CHAPTER 3

Molecular Virology of Enteric Viruses (with Emphasis on Caliciviruses)

Javier Buesa and Jesús Rodríguez-Díaz

1.0. CALICIVIRUSES

Human caliciviruses are members of the family Caliciviridae and are responsible for a majority of the outbreaks of acute nonbacterial gastroenteritis. In fact, they now are considered a common cause of sporadic cases of diarrhea in the community (Glass et al., 2000b; Koopmans et al., 2002; Lopman et al., 2002; Hutson et al., 2004). These viruses were implicated in as many as 95% of the reported viral gastroenteritis outbreaks examined over a 4.5-year period in the United States (Fankhauser et al., 2002), and similar high incidence rates have been found in other studies (Maguire et al., 1999; Glass et al., 2000b; Koopmans et al., 2000; Lopman et al., 2003). Common features of the Caliciviridae include the presence of a single major structural protein from which the capsid is constructed and 32 cup-shaped depressions on the surface of the virion arranged in an icosahedral symmetry. The name of the family was derived from the Latin word *calix*, which means cup or goblet, and refers to the surface hollows (Madeley, 1979). Another major feature of Caliciviridae is the absence of a methylated cap at the 5′ end of the viral RNA. Instead, a small protein (VPg) of ~10×10^3 to 12×10^3 kDa is covalently linked to the viral RNA and is considered essential for the infectivity of the RNA (Black et al., 1978; Ando et al., 2000).

Norwalk virus was the first human enteric calicivirus to be discovered, this after a gastrointestinal outbreak affecting both children and adults in an elementary school in Norwalk, Ohio (Kapikian et al., 1972). Over the next several years, other agents of epidemic viral gastroenteritis were described including Hawaii virus, Montgomery County agent, Snow Mountain virus, Southampton virus, Toronto virus, and so forth. An interim scheme to classify these viruses on a morphological and physicochemical basis was proposed by Caul and Appleton (1982). Some of these viruses were antigenically related to Norwalk virus by IEM (immune electron microscopy) and cross-challenge studies, whereas others, like the Sapporo virus (Chiba et al., 1979), were found to be antigenically distinct (Nakata et al., 1996).

Human caliciviruses have been classified into two distinct genera namely, Norovirus (previously called "Norwalk-like viruses" (NLVs) or "small round structured viruses") and Sapovirus (formerly "Sapporo-like viruses," or SLVs) (Green et al., 2000b; Mayo, 2002). The noroviruses and sapoviruses form distinct phylogenetic clades within Caliciviridae (Berke et al., 1997), and certain features of their viral genome organization distinguish them from

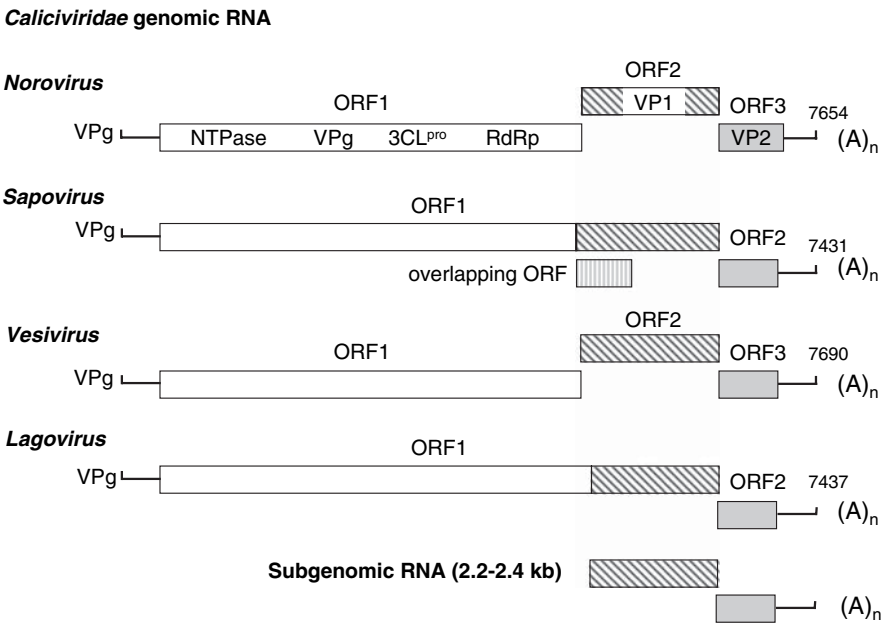

Figure. 3.1 Genome organization of the four different genera of Caliciviridae. The genome of Norovirus and Vesivirus has three open reading frames (ORFs) that encode the nonstructural proteins, the major capsid protein (VP1), and a minor structural basic protein (VP2). The genera Sapovirus and Lagovirus encode the capsid protein contiguous with the large nonstructural polyprotein (ORF1). An additional small overlapping ORF in a +1 frameshift has been described in certain strains of sapoviruses. A subgenomic RNA that covers the entire 3′ end of the genome, from the capsid gene to the 3′ end, has been detected in calicivirus-infected cells.

each other and from other genera of the family (Fig. 3.1). These viruses also differ in their epidemiology and host range. For example, noroviruses infect all age groups and are usually responsible for outbreaks of acute gastroenteritis frequently associated with contaminated food or water. Sapoviruses, on the other hand, are associated with sporadic cases of acute gastroenteritis and mainly infect infants and young children (Green et al., 2001), although outbreaks in adults have also been described (Noel et al., 1997).

Enteric caliciviruses of animals, associated with gastroenteritis in pigs, calves, chickens, mink, dogs, and cats, have also been described (Guo et al., 1999b; Liu et al., 1999a; van der Poel et al., 2000; Guo and Saif, 2003). The Caliciviridae has two additional genera, Lagovirus and Vesivirus, each of which includes caliciviruses that infect animals such as the rabbit hemorrhagic disease virus (RHDV) and the vesicular exanthema of swine virus (VESV), respectively. The proposed nomenclature for calicivirus strains is host species from which the virus was isolated/virus genus/virus name/strain

designation/year of isolation/country of origin. The type strain for Norovirus and Vesivirus are Norwalk virus (Hu/NV/Norwalk/8fIIa/1968/US) and VESV (Sw/VV/VESV/A48/1948/US), respectively (Atmar and Estes, 2001).

On examination by negative contrast electron microscopy (EM), caliciviruses show characteristic cup-shaped structures on their surfaces. These cuplike depressions are more prominent in sapoviruses, leading to the characteristic six-pointed "star of David" appearance, especially when viewed along the major two-, three-, and fivefold axes of symmetry. The noroviruses have a polyadenylated, positive-sense, single-stranded RNA genome with three major open reading frames (ORFs) (Jiang et al., 1993b; Lambden et al., 1993). The virions have a buoyant density of 1.33 to 1.41 g/cm^3 in cesium chloride ($CsCl_2$) (Caul and Appleton, 1982; Madore et al., 1986) and usually lack the distinctive calicivirus cuplike morphology when viewed by EM. The sapoviruses have a polyadenylated, positive-sense, single-stranded RNA genome with two (or three) major ORFs (Liu et al., 1995), have a buoyant density of 1.37 to 1.38 g/cm^3 in CsCl (Terashima et al., 1983), and often possess distinctive calicivirus cup-like morphology (Madeley, 1979).

The molecular era of norovirus research started with the successful cloning of the genomes of Norwalk and Southampton viruses obtained from stool samples (Jiang et al., 1990; Lambden et al., 1993). Despite numerous attempts, human calicivirus infections have not been induced in experimental animals nor have these viruses been propagated successfully in cultured cells, thus hampering many aspects of their research (Duizer et al., 2004). Unlike human caliciviruses, some animal caliciviruses, including primate calicivirus (Smith et al., 1983), feline calicivirus (FCV) (Love and Sabine, 1975), and San Miguel sea lion virus (SMSV) (Smith et al., 1973), have been successfully propagated in cell cultures. These viruses have provided a direct approach for the study of virus infections, genome transcription, viral protein translation, and virus replication (Green et al., 2002). In addition, information gained by the study of caliciviruses that grow efficiently in cell cultures, such as FCV and VESV, or that have an animal model and a limited cell culture system (such as RHDV), has been important in the identification of features that are likely to be shared among members of the Caliciviridae (Marin et al., 2000; Morales et al., 2004).

1.1. Structure, Composition, and Molecular Biology

The Norwalk virus capsid is composed of a single major structural protein, known as VP1, and a few copies of a second small basic structural protein named VP2 (Prasad et al., 1999; Glass et al., 2000a; Green et al., 2001). Cloning and expression of norovirus proteins VP1 and VP2 in insect cells using the baculovirus expression system resulted in the self-assembly of the viral capsid and the production of recombinant virus-like particles (rVLPs) that were antigenically and structurally similar to the native virions (Jiang et al., 1992; Green et al., 1997; Hale et al., 1999; Kobayashi et al., 2000).

The three-dimensional structure of Norwalk rVLPs was first determined by cryo-electron microscopy and computer-image processing to a resolution

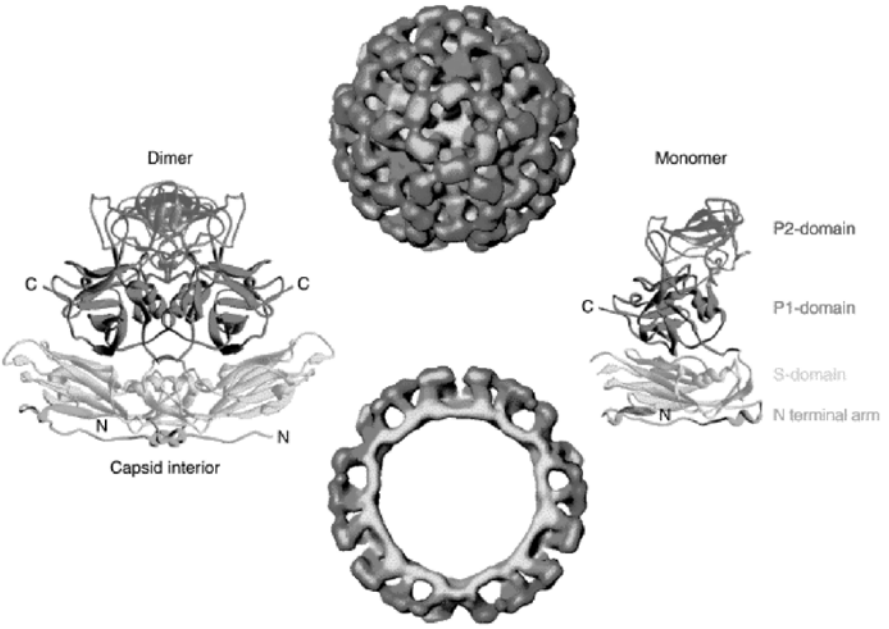

Figure. 3.2 The structure of Norwalk virus-like particles (NV VLPs) has been solved by cryo-electron microscopic reconstruction to 22 Å (top, surface representation; bottom, cross section) and by x-ray crystallography to 3.4 Å. The NV VLPs have 90 dimers of capsid protein (left, ribbon diagram) assembled in T = 3 icosahedral symmetry. Each monomeric capsid protein (right, ribbon diagram) is divided into an N-terminal arm region (green) facing the interior of the VLP, a shell domain (S domain, yellow) that forms the continuous surface of the VLP, and a protruding domain (P domain) that emanates from the S domain surface. The P domain is further divided into subdomains, P1 and P2 (red and blue, respectively) with the P2 subdomain at the most distal surface of the VLPs. (Reproduced with permission from Prasad et al., 1999, and Bertolotti-Ciarlet et al., 2002; see color insert.)

of 22 Å. This analysis showed that the particles (38 nm in diameter by this technique) have a distinct architecture and exhibit T = 3 icosahedral symmetry (Fig. 3.2). The capsid contains 180 copies of the capsid protein assembled into 90 dimers with an arch-like structure. The arches are arranged in such a way that there are large hollows at the icosahedral five- and threefold positions, and these hollows are seen as cuplike structures on the viral surface (Prasad et al., 1994, 1996a, 1999). To form a T = 3 icosahedral structure, the capsid protein has to adapt to three quasi-equivalent positions, and the subunits located at these positions are conventionally referred to as A, B, and C. The only high-resolution (3.4 Å) structure is that of the Norwalk virus capsid determined by x-ray crystallography (Prasad et al., 1999).

