different serotype (designated 1, 2, 3 and 4) (Flores et al, 1990; Shaw, 1992). These reassortant vaccines are attenuated in humans, although they do infect and lead to immune responses in young children (Flores et al, 1987; Blacklow and Greenberg, 1991). Field trials with this vaccine are still in progress but preliminary reports have indicated that achieving a balanced response against all four serotypes remains a difficult task.

Viral-neutralizing proteins produced by recombinant baculovirus systems have been reported to be immunogenic in animals but a method of antigen delivery that could induce protective immunity at the mucosal level has not yet been demonstrated. Simultaneous expression of several rotavirus proteins in recombinant baculovirus systems has been shown to result in spontaneous assembly of virus-like particles, which have been proposed as vehicles for the delivery of customized rotavirus antigens to the intestinal mucosa or the systemic immune systems (Sabara et al, 1991). This and many other novel methods of antigen presentation are being explored.

NORWALK VIRUSES, NORWALK-LIKE VIRUSES AND OTHER CALICIVIRUSES

Introduction

While rotavirus predominantly affects infants and young children, Norwalk and Norwalk-like viruses primarily affect older children and adults. The Norwalk virus was first identified by Kapikian from the examination of stools with electron microscopy during an outbreak of illness in Norwalk, Ohio (Kapikian et al, 1972). Other Norwalk-like viruses have been discovered since then which share similarities of morphology, epidemiology and clinical disease. This group of viruses includes the Hawaii, Snow Mountain, Montgomery County and Otofuke viruses among many others. Other caliciviruses cause gastroenteritis in infants and young children which may be clinically and epidemiologically indistinguishable from rotavirus infection in children.

Serological biochemical, and, most recently, molecular biological data have made it clear that the Norwalk viruses, Norwalk-like viruses and other small, round, structured viruses are probably members of the Caliciviridae family (Greenberg and Matsui, 1992). Serologically, some patients infected

with caliciviruses mount an immune response to Norwalk virus (Cubitt et al, 1987).

Neither the Norwalk viruses, Norwalk-like viruses nor other caliciviruses can be cultured in vitro. In fact, Norwalk viruses can be propagated only in human volunteers and chimpanzees, which has restricted our knowledge about them (Christensen, 1989). This handicap may be partially overcome by molecular cloning and expression of the Norwalk genome.

Molecular biology and classification

Norwalk viruses are probably members of the *Caliciviridae*, because of their protein and nucleic acid properties (Greenberg and Matsui, 1992). Besides Norwalk and Norwalk-like viruses, there are at least three different strains of caliciviruses and others probably exist. They are RNA viruses approximately 30 nm in diameter, and have 32 cup-shaped depressions on the surface of the virion (Christensen, 1989; Blacklow and Greenberg, 1991). Norwalk viruses contain a single structural protein with a weight of 59 kDa.

A recent breakthrough in this field has been in the cloning and characterization of the genome of the Norwalk virus which contains a single-stranded RNA of positive sense with a size of at least 7.5 kb (Jiang et al, 1990). An antigenic region of a Norwalk gene product has been identified which may facilitate diagnosis in future (Matsui et al, 1991).

Epidemiology

Norwalk virus has been associated with outbreaks of diarrhoea in families, schools and colleges, nursing homes, communities, camps and cruise ships. Recently, it caused infections in troops during Operation Desert Shield (Hyams et al, 1991). Norwalk and similar viruses have accounted for more than 30% of the outbreaks of viral gastroenteritis in several studies (Greenberg et al, 1979b, 1981; Christensen, 1989; Blacklow and Greenberg, 1991). Other caliciviruses may account for 3% of episodes of diarrhoea in children attending daycare centres in the USA (Matson et al, 1989). Sources of infection have included municipal water systems, swimming pools, contaminated seafood and person-to-person transmission.