Each subunit or monomeric capsid protein folds into an N-terminal region facing the inside of the capsid, a shell (S) domain that forms the

continuous surface of the VLP, and a protruding (P) domain that forms the protrusions (Fig. 3.2). A flexible hinge of eight amino acids links the S and P domains. The P domain is located at the exterior of the capsid and is likely to contain determinants of genotype specificity. The NH_2-terminal (N) arm, located within the S domain, consists of residues 10 to 49 and faces the interior of the capsid. The part of the S domain that forms a β-barrel consists of amino acids 50 to 225. The entire S domain (amino acids 1 to 225) corresponds to the N-terminal region of the capsid protein that is relatively conserved among noroviruses in sequence comparisons.

Amino acid residues 226 to 530 form the P domain, which corresponds to the C-terminal half of the capsid protein and forms the arch-like structures extending from the shell. The S domain is required for the assembly of the capsid and participates in multiple intermolecular interactions of dimers, trimers, and pentamers, whereas the P domain is mainly involved in dimeric interactions (Prasad et al., 1999). The P domain is considered to have subdomains, P1 formed by amino acids 226 to 278 and 406 to 530, and P2 encompassing amino acids 279 to 405. The P2 subdomain is the most variable region of the capsid protein among noroviruses (Hardy et al., 1996) and is believed to play an important role in immune recognition and receptor interaction. The isolated P domain forms dimers and binds to histo-blood group antigen receptors, not requiring the formation of VLPs (Tan et al., 2004). In addition, a binding pocket in the P domain is responsible for viral receptor binding, and formation of this pocket involves only intramolecular interactions (Tan et al., 2003). Prasad et al. (1999) have demonstrated that, although the S domain has a canonical eight-stranded β-barrel structure, the P2 subdomain in the P domain has a fold similar to that observed in domain 2 of the elongation factor Tu (EF-Tu), a structure never before seen in a viral capsid protein. Moreover, the fold of the P1 subdomain is unlike any other polypeptide observed so far (Bertolotti-Ciarlet et al., 2003).

A comparison of the capsid proteins from various caliciviruses reveals significant variations both in their sequences and in their sizes. In general, the capsid proteins of human caliciviruses are smaller than those of animal caliciviruses (Chen et al., 2004). The Norwalk virus recombinant capsid protein can also self-assemble into smaller VLPs (23 nm) with suspected T = 1 symmetry that is thought to be composed of 60 copies of the capsid protein (White et al., 1997).

It has been postulated that the N-terminal region of the capsid protein or the genomic RNA acts as a switching region that controls variations in the conformation of the coat protein of T = 3 viruses (Rossmann and Johnson, 1989). Norovirus particles are different from other T = 3 viruses because recombinant capsid protein readily forms rVLPs without RNA (Prasad et al., 1999). It has been suggested that the determinants for the T = 3 capsid assembly for Norwalk virus may lie outside of the N-terminus and that the interaction between subunits B and C is not mandatory for the formation of the capsid (Bertolotti-Ciarlet et al., 2002).

Caliciviruses have a linear, single-stranded, positive-sense RNA genome of 7.5–7.7 kb (Green et al., 2001) (see Fig. 3.1). The RNA genome of Norwalk

virus, the prototype strain for the genus Norovirus, is 7,654 nucleotides in length and is polyadenylated at the 3′ end (Jiang et al., 1993b). The lack of a cap structure typical of eukaryotic mRNA and the absence of an internal ribosomal entry site suggest that the VPg protein may play a role in the initiation of protein synthesis on calicivirus RNA through unique protein-protein interactions with the cellular translation machinery (Daughenbaugh et al., 2003). Removal of VPg from calicivirus RNA by proteinase K digestion results in loss of infectivity and dramatically reduces translation of FCV RNA *in vitro* (Herbert et al., 1997). The genome of calicivirus is organized into two or three major ORFs. The nonstructural proteins encoded in the calicivirus ORF1 were predicted on the basis of their sequence similarities with picornavirus nonstructural proteins (Neill, 1990). Amino acid sequence motifs in common with the poliovirus 2C NTPase, 3C protease, and 3D RNA-dependent RNA polymerase (RdRp) were readily identified and provided templates for further characterization of the calicivirus nonstructural proteins. Proteolytic mapping and enzymatic studies of *in vitro*–translated polyprotein or recombinant protein expression have confirmed the presence of an NTPase (p41), a 3C-like protease ($3CL^{pro}$), an RdRp, and the location in the polyprotein of the genome-linked protein VPg (Liu et al., 1996; Dunham et al., 1998; Pfister and Wimmer, 2001). The proposed six nonstructural proteins encoded in the norovirus ORF1 defined so far proceed N to C terminus, p48-NTPase-p22-VPg-$3CL^{pro}$-RdRp (Ettayebi and Hardy, 2003). It has been recently reported that the 3C-like proteinase ($3CL^{pro}$) inhibits host cell translation by cleavage of poly(A)-binding protein (PABP), a key protein involved in the translation of polyadenylated mRNAs (Kuyumcu-Martinez et al., 2004).

In the genera Norovirus and Vesivirus, the capsid structural protein VP1 is encoded in a separate ORF (ORF2), whereas viruses in the genera Sapovirus and Lagovirus encode the capsid protein contiguous with the large nonstructural polyprotein (ORF1) (see Fig. 3.1). Viruses in the latter two genera have two major ORFs (ORF1 and ORF2). In the genus Sapovirus, the ORF1/2 junction consists of a one- or four-nucleotide overlap between the stop codon of ORF1 and the first AUG codon of ORF2. A third ORF has been described in certain strains that overlaps the capsid protein gene in a +1 frameshift, which is not found in the Norovirus genome (Liu et al., 1995; Guo et al., 1999b; Clarke and Lambden, 2000). The presence of a conserved translation initiation motif GCAAUGG at the 5′ end of this overlapping ORF suggests that a biologically active protein may be encoded in this ORF (Schuffenecker et al., 2001). Viruses in the genera Norovirus and Vesivirus have three major ORFs (ORF1, ORF2, and ORF3). In noroviruses, the first and third ORFs are in the same reading frame and Norwalk virus ORF3 encodes a 212-amino-acid minor structural protein of the virion (Glass et al., 2000a). All calicivirus genomes begin with a 5′-end terminal GU. This 5′ end sequence is repeated internally in the genome and most likely corresponds to the beginning of a subgenomic-sized RNA transcript (2.2–2.4 kb) that is coterminal with the 3′ end of the genome and that has been observed in FCV-

and RHDV-infected cells as well as packaged into virions (Herbert et al., 1996). A comparison of the 5′-end sequences of representative viruses within each of the four genera and the corresponding repeated internal sequences suggests that this is a characteristic feature of caliciviruses. The synthesis of a subgenomic RNA in calicivirus-infected cells is a major difference between the replication strategy of caliciviruses and picornaviruses, although several of the replicative enzymes share distant homology (Green et al., 2001). It is not clear whether caliciviruses have a picornavirus-like internal ribosomal entry site (IRES) for the initiation of translation but is considered unlikely because translation of the FCV capsid precursor protein begins at the first AUG of the subgenomic RNA (Sosnovtsev and Green, 2003).

1.2. Molecular Diversity of Caliciviruses

Early studies demostrating the great variability of noroviruses soon led to the belief that it was important to distinguish between strains to better understand their epidemiology. Because no antigenic analysis of norovirus strains was available due to the lack of immune reagents, genome characterization by sequence analysis was used to provide an interim system of genotyping (Koopmans et al., 2003). As the genotypes ideally would correlate with serotypes, the sequence of the major structural protein gene was used as the basis for phylogenetic analyses (Ando et al., 2000; Koopmans et al., 2001). To determine which areas of the genome should be analyzed, sequenced regions in the RNA-dependent RNA polymerase, the capsid gene, and part of the 3′ ORF were compiled and analyzed (Green et al., 2000a; Vinje et al., 2000). By a similar approach, Ando et al. (2000) proposed a numerical system for genotypes based on phylogenetic grouping according to genetic relatedness in the major capsid protein.

Noroviruses were classified into two genetic groups, GI and GII, on the basis of sequence homologies across highly conserved regions of the genome, such as the RNA-dependent RNA polymerase and the capsid gene, although subdivision into five genetic groups or genogroups has been proposed recently (Karst et al., 2003) (Table 3.1). Molecular characterization of bovine enteric caliciviruses suggests that they be included into a proposed GIII group, which so far contains only viruses found in cattle (Ando et al., 2000; Oliver et al., 2003; Smiley et al., 2003). The porcine noroviruses cluster within GII (Sugieda and Nakajima, 2002). Phylogenetic analysis places at least two human noroviruses within a proposed genogroup IV: strains Alphatron (GenBank accession no. AF195847) and Ft. Lauderdale (GenBank accession no. AF414426) (Fankhauser et al., 2002).