In the USA approximately 5% of subjects prior to the age of 12 will be seropositive for immunoglobulin to the Norwalk virus. By the age of 35 years, 45% of subjects will be seropositive, and by 65 years, 60% will be seropositive (Blacklow et al, 1979). Serological evidence of exposure occurs earlier in less developed nations (Greenberg et al, 1979a).

The disease is spread by the faecal–oral route and has an incubation period of 12–48 h (Blacklow and Greenberg, 1991). Even after recovery from the illness, infectious viral particles can be excreted in the faeces (White et al, 1986; Reid et al, 1988). Infants and young children are mainly affected by the other caliciviruses, which may account for occasional epidemics in institutions and schools. Although there is a peak incidence in the winter months, infections with this virus can occur throughout the year. The incubation period is from 1 to 3 days, and transmission probably occurs

through the faecal–oral route (Christensen, 1989; Blacklow and Greenberg, 1991).

Clinical features

The symptoms of calicivirus infection are generally mild and of short duration but can be as severe as those of rotavirus infection (Cubitt, 1990). Diarrhoea and vomiting are the most common features, while many patients will also have respiratory symptoms and fever. The diarrhoea tends to not contain blood, mucus or leukocytes. Gastric emptying is markedly delayed in this disease, contributing to nausea and vomiting typically associated with Norwalk virus infection (Meeroff et al, 1980). Other associated features which do not distinguish Norwalk infection from other non-bacterial agents or from Norwalk-like viruses include diarrhoea, fever, myalgia and headache, which typically last 24–48 h (Blacklow and Greenberg, 1991).

The mucosal lesion occurs in the proximal small intestine. Mucosal biopsies from infected individuals have revealed partial villous flattening and broadening, disorganization of the epithelial lining cells, and infiltration of the lamina propria by mononuclear cells (Agus et al, 1973; Schreiber et al, 1973). These changes return to normal within 2 weeks. Jejunal brush border enzyme activity, including alkaline phosphatase, sucrase, trehalase and others, has been shown to be decreased, and malabsorption of D-xylose, lactose and fat occurs transiently during infection (Agus et al, 1973; Blacklow and Greenberg, 1991).

Immunology

Immunoglobulin directed against calicivirus seems to develop in most people during early childhood and it is probably protective (Nakata et al, 1985, 1988). Homologous immunity to the Norwalk virus occurs, at least in the short term, while heterologous immunity occurs in some situations. Volunteers infected with the Norwalk virus were protected from illness when challenged with the same virus 14 weeks later (Blacklow et al, 1972; Wyatt et al, 1974), but in the study by Wyatt et al, half the patients initially given the Norwalk agent became ill when challenged 7–15 weeks later with the Hawaii agent, indicating serological diversity (Wyatt et al, 1974). Those investigators also showed that challenge with the Norwalk virus was protective against future challenge with the Montgomery County agent, though the reverse was not always true.

The immunity associated with infection by the Norwalk agent is short-lived (Johnson et al, 1990). Within 42 months after initial exposure, rechallenge led to recurrent illness. This applied even if immunoglobulin titres were elevated prior to rechallenge, suggesting that antibodies alone may not be sufficient for protection (Parrino et al, 1977). When the same patients were subsequently re-exposed shortly after the second challenge, four of five remained well. One group of patients in this study retained long-term immunity despite low levels of antibody to the Norwalk virus, suggesting that factors other than circulating immunoglobulins are respon-

sible for protection. It is possible that there exists a receptor for the Norwalk virus on enterocytes, the absence of which allows individuals to remain relatively resistant to infection (Parrino et al, 1977; Christensen, 1989). Perhaps local antibodies at the mucosal surface are not well reflected by measurements of serum antibodies. Further investigation will be vital for the development of a rational vaccine strategy.

Diagnosis

Failure to propagate the Norwalk and Norwalk-like viruses in tissue culture has made it difficult to obtain the necessary reagents for epidemiological studies and for clinical diagnosis. Electron microscopic identification of the virus in the stool was initially used diagnostically. The recent development of immunoassays has facilitated diagnosis, with reagents capable of detecting all subtypes due to the presence of group-specific antigens (Cubitt et al, 1987; Blacklow and Greenberg, 1991).