The recently described murine norovirus has been included into a proposed GV group, the members of which are closer to GII than those of GI by sequence alignment (Karst et al., 2003). In the major capsid protein VP1, human norovirus strains within the same genogroup share at least 60% amino acid sequence identity, whereas most GI strains share less than 50% amino acid identity with GII strains (Green et al., 2001; Koopmans et al., 2003). Within genogroups, noroviruses can be further divided into at least 22

Table 3.1 Current genogroups and genotypes of noroviruses [adapted from Green et al. (2001) and Koopmans et al. (2003)]

Genogroup	*Genotype*	*Prototype strain*	*GenBank accession number (capsid gene)*	*Other strains*
I	1	Norwalk/1968/UK	M87661	KY/89/JP
	2	Southampton/1991/UK	L07418	White Rose, Crawley
	3	Desert Shield/395/1990/SA	U04469	Birmingham 291
	4	Chiba 407/1987/JP	D38547	Thistle Hall; Valetta, Malta
	5	Musgrove/1989/UK	AJ277614	Butlins
	6	Hesse 3/1997/GE	AF093797	Sindlesham, Mikkeli, Lord Harris
	7	Winchester/1994/UK	AJ277609	Lwymontley
II	1	Hawaii/1971/US	U07611	Wortley, Girlington
	2	Melksham/1994/UK	X81879	Snow Mountain
	3	Toronto 24/1991/CA	U02030	Mexico, Auckland, Rotterdam
	4	Bristol/1993/UK	X76716	Lordsdale, Camberwell, Grimsby
	5	Hillingdon/1990/UK	AJ277607	White river
	6	Seacroft/1990/UK	AJ277620	
	7	Leeds/1990/UK	AJ277608	Gwynedd, Venlo, Creche
	8	Amsterdam/1998/NL	AF195848	
III		Jena	AJ011099	Bovine strains
IV	1	Alphatron/1998/NL	AF195847	Ft. Lauderdale
V		MNV-1	AY228235	Murine norovirus 1

genetic clusters of genotypes based on genetic homology in the complete ORF2 sequence (Koopmans et al., 2000; Green et al., 2001; Vinje et al., 2004). Within these broad groupings there are many different genetic variants of noroviruses circulating in the community (Jiang et al., 1996; Green et al., 2000a; Buesa et al., 2002; Lopman et al., 2003, 2004). Picornavirus serotypes generally have >85% amino acid identity across the VP1 gene, which is in the range of the cutoff for calicivirus genotypes (>80% amino acid identity) (Oberste et al., 1999).

Serologically, the norovirus genotypes have been classified into different antigenic groups on the basis of solid-phase immuneelectron microscopy (SPIEM) studies (Vinje et al., 2000) or by enzyme immunoassays using type-specific antibodies generated against recombinant capsid proteins (Atmar and Estes, 2001). The molecular diagnosis of noroviruses relies mainly on a relatively small sequence in the RNA polymerase region (Ando et al., 1995; Vinje and Koopmans, 1996; Jiang et al., 1999b) or the 3′ end of ORF1 (Fankhauser et al., 2002). Although robust for generic norovirus detection, RT-PCR (reverse transcription–polymerase chain reaction) assays targeting small regions of the genome that do not code for structural viral proteins will

neither reflect the epitopes of the viruses important for attachment to the host cell nor correlate with their antigenic properties. Therefore, neither RNA polymerase nor 3′ end of ORF1 is suitable for genotyping, although, in combination with capsid typing, they may become important for identifying norovirus recombinants (Vinje et al., 2000; Vinje et al., 2004).

The Sapovirus capsid gene is fused to and is in frame with the polyprotein gene (Lambden et al., 1994; Numata et al., 1997). All Sapovirus strains except a human strain, London/92, and a porcine strain, PEC Cowden, contain an additional ORF predicted in +1 frame, overlapping the N terminus of the capsid gene (Guo et al., 1999b; Jiang et al., 1999a; Clarke and Lambden, 2001). By analogy with noroviruses, the sapoviruses were previously divided into four genotypes, belonging to two genogroups (Liu et al., 1995; Noel et al., 1997; Clarke and Lambden, 2000). More recently, human sapoviruses have been classified into four or five genogroups (Schuffenecker et al., 2001; Farkas et al., 2004) (Table 3.2). In addition, the porcine enteric calicivirus (PEC) strain Cowden has been shown to be related to the Sapovirus genus and to belong to a differentiated cluster (Guo et al., 1999b). Based on capsid sequences, it has recently been proposed to classify the currently known sapoviruses into nine genetic clusters within five genogroups, including one genogroup represented by the PEC strain Cowden (Farkas et al., 2004).

The occurrence of recombination among human caliciviruses in nature was suspected when it was found that the capsid nucleotide sequences of Snow Mountain virus and Melksham virus were almost identical (94%) but their RNA polymerase sequences were significantly different (79%). When Melksham virus was compared with Mexico virus and the Japanese strain

Table 3.2 Current genogroups and genotypes of sapoviruses [adapted from Schuffenecker et al. (2001) and Farkas et al. (2004)]

Genogroup/cluster	*Prototype strain*	*GenBank accession number (capsid gene)*	*Other strains*
GI/1	Sapporo/82	U65427	Houston/86, Plymouth/92, Manchester/93, Lyon30388/98
GI/2	Parkville/94	U73124	Houston/90
GI/3	Stockholm/97	AF194182	Mexico14917/00
GII/1	London/92	U95645	Lyon/598/97
GII/2	Mexico340/90	AF435812	
GII/3	Cruise ship/00	AY289804	
GIII	PEC/Cowden	AF182760	
GIV	Houston7-1181/90	AF435814	
GV	Argentina39	AY289803	

Oth-25, no significant difference was found in sequence identities of the capsid and RNA polymerase regions and hence Snow Mountain virus was considered a recombinant virus (Hardy et al., 1997). Caliciviruses contain subgenomic RNA that covers the entire 3′ end of the genome from the capsid gene to the 3′ end (Fig. 3.1). It has been hypothesized that the subgenomic RNA could act as an independent unit participating in the recombination events. It remains unknown how subgenomic RNA is involved in virus replication, but it is clear that both genomic and subgenomic RNAs share highly conserved 5′ end sequences and that both RNAs are assembled into virions. It is possible that interaction between genomic and subgenomic RNAs occurs by the same mechanism as that of genomic-genomic interaction, thereby significantly increasing the chance of recombination events (Jiang et al., 1999a). If RNA recombination is a common phenomenon among caliciviruses, a high diversity might be expected in Caliciviridae, which would facilitate the emergence of new variants and make genotyping more difficult. In addition, recombination may permit caliciviruses to escape host immunity, analogous to antigenic shifts in influenza viruses, but by a different molecular mechanism. It has also been reported that accumulation of mutations in the protruding P2 domain of the capsid protein may result in predicted structural changes, including disappearance of helix structure of the protein, and thus a possible emergence of new phenotypes (Nilsson et al., 2003).

1.3. Virus Replication

Studies on the replication strategy of human caliciviruses have been hindered by the lack of an efficient cell culture system. Nevertheless, expression of recombinant proteins from cDNA clones has allowed the generation of proteolytic processing maps for the nonstructural proteins of several caliciviruses, like Southampton virus (a norovirus) and RHDV (a lagovirus) (Liu et al., 1996; Wirblich et al., 1996). Analysis of individual recombinant proteins from these noncultivable caliciviruses allowed the identification of NTPase and 3C-like cysteine protease activities for RHDV and noroviruses (Liu et al., 1999b) and a 3D-like RNA-dependent RNA polymerase for RHDV (López-Vazquez et al., 1998). Studies on the replication mechanisms of cultivable caliciviruses, such as feline calicivirus (FCV), have contributed to a better understanding of the basic features of calicivirus replication (Sosnovtsev and Green, 2003). FCV replicates by producing two major types of polyadenylated RNAs: a positive-sense genomic RNA of approximately 7.7 kb and a subgenomic RNA of 2.4 kb (Herbert et al., 1996). The genomic RNA serves as a template for the synthesis of nonstructural protein encoded by ORF1, and the subgenomic RNA serves as a template for the translation of structural proteins (Carter, 1990).

1.4. Virus-Cell Interactions

Human and animal enteric caliciviruses are assumed to replicate in the upper intestinal tract, causing cytolytic infection in the villous enterocytes but not in the crypt enterocytes of the proximal small intestine. Biopsies of the

jejunum taken from experimentally infected volunteers who developed gastrointestinal disease after oral administration of Norwalk, Montgomery County, or Hawaii viruses showed histopathologic lesions, consisting of blunting of the villi, crypt cell hyperplasia, infiltration with mononuclear cells, and cytoplasmic vacuolization (Blacklow et al., 1972; Dolin et al., 1975). Experiments with recombinant Norwalk VLPs and human gastrointestinal biopsies showed binding of the rVLPs to epithelial cells of the pyloric region of the stomach and to enterocytes on duodenal villi. Attachment of these rVLPs occurred only to cells as well as to saliva from histo-blood group antigen-secreting individuals (Marionneau et al., 2002). It was previously known that RHDV attaches to H type 2 histo-blood group oligosaccharide present on rabbit epithelial cells (Ruvöen-Clouet et al., 2000). Significant attachment and entry of Norwalk rVLPs to differentiated Caco-2 cells has also been demonstrated (White et al., 1996). Differentiated Caco-2 cells resemble mature enterocytes, express the H antigen, and were derived from a group O individual (Amano and Oshima, 1999).

To date, the Cowden strain of porcine enteric calicivirus (PEC) is the only cultivable enteric calicivirus (Flynn and Saif, 1988). However, in order to replicate, it requires the incorporation of an intestinal content preparation (ICP) from uninfected gnotobiotic pigs as a medium supplement. Different porcine intestinal enzymes such as trypsin, pancreatin, alkaline phosphatase, enterokinase, elastase, protease, and lipase were tested as medium supplements, but none of them alone promoted the growth of PEC in cell cultures (Parwani et al., 1991). It was speculated that some enzymes or factors in porcine ICP could activate the viral receptor, promote signaling of host cells, or may cleave viral capsid for successful uncoating (Guo and Saif, 2003).

Although noroviruses are highly infectious (it has been estimated that less than 10 virions may be enough to infect an adult), studies with volunteers have shown that some subjects remain uninfected despite having been challenged with high infectious doses (Matsui and Greenberg, 2000). These individuals may remain disease-free because of innate resistance or because of preexisting immunity to the virus (Lindesmith et al., 2003). An increased risk of Norwalk virus infection has been associated with blood group O; Norwalk VLPs bind to gastroduodenal cells from individuals who are secretors (Se+) but not to those from nonsecretors (Se−) (Hutson et al., 2002; Marionneau et al., 2002). The gene responsible for the secretor phenotype, *FUT2*, encodes an $\alpha(1,2)$ fucosyltransferase that produces the carbohydrate H type 1 found on the surface of epithelial cells and in mucosal secretions (Lindesmith et al., 2003). The form of H type 1 secreted depends on additional glycosyltransferases, including the Lewis, A and B enzymes present in epithelial and red blood cells (Marionneau et al., 2001). The recent discovery that noroviruses attach to cells in the gut only if the individuals express specific, genetically determined carbohydrates is a breakthrough in understanding norovirus-host interactions and susceptibility to norovirus disease (Lindesmith et al., 2003; Hutson et al., 2004).

Recent studies also suggest that some animal caliviviruses may cross the species barrier and potentially infect humans. The hypothetical existence of animal reservoirs and the possibility of interspecies transmission have been suggested by phylogenetic links of bovine and porcine viruses within the genera Norovirus and Sapovirus, respectively (Clarke and Lambden, 1997; Dastjerdi et al., 1999; Liu et al., 1999a; van Der Poel et al., 2000). However, information concerning the frequency of interspecies transmission among caliciviruses is limited.

It has recently been demonstrated that gnotobiotic pigs inoculated by the intravenous route with wild-type PEC Cowden developed diarrhea and also presented histological lesions in the duodenum and jejunum similar to those observed in gnotobiotic pigs inoculated orally with wild-type PEC (Guo et al., 2001). Moreover, PEC RNA and low titers of virus antigen were detected in sera from pigs inoculated with wild-type PEC both orally and intravenously (Guo and Saif, 2003). Thus, viremia may occur after natural PEC infection, and acute sera may contain infectious virus. It has been hypothesized that human calicivirus infections might also induce viremia, interacting with erythrocytes in the blood (Guo and Saif, 2003). If this is true, how the enteric caliciviruses reach the bloodstream from the gut and vice versa remains unexplained.

2.0. ROTAVIRUSES

Rotaviruses are the leading etiologic agents of viral gastroenteritis in infants and young children worldwide and in the young of a large variety of animal species (Kapikian, 2001). Rotavirus infections in humans continue to occur throughout their lives, but the resulting disease is mild and often asymptomatic (Bishop, 1996). In addition to sporadic cases of acute gastroenteritis, outbreaks of rotavirus diarrhea in school-aged children and adults have increasingly been reported (Griffin et al., 2002; Mikami et al., 2004).

Rotaviruses are responsible for an estimated 500,000 deaths each year in developing countries (Parashar et al., 2003). There is an urgent need to develop an effective vaccine and to implement therapeutic strategies to prevent and treat these infections. A better understanding of the molecular mechanisms of rotavirus replication and interaction with the host cell and of antigenic variability of these viruses are fundamental to achieving these goals.

Rotaviruses are classified into at least five groups (A to E), and there are possibly two more groups (F and G) based on the reactivities of the VP6 middle layer protein (Estes, 2001). Group A rotaviruses are most commonly associated with human infections (Kapikian, 2001). Within group A, four subgroups (I, II, I + II, and non-I/non-II) are recognized based on the reactivity of VP6 with two monoclonal antibodies. The two outer capsid proteins, VP7 and VP4, form the basis of the current dual classification system of group A rotaviruses into G and P types (Estes, 2001). At least 14 different G-serotypes

and 20 P-types have been identified among human and animal rotaviruses, depending on VP7 and VP4, respectively. G serotypes correlate fully with G genotypes as determined by sequence analysis of the VP7 gene. However, to date only 12 of the 20 P genotypes have been correlated with P serotypes (Estes, 2001). Because VP4 and VP7 are coded for by different RNA segments (segment 4 and segments 7–9, respectively), various combinations of G- and P-types can be observed both in humans and in animals. Viruses carrying G1P[8], G2[P4], G3[P8], and G4[P8] represent more than 90% of human rotavirus strains cocirculating in most countries, although other G and P combinations are being isolated in increasing numbers (Cunliffe et al., 1999; Buesa, 2000; Iturriza-Gomara et al., 2000; Adah et al., 2001; Armah et al., 2003; Zhou et al., 2003).

Group B rotaviruses are common animal pathogens infecting pigs, cows, sheep, and rats but have also been found to infect humans (Sanekata et al., 2003). Group C rotaviruses are commonly found in animals including pigs and dogs and can cause outbreaks in humans (Castello et al., 2000).

2.1. Structure of the Virion

Rotavirus particles are of icosahedral symmetry, have three concentric layers of protein, and measure ~1,000 Å in diameter including the spikes (Estes, 2001). The core layer is formed by a protein known as VP2 (which surrounds the entire viral genome) and proteins VP1 and VP3, which are transcription enzymes and are attached as a heterodimeric complex to the inside surface of VP2 at the fivefold symmetry positions (Prasad et al., 1996b) (Fig. 3.3). VP1, the RNA-dependent RNA polymerase, interacts with VP3, guanylytransferase, and methylase (Liu et al., 1992). This innermost layer is composed of 120 copies of VP2, a RNA-binding protein (Labbé et al., 1991). The addition of VP6 to the VP2 layer produces double-layered particles (DLP). VP6 forms 260 trimers interrupted by 132 aqueous channels of three different kinds in relation to the capsid's symmetry. The outer capsid of the triple-layered particles (TLP) is composed of two proteins, VP7 and VP4.

The smooth surface of the virus is made up of 260 trimers of VP7, and 60 spikes emerging from the viral surface consist of dimers of VP4 (Prasad et al., 1988; Yeager et al., 1990). Rotavirus DLP and TLP contain 132 porous channels that allow the exchange of compounds to the inside of the particle. There are 12 type I channels, each located at the icosahedral fivefold vertices of the TLP and DLP. Each of the type I channels is surrounded by five type II channels, and finally 60 type III channels are placed at the hexavalent positions immediately neighboring the icosahedral threefold axis (Prasad et al., 1988; Yeager et al., 1990).

During cell entry, the TLP loses the VP7 and VP4 proteins, and the resulting DLP becomes transcriptionally active inside the cytoplasm (Estes, 2001). VP4 is a nonglycosylated protein of 776 amino acids and has essential functions in the virus cell cycle, including receptor binding, cell penetration, hemagglutinin activity, and permeabilization of cellular membranes (Fiore et al., 1991; Burke and Desselberger, 1996; Gilbert and Greenberg, 1998;

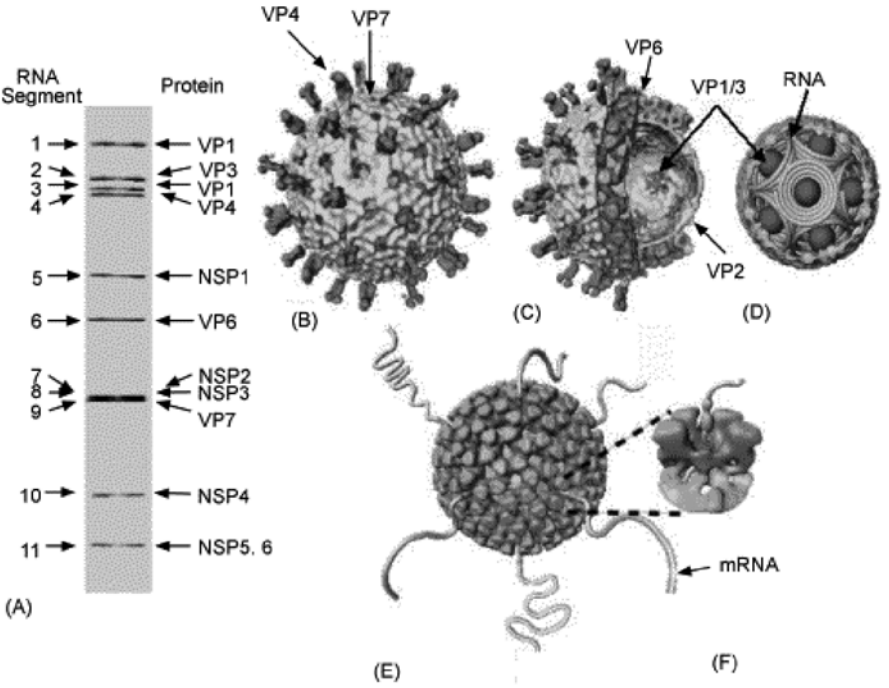

Figure. 3.3 Architectural features of rotavirus. (A) PAGE gel showing 11 dsRNA segments composing the rotavirus genome. The gene segments are numbered on the left and the proteins they encode are indicated on the right. (B) Cryo-EM reconstruction of the rotavirus triple-layered particle. The spike proteins VP4 are colored in orange and the outermost VP7 layer in yellow. (C) A cutaway view of the rotavirus TLP showing the inner VP6 (blue) and VP2 (green) layers and the transcriptional enzymes (shown in red) anchored to the VP2 layer at the fivefold axes. (D) Schematic depiction of genome organization in rotavirus. The genome segments are represented as inverted conical spirals surrounding the transcription enzymes (shown as red balls) inside the VP2 layer in green. (E and F) Model from cryo-EM reconstruction of transcribing DLPs. The endogenous transcription results in the simultaneous release of the transcribed mRNA from channels located at the fivefold vertex of the icosahedral DLP. (Reproduced with permission from Jayaram et al., 2004; see color insert.)

Denisova et al., 1999; Zárate et al., 2000b). VP4 is post-transcriptionally cleaved into the larger VP5* and the smaller VP8* subunits, and cleavage of VP4 enhances viral infectivity by several-fold. It has been shown that trypsin cleavage confers icosahedral ordering on the VP4 spikes, which is essential for the virus to enter the cell (Crawford et al., 2001). Moreover, biochemical studies of recombinant VP4 indicate that proteolysis of monomeric VP4 yields dimeric VP5* (Dormitzer et al., 2001).

VP7 is a calcium-binding glycoprotein of 326 amino acids, with nine variable regions contributing to type-specificity (Nishikawa et al., 1989; Hoshino

et al., 1994). In addition, VP7 interacts with integrins αxβ2 and α4β1 (Coulson et al., 1997; Hewish et al., 2000) and induces polyclonal intestinal B-cell activation during rotavirus infection (Blutt et al., 2004).

When the DLP is located intracellularly, it becomes transcriptionally competent, and new mRNA transcripts are translocated from the particle through type I channels at the fivefold axes (Lawton et al., 1997). Prasad et al. (1988) were the first to propose that these channels in the VP6 layer could be used by mRNA to exit. Cryo-EM studies have confirmed that DLPs maintain their structural integrity during the process of transcription. In a pseudoatomic model of the T = 13 VP6 layer (Mathieu et al., 2001), a β-hairpin motif of VP6 with a highly conserved sequence that protrudes into the mRNA exit channel may play a functional role in the translocation of the nascent transcripts (Lawton et al., 2000). A detailed mutational analysis of the VP6 layer has helped to elucidate the determinants of VP6 required for its assembly on VP2 and how VP6 may affect endogenous transcription (Charpilienne et al., 2002).

2.2. The Genome

The genome of rotavirus (simian rotavirus strain SA11) consists of 11 segments of double-stranded RNA (dsRNA) with conserved 5′ and 3′ ends, ranging from 667 bp (segment 11) to 3,302 bp (segment 1) in size, totaling 6,120 kDa. Six structural proteins (VP1, VP2, VP3, VP4, VP6, VP7) and six nonstructural proteins (NSP1 to NSP6) are encoded. The coding assignments, functions, and many properties of the proteins encoded by each of the 11 genome segments are now well established (Table 3.3). Protein assignments have been determined by *in vitro* translation using mRNA or denatured dsRNA and by analyses of reassortant viruses (Estes and Cohen, 1989; Estes, 2001). Cryo-EM analysis of rotavirus has provided first visualization of the structural organization of the viral genome (Prasad et al., 1996b). The dsRNA forms a dodecahedral structure in which the RNA double helices, interacting closely with the VP2 inner layer, are packed around the transcription complexes located at the icosahedral vertices (Fig. 3.3D). VP2 has RNA binding properties and may be responsible for the icosahedral ordering and for the closely interacting portions of the RNA. A study by Pesavento et al. (2001) demonstrated reversible condensation and expansion of the rotavirus genome within the capsid interior under various chemical conditions. This condensation is concentric with respect to the particle center, and a dark mass of density is consistently seen in the center of each of the particles. At high pH in the presence of ammonium ions, the genome condenses to a radius of 180 Å from the original radius of 220 Å, and when brought back to physiological pH the genome expands to its original radius (Pesavento et al., 2001). This study suggests that VP2, through its RNA-binding properties, plays an important role in maintaining appropriate spacing between the RNA strands in the native expanded state. A plausible and elegant model for the structural organization of the genome that emerges from these studies is that each dsRNA segment is spooled around a transcription enzyme

complex at the fivefold axes, inside the innermost capsid layer (Prasad et al., 1996b; Pesavento et al., 2001, 2003).