Circulating titres of Norwalk-specific immunoglobulins A and M peak within 2 weeks after the onset of symptoms (Erdman et al, 1989). The ELISA for stool antigen and serum IgM specific to Norwalk virus are markers of recent infection, but they are presently available mainly in research laboratories (Herrmann et al, 1985; Erdman et al, 1989; Blacklow and Greenberg, 1991). One group of workers has found that antigen excreted in the stool was more specific than serology in detecting infection (Fleissner et al, 1989).

ENTERIC ADENOVIRUS

Introduction

Enteric adenoviruses are specific serotypes (usually types 40 and 41) of adenovirus which predominantly lead to symptoms of gastroenteritis (Blacklow and Greenberg, 1991). Serotype 31 may produce a clinical syndrome indistinguishable from that caused by serotypes 40 and 41, although it is one of the non-fastidious serotypes (Krajden et al, 1990).

Classification and molecular biology

Adenoviruses are 70–75 nm, non-enveloped, icosahedral, double-stranded DNA viruses made up of 252 capsomeres. The outer capsid is made up of the 240-nonvertex hexon capsomeres and the 12-penton base capsomeres. From the base of each penton protrudes a fibre vertex projection, all surrounding the double-stranded DNA core (Christensen, 1989). The different serotypes are determined by antigens located on the hexon, while subgroup specificities are determined by the penton capsomeres, and subgroup and type specificities are determined by the fibres (Christensen, 1989). Serotypes 40 and 41 are not easily propagated in cell culture.

Several different classifications have been proposed based on, for example, the virion polypeptide. Serotypes 1 to 39 fall into subgroups A to D, except for serotype 4, whose pattern does not fit and which has thus been placed into subgroup E. The fastidious serotypes do not fit subgroups A to E, and are placed into separate subgroups (Wadell, 1984). Using restriction endonuclease analysis, serotypes 40 and 41 appear to be different and thus are given separate subgroups, although they appear to have several cleavage sites in common (de Jong et al, 1983; Uhnoo et al, 1983; Takiff et al, 1984). However, they have biological similarities and are indistinguishable by haemagglutination inhibition (Kidd, 1984; Christensen, 1989). Consequently, some authors have recommended that the two be classified together.

Epidemiology

Enteric adenovirus leads to endemic disease of infants and young children, and may be the second most common cause of paediatric viral gastroenteritis after rotavirus. It primarily affects children under the age of 2 years and seems to have no seasonal predisposition (Uhnoo et al, 1984; Kim et al, 1990; Blacklow and Greenberg, 1991). The incubation period is longer than for the other gastroenteritis viruses, about 8–10 days, and the mode of transmission is generally person-to-person (faecal–oral) (Blacklow and Greenberg, 1991).

Clinical features

Enteric adenovirus infection leads to severe prolonged diarrhoea lasting up to 12 days, and can be associated with vomiting and fever (Uhnoo et al, 1984, 1990). Dehydration tends to be less severe than during rotavirus infection although fatalities have been reported (Whitelaw et al, 1977). Some controversy exists as to the association of enteric disease with respiratory symptoms. In one study, almost all patients with diarrhoea and adenovirus antigen in the stool had respiratory symptoms, including cough, wheezing or rhinorrhoea (Yolken et al, 1982a); others report only a rare occasion with respiratory symptoms (Uhnoo et al, 1986). The role of the fastidious adenovirus subtypes in the development of these symptoms is unclear, as some of the earlier studies used antibodies to adenovirus group antigens for diagnosis, and thus the causative agent may in fact have been non-fastidious organisms excreted in lower numbers in the stool (Christensen, 1989). This may have been associated with the respiratory symptoms, as simultaneous infection with different enteric and respiratory tract adenoviruses is known to occur.