2.3. Mechanisms of Evolution and Strain Diversity

Rotaviruses, like many other RNA viruses, display a great degree of diversity. As well as showing different G and P types and a variety of combinations, there is also intratypic variation. Three mechanisms, singly or in combination, are important for the evolution and diversity of rotaviruses, although it is yet unclear what their relative contributions are to the burden of the disease.

2.3.1. Antigenic Drift

The rate of mutation within rotavirus genes is relatively high because RNA replication is error-prone. The mutation rate has been calculated to be $<5 \times 10^{-5}$ per nucleotide per replication, which implies that on average a rotavirus genome differs from its parental genome by at least one mutation (Blackhall et al., 1996). Point mutations can accumulate and give rise to intratypic variation as identified by the existence of lineages within the VP7 and VP4 genes of particular G and P types. Point mutations can lead to antigenic changes, which may result in the emergence of escape mutants (Palombo et al., 1993; Cunliffe et al., 1997; Maunula and von Bonsdorf, 1998; Iturriza-Gómara et al., 2002).

2.3.2. Antigenic Shift

Shuffling of gene segments through reassortment can occur during dual infection of one cell. Reassortment can therefore contribute to the diversity of rotaviruses, and there is increasing evidence that reassortment takes place *in vivo* (Ramig, 2000). There is evidence that reassortment, through alteration in protein-protein interactions, possibly leads to changes in conformational epitopes and may contribute to the evolution of antigenic types (Chen et al., 1992; Lazdins et al., 1995). Interspecies transmission and subsequent reassortment have enormous potential to increase the diversity of cocirculating rotaviruses. In addition, human rotavirus genes encoding proteins to which the human population is immunologically naïve may allow a rapid spread of the reassortant strain (Iturriza-Gomara et al., 2000).

2.3.3. Gene Rearrangement

The concatemerization or truncation of genome segments and their ORFs has the potential to contribute to the evolution of rotaviruses through the production of new proteins with altered functions (Desselberger, 1996).

2.4. Genome Replication

The RNA polymerase activity of DLPs catalyzes synthesis of 11 mRNAs of rotaviruses, which range in size from ~0.7 to 3.3 kb. With the exception of gene 11, they are monocistronic (Estes, 2001). The nascent transcripts are extruded through channels present at the fivefold axes of the DLPs (Lawton et al., 1997). The 5′- and 3′-untranslated regions (UTRs) are 9 to 49 and 17 to 182 nucleotides in length, respectively. The viral mRNAs serve as

templates for the synthesis of minus-strand RNA to form dsRNA molecules (Chen et al., 1994). The synthesis of viral dsRNA and the assembly of cores and DLPs occur in viroplasms in the cytoplasm (Estes, 2001). There are three species of RNA-containing replication intermediates (RIs) in the infected cells: pre-core RIs, which contain the structural proteins VP1 and VP3; core-RIs, with VP1, VP2, and VP3; and double-layered RIs, which contain VP1, VP2, VP3, and VP6. The 11 genomic segments are produced and packaged in equimolar amounts in rotavirus-infected cells, demonstrating that RNA packaging and replication are coordinated processes (Patton and Gallegos, 1990). The absence of naked dsRNA in infected cells suggests that packaging takes place before replication (Patton et al., 2003).

The only primary sequences that are conserved among the rotavirus mRNAs are located within the UTRs. Because all 11 mRNAs are replicated by the same VP1-VP2-VP3 polymerase complex, the viral mRNAs almost certainly must share common *cis*-acting signals recognized by the polymerase (Patton et al., 2003). The most remarkable feature of the 3′ ends of rotavirus mRNAs and of other members of the Reoviridae is the absence of a poly(A) tail. Instead, all rotavirus genes and mRNAs end with the same short sequence UGACC, which is conserved among all the segments in group A rotaviruses. Site-specific mutagenesis has revealed that it is the 3′-CC of the 3′ consensus sequence that is most critical for minus-strand synthesis (Chen et al., 2001).

In addition, it has been demostrated that the promoter for minus-strand synthesis is formed by base-pairing in *cis* of complementary sequences proximal to the 5′- and 3′-ends of the viral mRNAs (Chen and Patton, 1998). The 3′-consensus sequence also contains a *cis*-acting signal that acts as a translation enhancer, whose activity is mediated by NSP3 that specifically recognizes the last four to five nucleotides of the 3′-consensus sequence (Poncet et al., 1994). NSP3 also interacts with the initiation factor eIF4GI and facilitates the circularization of viral mRNAs in polysomes, thus increasing the efficiency of viral gene expression (Piron et al., 1998).

The development of a cell-free system that supports the synthesis of dsRNA from exogenous mRNA represents an important milestone in the study of rotavirus replication, providing a mechanism by which the elements in viral mRNAs that promote minus-strand synthesis can be analyzed. This system is based in virion-derived cores that have been disrupted or "opened" by incubation in hypotonic buffer (Chen et al., 1994). Although several investigators have tried to develop a reverse genetics system for rotaviruses, this goal has not been achieved so far (Patton et al., 2003). Our understanding of even the most basic mechanisms that occur during rotavirus replication would be greatly enhanced by the development of the reverse genetics approach.

2.5. Cell Infection

Rotaviruses have a specific tropism *in vivo* infecting primarily the mature enterocytes of the villi of the small intestine. Recent reports suggest that

extraintestinal spread of the virus takes place during infection, indicating a wider tropism than previously considered (Blutt et al., 2003; Mossel and Ramig, 2003). Rotaviruses can bind to a wide variety of cell lines, although only a subset of them (including cells of renal and intestinal origin, and transformed cell lines from breast, stomach, lung, and bone) is efficiently infected (Ciarlet et al., 2002a; López and Arias, 2004). Most studies on rotavirus replication have been carried out using the simian kidney epithelial MA104 cells, which are routinely used to produce progeny virus. However, new investigations into the pathophysiological mechanisms of rotavirus infection are now being performed using *in vitro* polarized cells such as the human intestinal HT-29 and Caco-2 cells (Servin, 2003).

Rotaviruses enter the cell by a complex multistep process in which different domains of the viral surface interact with different cell surface molecules that act as receptors for the attachment of the viruses (Guerrero et al., 2000; Ciarlet and Estes, 2001; López and Arias, 2004). Some animal rotavirus strains interact with sialic acid (SA) residues to attach to the cell surface, and the infectivity of these strains is diminished by the treatment of cells with neuraminidase (NA). By contrast, many animal strains and some human strains are NA-resistant (Ciarlet and Estes, 1999). The interaction of rotavirus with SA has been shown to depend on the VP4 genotype of the virus and not the species of origin (Ciarlet et al., 2002b). Ganglioside GM3 has been suggested to act as the SA-containing receptor for the porcine rotavirus strain OSU (Rolsma et al., 1998), and ganglioside GM1 (NA-resistant) has been identified as the receptor for the NA-resistant human rotavirus strains KUN and MO (Guo et al., 1999a). The VP8* domain of VP4 is involved in interactions with SA, whereas VP5* is implicated in interactions with integrins. The interaction of rotavirus with integrin $\alpha 2\beta 1$ has been shown to be mediated by the DGE integrin-recognition motif, located at amino acids 308 to 310 of VP4, within VP5* (Zárate et al., 2000a). VP4 also contains the tripeptide IDA at amino acids 538 to 540, which is ligand motif for integrin $\alpha 4\beta 1$ (Coulson et al., 1997). However, the functionality of this site has not been demonstrated. Integrin $\alpha v\beta 3$ is also involved in the cell entry of several rotavirus strains at a postattachment step (Graham et al., 2003). Besides, cell-surface heat shock cognate protein hsc70 has also been implicated as a postattachment receptor for both NA-sensitive and NA-resistant rotavirus strains (Guerrero et al., 2002). Studies with polarized epithelial cell lines show that the viral entry of SA-dependent strains is restricted to the apical membrane, whereas SA-independent strains enter either apically or basolaterally (Ciarlet et al., 2001).

It has been suggested that lipid rafts might play an important role in the cell entry of rotavirus (Isa et al., 2004), probably serving as platforms to allow an efficient interaction of cell receptors with the viral particle (Manes et al., 2003; López and Arias, 2004). Rotavirus infection in polarized, fully differentiated Caco-2 cells is followed by a defect in brush-border hydrolase expression (Jourdan et al., 1998). Sucrase-isomaltase activity and apical expression are specifically decreased by rotavirus infection without any

apparent cell destruction (Jourdan et al., 1998). In addition, viral infection induces an increase in intracellular calcium concentration, damages the microvillar cytoskeleton, and promotes structural and functional injuries at the tight junctions in cell-cell junctional complexes of cultured Caco-2 cells without damaging the integrity of the monolayers (Brunet et al., 2000; Obert et al., 2000).

2.6. The NSP4 Enterotoxin

The rotavirus nonstructural glycoprotein NSP4 was shown to function as an intracellular receptor that mediates the acquisition of a transient membrane envelope as subviral particles bud into the endoplasmic reticulum (ER). It has been demonstrated that NSP4 binds intracellularly newly made DLPs, interacting with VP6. This receptor role of NSP4 is confirmed by the observation that DLPs bind to ER membranes containing only NSP4 (Taylor et al., 1993; Estes, 2001). Many structural motifs or protein regions have been implicated in the NSP4 biological function. Amino acids 17 to 20 from the C-terminus extreme are necessary and sufficient for inner capsid particle binding (O'Brien et al., 2000) and the region involved in the retention of the NSP4 protein into the endoplasmic reticulum has been mapped between the amino acids 85 and 123 in the cytoplasmatic region of the protein (Mirazimi et al., 2003). Residues at positions 48 to 91, a region that includes a potential cationic amphipatic helix, have been shown to be involved in membrane destabilization (Tian et al., 1996; Browne et al., 2000). Purified NSP4 or a peptide corresponding to NSP4 residues 114–135 induce diarrhea in young mice after an increase in intracellular calcium levels, suggesting a role for NSP4 in rotavirus pathogenesis (Tian et al., 1994; Ball et al., 1996; Horie et al., 1999). *In vitro* studies have shown that after rotavirus replication in cells, a functional 7-kDa peptide of NSP4 (amino acids 112–175) is released into the medium from virus-infected cells by a nonclassic, Golgi-independent cellular secretory pathway (Zhang et al., 2000). This endogenously produced peptide binds to an as yet unidentified apical membrane receptor to mobilize intracellular calcium through phospholipase C signaling.

NSP4, or its active $NSP4_{114–135}$ peptide, induces age-dependent diarrhea and age-dependent chloride permeability changes in mice lacking the cystic fibrosis transductance regulator (CFTR) that exhibit no functional cAMP-dependent secretory pathway (Morris et al., 1999). These observations indicate that NSP4 is a novel secretory agonist, because the classical secretagogues carbachol and the diterpene forskolin that induce chloride changes by activating cyclic adenosine monophosphate, instead of by mobilizing $[Ca^{2+}]_i$ as a secondary mediator, fail to cause disease in CFTR knockout mice (Morris et al., 1999). NSP4 or its active peptide may induce diarrhea in neonatal mice through the activation of an age- and Ca^{2+}-dependent plasma membrane anion permeability distinct from CFTR. The molecular identity of the responsible channel remains to be determined (Morris and Estes, 2001).

It has been suggested that NSP4 may directly inhibit the functioning of the cellular Na^+-dependent glucose transporter SGLT-1 (Halaihel et al., 2000). In addition, extracellular and/or intracellular NSP4 may contribute to diarrheal pathogenesis by altering the dynamics of intracellular actin distribution and intercellular contacts (Ciarlet and Estes, 2001). NSP4 can affect the cytoskeleton in polarized epithelial cells, but how these pleiotropic properties of NSP4 influence the function of NSP4 in morphogenesis or pathogenesis remains unclear (Tafazoli et al., 2001). The significance of immune response to NSP4 in protection against rotavirus infection in humans is still unknown, although it has been shown that NSP4 elicits both humoral and cell-mediated immune responses (Johansen et al., 1999; Ray et al., 2003).

3.0. ASTROVIRUSES

Human astroviruses are nonenveloped viruses with a positive-sense, single-stranded, polyadenylated RNA genome about 6,800 nucleotides in length (Matsui, 1997). Astroviruses are members of the Astroviridae family and were originally described in 1975 in association with outbreaks of gastroenteritis in newborns (Appleton and Higgins, 1975). Astroviruses produce infections mainly in young children, although illness rates increase again in the elderly (Lewis et al., 1989). However, they also cause disease in adults and immunocompromised patients (Cubitt et al., 1999; Coppo et al., 2000; Liste et al., 2000). Persistent gastroenteritis in children with no background disease has also been reported, mainly in association with serotype 3 strains (Caballero et al., 2003). Astrovirus infections occur worldwide, and their incidence ranges from 2% to 9% in both developing and developed countries (Gaggero et al., 1998; Bon et al., 1999; Mustafa et al., 2000; Guix et al., 2002). Astroviruses are transmitted by the fecal-oral route; outbreaks have been associated with consumption of sewage-polluted shellfish and ingestion of water from contaminated sources (Pintó et al., 1996; Pintó et al., 2001). The virus is frequently shed in stools in significant numbers at the onset of the illness, which is in contrast with low numbers of calicivirus produced in the stools of infected patients. Unlike caliviruses, astroviruses replicate *in vitro* in cell lines, and hence detailed studies on their replication are available (Matsui, 1997; Willcocks et al., 1999; Matsui and Greenberg, 2001). Human astroviruses were originally isolated in HEK cells and were subsequently adapted to grow in LLCMK2 cells in trypsin-containing media, although without demonstrable cytopathic effect (CPE). The colonic carcinoma cell line Caco-2, in contrast with LLCMK2 cells, is directly susceptible to fecally derived astrovirus and displays CPE as early as 2 days postinfection (Willcocks et al., 1990; Pinto et al., 1994).

The average diameter of astrovirus particles is approximately 28 nm (Madeley, 1979), although it may vary depending on the source of the virus and the method of preparation for EM (Woode et al., 1984). More detailed ultrastructural analysis of human astrovirus serotype 2 grown in LLCMK2

cells in the presence of trypsin revealed particles with icosahedral symmetry and an array of spikes emanating from the surface (Risco et al., 1995). These particles had an external diameter of 41 nm (including spikes). They did not display the star-like surface characteristically found on fecally shed virus. However, the star-like morphology was inducible after alkaline treatment (pH 10). Intact virions generally band at densities of 1.35 to 1.37 g/ml in CsCl gradients (Caul and Appleton, 1982), although banding at densities of 1.39–1.40 has also been reported (Konno et al., 1982; Midthun et al., 1993). The astroviruses band at a density of 1.32 g/ml in potassium tartrate–glycerol gradient, which preserves better than CsCl the structural integrity of the virus (Ashley and Caul, 1982).

The RNA genomes of several cell culture–adapted human astrovirus strains have been cloned and sequenced (Jiang et al., 1993a; Lewis et al., 1994; Willcocks et al., 1994; Geigenmuller et al., 1997), providing new perspectives for studying the molecular biology of astroviruses. The organization of the genome includes three long open reading frames (ORFs), designated ORF1a, ORF1b, and ORF2. ORF1a (~2700 nt) is located at the 5′ end of the genome and contains transmembrane helices, a 3C-like serine protease motif, a putative protease-dependent cleavage site, and a nuclear localization signal (Jiang et al., 1993a; Willcocks et al., 1994). ORF1b (~1550 nt) contains a RNA-dependent RNA polymerase motif, whereas ORF2 encodes the structural proteins (Matsui and Greenberg, 2001). The nonstructural proteins of the virus are translated from the genomic viral RNA as two polyproteins (Jiang et al., 1993a). One of them contains only ORF1a and the other includes ORF1a/1b and is translated via –1 ribosomal frameshifting event between ORF1a and ORF1b (Jiang et al., 1993a). Both proteins are proteolitically processed, generating a variety of polypeptides, although it is unclear whether the viral protease is responsible for all the cleavages (Geigenmuller et al., 2002a, 2002b; Kiang and Matsui, 2002).

A subgenomic RNA (approximately 2,400 nucleotides) that contains ORF2 is found in abundance in the cytoplasm of astrovirus-infected cultured cells (Monroe et al., 1991). The subgenomic RNA is translated as a 87-kDa capsid precursor protein that is believed to give rise to three to five smaller mature capsid proteins in a process that involves trypsin and a putative cellular protease (Monroe et al., 1991; Bass and Qiu, 2000; Mendez et al., 2002). The 87-kDa capsid protein is rapidly converted intracellularly to a 79-kDa form, which is found in smaller amounts in the cell supernatants. Bass and Qiu (2000) identified a trypsin cleavage site in a highly conserved region of the ORF2 product. Trypsin-free particles were minimally infectious in cultured Caco-2 cells but became highly infectious after trypsin treatment but not chymotrypsin treatment. This trypsin-enhanced infectivity correlated with conversion of the 79-kDa capsid protein to three smaller peptides of approximately 26, 29, and 34 kDa. However, the apparent molecular weight reported for the smaller mature proteins has depended on the astrovirus serotype and whether the virus studied was derived from infected cultured cells or from stools (Matsui and Greenberg, 2001).

Table 3.3 Genome segments and proteins of simian rotavirus SA11: Coding assignments, functions and biological properties of the encoded proteins. Adapted from Estes (2001) and Ramig and Estes (2003)

Genome segment size (bp)	*ORFs*	*Gene product(s)*	*Protein size aa (Da)*	*Location in virus particle*	*Functions and properties*
1 (3,302)	18-3282	VP1	1,088 (125,000)	Inner capsid 5-fold axis	RNA-dependent RNA polymerase Part of minimal replication complex Virus specific 3′-mRNA binding Part of virion transcription complex with VP3
2 (2,690)	17-2659	VP2	881 (102,431)	Inner capsid	Core matrix protein Non-specific ss and dsRNA-binding activity Myristylated Assembly and RNA-binding activity Part of minimal replication complex Leucine zipper
3 (2,591)	50-2554	VP3	835 (98,120)	Inner capsid, 5-fold axis	Guanylyltransferase Methyltransferase Located at the vertices of the core Part of virion transcription complex with VP1 Non-specific ssRNA binding
4 (2,362)	10-2337	VP4	776 (86,782)	Outer capsid spike	VP4 dimers form outer capsid spike Interacts with VP6
	—	VP5*	529 (60,000)		Virus infectivity enhanced by trypsin cleavage of VP4 into VP5* and VP8*
	—	VP8*	247 (28,000)		Hemagglutinin and cell attachment protein P-type neutralization antigen VP5* permeabilizes membranes Protection
5 (1,611)	31-1515	NSP1	495 (58,654)	Nonstructural	Associates with cytoskeleton Extensive sequence diversity between strains Two conserved cysteine-rich zinc-finger motifs Virus specific 5′-mRNA binding Interacts with host IFN regulatory factor 3
6 (1,356)	24-1214	VP6	397 (44,816)	Middle capsid	Major virion protein Middle capsid structural protein Homotrimeric structure Subgroup antigen

					Myristoylated Protection (mechanism?)
7 (1,105)	26-970	NSP3	315 (34,600)	Nonstructural	Homodimer Virus-specific 3′-mRNA binding Binds eIF4G1 and circularizes mRNA on initiation complex Involved in translational regulation and host shut-off
8 (1,059)	47-997	NSP2	317 (36,700)	Nonstructural	NTPase activity Helix destabilization activity Non-specific ssRNA-binding Involved in viroplasm formation with NSP5 Functional octamer Binds NSP5 and VP1 Induces NSP5 hyperphosphorylation
9 (1,062)	49-1026	VP7	326 (7,368)	Outer capsid glycoprotein	Outer capsid structural glycoprotein G-type neutralization antigen N-linked high mannose glycosylation and trimming RER transmembrane protein, cleaved signal sequence Ca^{2+} binding Protection
10 (751)	41-569	NSP4	175 (20,290)	Nonstructural	Enterotoxin Receptor for budding of double-layer particle through ER membrane RER transmembrane glycoprotein Ca^{2+}/Sr^{2+} binding site N-linked high mannose glycosylation Protection Host cell $[Ca^{2+}]_i$ mobilization
11 (667)	22-615	NSP5	198 (21,725)	Nonstructural	Interacts with VP2, NSP2 and NSP6 Homomultimerizes O-linked glycosylation (Hyper-) phosphorylated Autocatalytic kinase activity enhanced by NSP2 interaction Non-specific ssRNA binding
	80-355	NSP6	92 (11,012)	Nonstructural	Product of second, out-of-frame ORF Interacts with NSP5 Localizes to viroplasm

Recently, astrovirus virus-like particles (VLPs) have been generated by cloning the cDNA corresponding to ORF2 from a human astrovirus serotype 2 into vaccinia virus (Dalton et al., 2003). Protein composition of these purified VLPs revealed no substantial difference from that of authentic astrovirus virions when analyzed by Western blotting. Trypsin cleavage seems to be necessary to process the capsid polyprotein into mature structural proteins.

Human astroviruses are classified into eight serotypes (HAstV-1 to HAstV-8) according to the antigenic reactivity of the capsid proteins (Lee and Kurtz, 1982, 1994; Taylor et al., 2001). Molecular analysis of a region at the 5′ end of ORF2 can be used to confirm antigenic typing or to characterize strains with ambiguous serotyping results (Noel et al., 1995; Belliot et al., 1997; Monroe et al., 2001). HAstV-1 is the most prevalent of the human astroviruses that cause actue gastroenteritis, whereas HAstV-6 and HAstV-7 have rarely been isolated (Lee and Kurtz, 1994; Noel and Cubitt, 1994; Gaggero et al., 1998; Unicomb et al., 1998).