Immunology

Little is known of the development of local and circulating immunoglobulins to serotypes 40 and 41 adenovirus. Once a patient is exposed to the agent, immunoglobulins probably provide long-term clinical immunity but the data

are limited (Blacklow and Greenberg, 1991). Roles for other aspects of immunity are presently speculative.

Diagnosis

Diagnostic techniques are not readily available except in specialized research laboratories. The use of Graham 293 cells has aided in the ability to propagate the virus in vitro, thus providing increased quantities of antigen (Christensen, 1989). Specific diagnosis was initially by electron microscopic examination of the stool for virus particles or extraction and analysis of DNA from the stool. Immunoassays using monoclonal antibodies specific for subtypes 40 and 41 have now facilitated diagnosis (Wood et al, 1989). Antigenic variation in surface epitopes may affect the sensitivity of the test, necessitating careful selection of a monoclonal antibody directed against a common antigen. One study showed that DNA restriction analysis was better than serum neutralization for identification of the enteric adenovirus serotypes in stool specimens (Brown, 1990).

The sensitivity of the ELISA seems to be less than that of both electron microscopy and virus isolation, although this may be related to the amount of virus particles present in stool samples (Martin and Kudesia, 1990). In addition, a latex agglutination test has recently been developed with good sensitivity (Sanekata et al, 1990). Both tests may yet prove to be very accurate and rapid methods in the diagnosis of adenovirus infection.

ASTROVIRUSES

Introduction

First discovered in 1975 by electron microscopy in the stools of infants, astroviruses account for approximately 5% of infantile gastroenteritis (Madeley and Cosgrove, 1975; Christensen, 1989). These viruses were named for the unique stellate configuration on the surface of many of the viral particles and five human serotypes have been identified (Greenberg and Matsui, 1992).

Epidemiology

Astrovirus is most commonly seen in infants and children up to the age of 7 years, and has also been associated with outbreaks of diarrhoea in nursing homes (Blacklow and Greenberg, 1991). The infection has an incubation period of 24–36 h and symptoms last for up to 4 days (Christensen, 1989). Astrovirus illness occurs throughout the year, although there is a peak incidence during the winter months, similar to rotavirus (Greenberg and Matsui, 1992).

In one study faeces from a large number of children with diarrhoea, as well as a control population, were screened with an enzyme immunoassay (EIA) for rotavirus, astrovirus and adenovirus. Astrovirus was second only

to rotavirus in terms of frequency of identification in the stool of those with diarrhoea (Herrmann et al, 1991).

Classification and molecular biology

The virion is 27–32 nm and is composed of single-stranded RNA of positive sense, with a size of 7.9 kb. The number of astrovirus structural proteins is unclear. The electron microscopic appearance is that of a five- or six-pointed star, with a distinct outer margin forming a rim (Greenberg and Matsui, 1992). There are five human serotypes which can each be cultured in vitro (Blacklow and Greenberg, 1991). These can be propagated in human kidney cells with trypsin-containing medium, and more recently in a continuous colonic carcinoma cell line (CaCo-2) (Lee and Kurtz, 1981; Willcocks et al, 1990). The Marin County agent (which caused an outbreak of diarrhoea in a convalescent hospital in California) is an astrovirus, although it had first been thought to be a Norwalk-like virus (Herrmann et al, 1987).

Clinical features

The incubation period of astrovirus infection is 3–4 days. The virion has been detected in duodenal biopsy specimens in the lower portion of the intestinal villi (crypts) as well as in the exposed surface epithelium (Phillips et al, 1982). Watery diarrhoea is the most prominent symptom and usually lasts from 1 to 4 days. Vomiting and fever each occur in approximately one-third of affected patients, while abdominal pain occurs in nearly half (Kurtz et al, 1977; Konno et al, 1982). Clinical illness lasts from several days to 1 week in most patients, although the spectrum varies considerably among individuals, with some experiencing diarrhoea for up to 2 weeks.