4.0. ENTEROVIRUSES

4.1. Polioviruses

Enteroviruses replicate in the gastrointestinal tract, but the resulting infection is frequently asymptomatic. Symptoms, when they occur, range from paralysis to fever. Enteroviruses, named after the site of replication, rarely cause gastroenteritis. In addition, in many cases the enterovirus isolated might merely have been a passenger virus unrelated to the disease (Melnick, 1996).

The capsids of enteroviruses (family Picornaviridae) are composed of four structural proteins (VP1–VP4) arranged in 60 repeating protomeric units with icosahedral symmetry. Among the family members, the capsid proteins are arranged similarly, but the surface architecture varies. These differences account for not only the different serotypes but also the different modes of interaction with cell receptors. The basic building block of the picornavirus capsid is the protomer, which contains one copy of each structural protein. The capsid is formed by VP1 to VP3, and VP4 lies on its inner surface. VP1, VP2, and VP3 have no sequence homology, yet all three proteins have the same topology: they form an eight-stranded antiparallel "β-barrel" core structure (Racaniello, 2001). The external loops that connect the beta strands are responsible for differences in the antigenic diversity of enteroviruses. Neutralization sites are more densely clustered on VP1. The structures of many human picornaviruses have been resolved, indicating that they share a number of conserved structural motifs. For example, the capsids of polioviruses, rhinoviruses, and coxsackieviruses have a groove, or canyon, surrounding each fivefold axis of symmetry. In contrast, cardioviruses and aphthoviruses do not have canyons (Racaniello, 2001). Immediately beneath the canyon floor of each protomer is a hydrophobic pocket occupied by a

lipid moiety. These molecules, termed *pocket factors*, have been shown to stabilize the capsid, and their removal from the pocket is a necessary prerequisite to uncoating. On the basis of the electron densities and uncoating studies, the pocket factors are thought to be short-chain fatty acids (Smyth et al., 2003).

The enterovirus positive-sense RNA genome is approximately 7.4 kb long and serves as a template for both viral protein translation and virus replication (Racaniello, 2001). The 5′ end is covalently linked to a VPg protein (for genome-linked virus protein). The genome is organized into a long (~740 nucleotides) nontranslated region (5′NTR), which contains the internal ribosome entry site (IRES) and precedes a single ORF. The ORF is subdivided into three regions, P1 to P3. The P1 region codes for structural proteins, whereas P2 and P3 regions encode for nonstructural proteins essential for virus replication (2A–C, 3A–D). Translation of the ORF gives rise to a single large polyprotein that is post-translationally modified by virus encoded proteases. Immediately downstream of the protein-coding region is the 3′ nontranslated region (3′-NTR), which plays a role in viral RNA replication, followed by a terminal poly(A) tail (Racaniello, 2001).

The three serotypes of human poliovirus are considered a species within the genus Enterovirus. A redefinition of the criteria for species demarcation within the genus Enterovirus has recently been issued by the International Committee on Taxonomy of Viruses (ICTV). Based on these molecular and biochemical characteristics, Enteroviruses are now classified as human enteroviruses A through D, bovine enterovirus, and porcine enterovirus A and B (King et al., 2000).

Poliovirus infections, once responsible for high morbility and mortality throughout the world, are now under control, and their eradication is a priority for the World Health Organization (WHO). In the past 15 years, since the Global Polio Eradication Initiative was launched by WHO, the number of cases has fallen from an estimated 350,000 cases in 1988 to 1,919 in 2002. In the same time period, the number of polio-infected countries has been reduced from 125 to 7. There is a historic opportunity to forever stop the transmission of poliovirus. If the world seizes this opportunity and acts immediately, no child will ever again know the effects of this devastating disease (Dowdle et al., 2001).

4.2. Kobuviruses

Aichi virus, a cytopathic small round virus, was isolated for the first time in 1989 from fecal samples of patients involved in an oyster-associated gastroenteritis outbreak (Yamashita et al., 1991). Since then, several Aichi virus strains have been isolated in BS-C-1 cells from patients with gastroenteritis (Yamashita et al., 1995). The virus is commonly found in outbreaks of gastroenteritis in Japan and is often associated with the consumption of oysters (Yamashita et al., 1995). Genetic analysis of Aichi virus revealed that it belongs to the Picornaviridae family but that it is different from any other genus such as the Entero-, Rhino-, Cardio-, Aphtho-, Hepato-, Tescho-, or

Parechovirus (Yamashita et al., 1998). It has been recently proposed by the ICTV that this virus be assigned to a new genus named Kobuvirus (King et al., 1999). *Kobu* means "knob" in Japanese; the reference based on the characteristic morphoplogy of the virion.

Aichi virus has been cultivated in BS-C-1 and Vero cells, producing CPE characterized by detachment of cells. It does not replicate in other human cell lines such as HeLa, RD, or HEL cells. By EM, the virion shows a rough surface and measures around 30 nm in diameter. Three capsid proteins of 42, 30, and 22 kDa have been described (Yamashita et al., 1998). Aichi virus possesses a genome of single-stranded RNA of 8,280 nucleotides, excluding a poly(A) tract. It contains a large ORF with 7,299 nucleotides that encodes a polyprotein precursor of 2,432 amino acids, which is preceded by 744 nucleotides and followed by 237 nucleotides and a poly(A). The precise secondary structure of the 5′ nontranslated region (5′-NTR) has not been defined, although an internal ribosomal entry site (IRES) similar to that of other members of the Picornaviridae family has been reported (Sasaki et al., 2001). The complete nucleotide sequence of Aichi virus (GenBank accession no. AB010145) has been determined (Yamashita et al., 1998).

The organization of the deduced amino acid sequence of the polyprotein encoded by the Aichi virus genome is analogous to that of the other picornaviruses. Preceded by a leader (L) protein, there is the P1 region, which corresponds to the structural proteins (VP0, VP3, and VP1, with molecular weights of 42, 30, and 22 kDa, respectively) followed by the P2 and P3 regions, which contain sequences encoding the nonstructural proteins (2A–C, 3A–D). It has been reported that the 2A protein of Aichi virus contains conserved motifs that are characteristic of the H-rev107 family of cellular proteins involved in the control of cellular proliferation (Hughes and Stanway, 2000). Amino acid sequences of the 2C, 3C, and 3D regions are well aligned with the corresponding sequences of other picornaviruses. The 3B protein corresponds to the VPg protein. The 3C protein is the protease and contains conserved motifs characteristic of all picornaviruses (Yamashita and Sakae, 2003). The relationship of kobuviruses to other picornaviruses has been analyzed based on the 3D amino acid sequence (which corresponds to the RNA polymerase) and the polyprotein sequence (Hughes, 2004). Recently, bovine kobuvirus has been isolated and characterized (accession no. AB084788) (Yamashita et al., 2003). The genus Kobuvirus, including bovine kobuvirus and Aichi virus, cluster near the genera Teschovirus, Cardiovirus, Erbovirus, and Aphthovirus (Hughes, 2004).

5.0. HEPATITIS A VIRUS

Hepatitis A virus (HAV), the prototype of the genus Hepatovirus in the family Picornaviridae (Minor, 1991), is a hepatotropic virus that represents a significant problem for human health (Hollinger and Emerson, 2001). HAV infection is mainly propagated via the fecal-oral route, and although trans-

mission remains primarily from person to person, waterborne and foodborne outbreaks of the disease have been reported (De Serres et al., 1999; Hutin et al., 1999; Fiore, 2004). HAV was originally classified as enterovirus type 72 because its biophysical characteristics are similar to those of enteroviruses. However, later studies demonstrated that HAV nucleotide and amino acid sequences are different from those of other picornaviruses, as are the predicted sizes of several HAV proteins (Cohen et al., 1987b). This virus is difficult to cultivate in cell cultures and usually replicates very slowly without producing CPE. It is resistant to temperatures and drugs that inactivate other picornaviruses and is stable at pH 1. There is only a single serotype of human HAV with one immunodominant neutralization site (Lemon and Binn, 1983). However, a significant degree of nucleic acid variability has been observed among different isolates from different regions of the world (Robertson et al., 1992; Costa-Mattioli et al., 2001). The molecular basis of this genetic variability may be the high error rate of the viral RNA-dependent RNA polymerase and the absence of proofreading mechanisms (Sánchez et al., 2003).

The virion is composed of a genome of linear, single-stranded RNA of messenger sense polarity, approximately 7.5 kb in length, and a capsid containing multiple copies of three or four proteins named VP1, VP2, VP3, and a putative VP4, encoded in the P1 region of the genome (Coulepis et al., 1982; Racaniello, 2001). The presence of a fourth protein VP4 has been described repeatedly, but the reported molecular weight (7–14 kDa) does not correspond to that predicted from nucleic acid sequence data (1.5 or 2.3 kDa) (Weitz and Siegl, 1998). The P2 and P3 regions encode for nonstructural proteins associated with replication.

HAV has a buoyant density of 1.32 to 1.34 g/cm^3 in CsCl and sediments with 156S during sucrose gradient centrifugation (Coulepis et al., 1982). Infectious viral particles with higher (1.44 g/cm^3) as well as lower (1.27 g/cm^3) buoyant densities have also been reported (Lemon et al., 1985). Phylogenetic analysis based on a 168-base segment encompassing the VP1/2A junction region of the HAV genome has established the classification of human and simian isolates into seven different genotypes (I–VII), with genotypes I, II, III, and VII including human isolates (Robertson et al., 1992). About 80% of the human isolates belong to genotype I, which has been subdivided into two subgenotypes, IA and IB (Fujiwara et al., 2003).

5.1. The Genome

Detailed analysis of the HAV genome has been accomplished with cloned or RT-PCR–amplified cDNA. Cultured cells can be infected with RNA transcribed from cloned HAV cDNA (Cohen et al., 1987a). HAV contains a genome that differs from that of Caliciviridae in that the genes encoding the nonstructural proteins are located at the 3′ portion of the genome, whereas those encoding the structural proteins are located at the 5′ end (Weitz and Siegl, 1998). The HAV genome is divided into a 5′ nontranslated region of 735 nucleotides, a long open reading frame of 6,681 nucleotides encoding

a polyprotein of 2,227 amino acids, and a 3′ nontranslated region 63 nucleotides in length.