Immunology

Antibodies develop in more than two-thirds of children by the age of 4 years, but the protective capabilities are unknown (LeBaron et al, 1990a). Nevertheless, since outbreaks are relatively uncommon in the adult population, these immunoglobulins may be somewhat protective.

Diagnosis

Electron microscopic identification of the virus in the stool provided the only diagnostic modality in the past. Recently, an ELISA has been developed utilizing monoclonal antibodies which recognize all astrovirus serotypes in stool (Herrmann et al, 1990). In addition, two new astrovirus assays, a rapid biotin-avidin EIA and RNA probe hybridization have been developed (Moe et al, 1991).

Although the mainstay of diagnosis rests on identification of the virus, viral particles, or RNA in the stool, circulating immunoglobulins can also be measured. Both IgG and IgM have been detected by IEM, although all these techniques remain research and epidemiological tools (Christensen, 1989).

CORONAVIRUSES

The clear association of coronaviruses as causative agents in human gastroenteritis has not yet been established (Blacklow and Greenberg, 1991). Although several studies have correlated the presence of coronavirus-like particles (CVLPs) in the stool with the presence of gastroenteritis, others have not. In one study, the prevalence of CVLPs was greater in the control group (23.0%) than in those with diarrhoea (8.9%) (Gerna et al, 1985; Sitbon, 1985; Singh et al, 1989). Infections caused by coronavirus, not limited to gastrointestinal infections, tend to cluster in the drier autumn and winter months and are more common in the western USA; infections occur mainly in infants (Mortensen et al, 1985).

These viruses are 80–150 nm in diameter and are round or pleomorphic with club-shaped projections on the surface known as peplomers (Christensen, 1989). Most of these viruses are associated with respiratory infection.

The mean duration of illness is said to be 1 week, with diarrhoea occurring in nearly all patients. Vomiting and fever occur in approximately 50% and 60% respectively (Mortensen et al, 1985). The laboratory diagnosis of coronavirus depends on the identification of the virus in the appropriate body fluid, but this is not often pursued.

TREATMENT, PREVENTION AND VACCINATION

The treatment for enteric viral infections is supportive, with rehydration and electrolyte balance being the principal objectives. Except for rotavirus, there are no vaccines or vaccine candidates for gastroenteritis viruses, nor has passive immunization therapy been feasible except in immunocompromised hosts. Breast-feeding has not been clearly demonstrated to prevent rotavirus infections. The focus of Norwalk prevention efforts is to limit the spread of outbreaks once they occur. These measures include personal hygiene, avoidance of raw or undercooked shellfish, prohibiting food handlers with gastroenteritis to work, and adequate water and sewage treatment (Christensen, 1989). This group of viruses appears to be resistant to chlorine inactivation, thus making traditional decontamination procedures ineffective (Keswick et al, 1985). In addition, careful design of waste disposal systems and public water supplies may prevent outbreaks. Controlling faecal pollution of estuaries or restricting shellfish harvesting are important factors in limiting outbreaks, especially of Norwalk virus.

Specific drug treatments for viral gastroenteritis have not been devised. In a recent study the activity of halogeno-, cyano- and amidino-isoflavenes, isoflavans and flavans on the multiplication of human astroviruses was studied. All drugs caused a dose-dependent reduction of viral antigen synthesis as monitored by immunofluorescence, but the chloro derivatives were the most effective in this regard (Superti et al, 1990). The use of glycoproteins or glycolipids that compete with enterocytes for binding of rotavirus has been proposed as passive therapy or for transient prevention (Willoughby and Yolken, 1990; Willoughby et al, 1990).

Maintenance of personal and community hygiene is imperative for prevention. Many infections are asymptomatic and viral shedding can occur prior to the onset of symptoms (Herrmann et al, 1991; Cruz et al, 1992).

THE IMMUNOCOMPROMISED HOST

Cytomegalovirus (CMV)

Cytomegalovirus (CMV) has been identified in the gastrointestinal tract of nearly 8% of patients with HIV, and in 13% of patients with AIDS (Frances et al, 1989). This virus has caused severe or life-threatening disease in 2.2% of patients with AIDS (Jacobson and Mills, 1988; Smith et al, 1992). Its most common enteric manifestation is colitis, characterized by diarrhoea, haematochezia, abdominal pain and fever (Smith et al, 1992). Infection may occur in many areas of the gastrointestinal tract, including the oesophagus, stomach, small intestine, appendix, pancreas and biliary tree (Valerdiz-Casasola and Pardo-Mindan, 1991; Smith et al, 1992).

The diagnosis is usually made by histological detection of the replicating virion represented by viral cytoplasmic inclusions surrounded by inflammation (Smith et al, 1992). Some investigators consider that full colonoscopy with biopsies is necessary for accurate diagnosis because approximately 39% of patients with CMV colitis may only harbour the organism within their caecum (Dieterich and Rahmin, 1991).

The treatment of enteric CMV infection is Ganciclovir, which has been shown to reduce the signs and symptoms of infection in some patients, although the subsequent optimal maintenance regimen has not yet been determined (Chachoua et al, 1987). In bone-marrow transplant recipients Ganciclovir has been shown to suppress CMV replication during a 2-week treatment course, but there was no associated clinical or histological improvement noted when compared with supportive care (Reed et al, 1990).

Adenovirus

In general, it is relatively unusual for common gastroenteritis viruses to be clinically important in patients with HIV-associated disease. Nevertheless, adenovirus has recently been identified in the setting of chronic inflammatory infiltrate and necrotic epithelial cells in these patients (Janoff et al, 1991). Adenovirus and rotavirus had previously been identified in approximately 50% of homosexual AIDS patients, but the frequencies were the same regardless of presence or absence of diarrhoea and seemed incidentally related to the level of immunosuppression rather than the presence of disease (Cunningham et al, 1988).

Human immunodeficiency virus (HIV)

Intestinal abnormalities have been noted in the absence of any identifiable pathogen in patients with AIDS. Villous atrophy and crypt hyperplasia were

correlated with enteric infection in one study, whereas subjects without identifiable infection had normal crypt depth, slightly reduced villous surface area, and decreased numbers of mitotic figures per crypt. The latter group of patients was associated with deficiency or absence of jejunal brush border lactase activity in most of the patients (Ullrich et al, 1989). In many of these patients immunohistological tests for the presence of p24 antigen (Ullrich et al, 1989) and in situ hybridization have revealed HIV antigen in the intestinal epithelial cells or mononuclear cells (Heise et al, 1991). It has, therefore, been suggested that the HIV infection itself leads to an 'enteropathy' in the absence of any other identifiable pathogen. But, given the low percentage of patients with intestinal HIV infection, the small number of cells actually infected with the virus, and the absence of associated enteric inflammation, the aetiology of this 'AIDS enteropathy' remains unclear (Smith et al, 1992).

SUMMARY

Increased knowledge has been gained into the aetiology and pathogenesis of viral gastroenteritis during the past two decades. There are now thought to be four major subclassifications of gastroenteritis-causing viruses; these include rotavirus, enteric adenovirus, calicivirus, including Norwalk and Norwalk-like viruses, and astrovirus. The association of these agents with gastroenteritis has been made by their electron microscopic detection in stool and intestinal biopsy specimens from affected patients, the inability to detect the viruses after recovery from disease, and the subsequent development of immunoglobulin responses after infection; in some instances disease transmission was achieved in human volunteers. The association of these viral agents with gastroenteritis has facilitated the study of classification, epidemiology, immunity, diagnostic tests, methods of treatment and, most importantly, disease prevention strategies such as vaccine development for rotavirus. This chapter highlights the major features of these agents, with special attention being given to the pertinent molecular biology as well as current and future prospects for vaccination. Enteric viral infections of the gastrointestinal tract in patients with AIDS are also discussed.

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