The 5′-NTR contains a *cis*-acting internal ribosome entry site (IRES) that directs initiation of cap-independent translation directed to a particular AUG triplet several hundreds of nucleotides downstream (Glass et al., 1993; Brown et al., 1994; Ali et al., 2001; Borman et al., 2001; Kang and Funkhouser, 2002). It has been demonstrated that the IRES is located between nucleotides 151 and 734, and that it is able to direct internal initiation of translation in a cap-independent manner (Brown et al., 1994). However, a cap-dependent message effectively competes with the IRES of HAV (Glass et al., 1993). Translational efficiency of this IRES may be dependent on the availability of intact cellular proteins such as the p220 subunit of the eukaryotic initiation factor eIF-4F and requires association between the cap-binding translation initiation factor (eIF4E) and eIF4G (Ali et al., 2001; Borman et al., 2001). The 5′-NTR is the most highly conserved region in the HAV genome among all strains sequenced to date, with a 95% nucleotide identity. By contrast, the 3′-NTR region shows the highest (up to 20%) degree of variability. The presence of a 23-nucleotide-long *cis*-acting element has been described that specifically interacts with proteins involved in the establishment and maintenance of the persistent type of infection characteristic for replication of most HAV isolates in cell cultures. Several unidentified cytoplasmic and ribosomal proteins of infected cells bind to the 3′ end of HAV RNA, indicating an intimate and dynamic interaction between host proteins and viral RNA (Kusov et al., 1996). The poly(A) tail is also involved in formation of RNA/protein complexes.

In analogy with other picornaviruses, the coding region can be subdivided into P1, P2, and P3 regions, which specify proteins 1A–D, 2A–C, and 3A–D, respectively. The polyprotein is further processed to the four structural and seven nonstructural proteins by proteinases encoded in and around the 3C region (Probst et al., 1998). Proteins 1A–D correspond to structural proteins VP1–VP4. As in other picornaviruses, the 5′ end of HAV genome is covalently linked to VPg protein, which is specified by 3B, instead of the classical m7G cap structure (Weitz et al., 1986). The 2C gene carries a guanidine resistance marker and is assumed to play a role in viral RNA replication (Cohen et al., 1987b). The region 3D is considered the RNA-dependent RNA polymerase. This region shows the highest degree of homology (29%) with the corresponding sequence in the poliovirus type 1 protein (Cohen et al., 1987b). A region of 18 amino acids considered to be essential for an active polymerase is present in the 3D region. This sequence contains a conserved motif of two asparatate residues flanked by hydrophobic amino acids that might function as a GTP-binding domain (Kamer and Argos, 1984). Replication efficiency seems to be controled by amino acid substitutions in the 2B and 2C regions (Yokosuka, 2000).

5.2. Proteins

The genomes of all picornaviruses encode a single polyprotein, which is co- and post-translationally cleaved by virus-encoded proteinase(s). In contrast

with well-characterized proteolytic events in the polyprotein of Enterovirus and Rhinovirus, this processing has been difficult to characterize in Hepatovirus. It has been shown that the primary cleavage of HAV polyprotein occurs at the 2A/2B junction, which has been mapped by the N-terminal sequencing of 2B. This primary cleavage of the polyprotein is mediated by $3C^{pro}$ proteinase, which is the only proteinase known to be encoded by the virus (Martin et al., 1995; Gosert et al., 1996). The P1-2A capsid protein precursor is probably released from the nonstructural protein precursor (P3-2BC) as soon as $3C^{pro}$ is synthesized, as the full-length polyprotein has not been observed in these studies. A P1-2A precursor produced in a cell-free translation system has been shown to be cleaved *in vitro* by recombinant $3C^{pro}$ to generate VP0 (VP4–VP2), VP3, and VP1-2A (Malcolm et al., 1992). This VP1-2A polypeptide is unique to hepatoviruses; it associates with VP0 and VP3 to form pentamers, intermediates in the morphogenesis of HAV particles (Borovec and Andersen, 1993). The mature capsid protein VP1 is subsequently derived from the VP1-2A precursor later in the morphogenesis process. It has been hypothesized that the maturation of VP1 is dependent on $3C^{pro}$ processing of the VP1-2A precursor (Probst et al., 1998). However, using recombinant vaccinia viruses expressing relevant HAV substrates, it has also been shown that $3C^{pro}$ is incapable of directing the cleavage of VP1 from the VP1-2A precursor, indicating that maturation of VP1 could not depend on processing by $3C^{pro}$ proteinase (Martin et al., 1999).

5.3. Virus Replication

Hepatitis A virus typically has a protracted and noncytolytic replication cycle in cell cultures and fails to shut down host cell metabolism (Lemon and Robertson, 1993). Even after successful adaptation to grow in cell cultures, replication of HAV is a slow process that terminates in a state of persistent infection (de Chastonay and Siegl, 1987). Cytopathic HAV strains have been recovered only from persistently infected cell cultures. Maximal levels of viral RNA synthesis can be detected at 24 h after infection, and exponential production of progeny virus continues for up to 4 days postinfection. Lysis of infected cells may become apparent within 3 to 9 days postinfection, and yield of progeny virus rarely exceeds 10^7 $TCID_{50}$/ml (Siegl et al., 1984).

6.0. HEPATITIS E VIRUS

Hepatitis E virus (HEV), the prototype of the proposed Hepevirus, is a nonenveloped RNA virus, previously classified as a calicivirus but provisionally considered as a separate group of HEV-like viruses (Berke and Matson, 2000). Sequence comparison and phylogenetic analysis suggest that these viruses are more closely related to the Togaviridae (Koonin et al., 1992).

Hepatitis E virus is a major cause of epidemics and sporadic cases of acute hepatitis in Southeast and Central Asia, the Middle East, and many

areas of Africa and Mexico (Purcell and Emerson, 2001). Sporadic cases have also been identified in Europe, Japan, and the United States (Schlauder et al., 1998, 1999; Takahashi et al., 2001). Transmission of HEV infection during outbreaks primarily occurs through contaminated water. Provision of clean drinking water is considered the best preventive strategy. The illness is self-limited and no chronic HEV infection has been described, although high mortality rate has been observed in pregnant women, with death rate as high as 25% (Balayan, 1997). HEV has been isolated from humans and swine (Meng et al., 1997, 1998a; Hsieh et al., 1999; Garkavenko et al., 2001). Antibodies to the HEV capsid protein have been detected in many animal species, including rats and non-human primates (Kabrane-Lazizi et al., 1999a; Arankalle et al., 2001).

To date, four genotypes have been identified (Tam et al., 1991); a genotype 3 strain of HEV isolated from swine has been passed experimentally to monkeys, and a genotype 3 strain isolated from humans has been passed to swine (Meng et al., 1998b). Intriguingly, attempts to infect swine with other HEV human isolates have failed (Meng et al., 1998a). The question of whether HEV infection is a zoonosis is still being discussed (Meng, 2000). The viral genome is approximately 7.2 kb in length and has three partially overlapping ORFs: ORF1 encodes nonstructural proteins (1,693 amino acids), ORF2 encodes the capsid protein (660 amino acids), and ORF3 encodes a very small protein (123 amino acids) that has been shown to bind *in vitro* to a number of proteins involved in cellular signal transduction (Korkaya et al., 2001). The virus has not been propagated efficiently in cell cultures. Recently, various regions of the viral genome have been cloned and expressed *in vitro*. The ORF1 gene encodes the largest protein, which contains motifs characteristic of a methyl-transferase, a papain-like protease, a helicase, and an RNA-dependent RNA polymerase (Koonin et al., 1992). It has been demonstrated that the HEV genome contains a m^7G cap at its 5′ end (Kabrane-Lazizi et al., 1999b; Zhang et al., 2001). There is still controversy over whether the 5′ cap is critical for HEV replication. Although it has been shown that the cap is required for infectivity of recombinant genomes *in vivo*, uncapped viral genomes can replicate in transfected HepG2 cells (Panda et al., 2000; Emerson et al., 2001).

The full-length ORF2 gene encodes a 72-kDa capsid protein consisting of 660 amino acids. ORF2 is the major, if not the only protein in the virion, but its real size in infectious virions is not known. When synthesized in insect cells, the initial 72-kDa protein is processed to smaller proteins of approximately 63, 56, and 53 kDa (Robinson et al., 1998). It has been demostrated that antibodies against ORF2 antibodies can neutralize HEV (Mast et al., 1998; Meng et al., 2001). Persons with preexisting anti-ORF2 did not develop hepatitis E after exposure to HEV in an outbreak in Pakistan (Bryan et al., 1994). Similarly, anti-ORF2–positive monkeys did not develop hepatitis after challenge with infectious HEV (Tsarev et al., 1994; Arankalle et al., 1999). A number of antigenic determinants have been identified within ORF2 (Khudyakov et al., 1999; Li et al., 2000; Riddell et al., 2000). However, it

appears that only antibodies directed against the C-terminus of ORF2 are neutralizing (Mast et al., 1998). The ORF2-encoded 56-kDa protein, truncated at its N- and C-termini, served as a highly reactive antigen in detecting anti-HEV antibodies (Zhang et al., 1997).

The ORF3 protein contains only 123 amino acids, and its biological role has yet to be elucidated. Recombinant ORF3 protein expressed in eukaryotic cells is an immunogenic phosphoprotein that accumulates in the cytoplasm and associates with the cytoskeleton (Zafrullah et al., 1997). This association is dependent on amino acids 1 to 32 of ORF3, which comprise a hydrophobic domain. In addition, ORF3 contains two conserved proline-rich domains. By the yeast two-hybrid assay, it has been demonstrated that the second of these proline-rich domains binds to certain Src homology 3 domains present in a subset of cellular proteins. It has been hypothesized that ORF3 may be a viral regulatory protein involved in cellular signal transduction (Korkaya et al., 2001). Also, recombinant ORF3 protein has been shown to interact in yeast two-hybrid assays and by co-immunoprecipitation assays with the nonglycosylated form of the major capsid protein, ORF2 protein (Tyagi et al., 2002). These observations lead to the hypothesis that ORF3 has a well-regulated role in HEV structural assembly (Emerson and Purcell, 2003). One HEV vaccine candidate, a baculovirus-expressed protein spanning 112–607 amino acids of ORF2, was demonstrated to be safe and immunogenic in volunteers and is currently being evaluated in clinical trials in Nepal (Emerson and Purcell, 2003).

7.0. ENTERIC ADENOVIRUSES

Adenoviruses are nonenveloped, icosahedral viruses measuring 70 to 90 nm in diameter. They have a buoyant density in cesium chloride of 1.33 to 1.34 g/cm^3. The capsid is composed of 252 capsomeres, of which 240 are hexons and 12 are pentons. Inside the capsid is a single molecule of linear, double-stranded DNA (Shenk, 2001). Two genera are recognized within the Adenoviridae family: Mastadenovirus includes viruses that infect mammals, and Aviadenovirus contains viruses that infect birds (Benkö et al., 2000). Adenoviruses are species-specific and generally replicate only in cells derived from their native host. Human adenoviruses are associated with a variety of infectious diseases affecting the respiratory, urinary, and the gastrointestinal tracts and the eyes (Horwitz, 2001). To date, 51 serotypes of human adenoviruses have been recognized, which are classified into six subgroups, A to F, based on immunological properties, oncogenicity in rodents, DNA homologies, and morphological properties (Benkö et al., 2000).

Enteric adenoviruses were originally identified in stool samples of infants with acute gastroenteritis (Flewett et al., 1973) and have been consistently associated with gastroenteritis in children through epidemiological and clinical studies (Uhnoo et al., 1983; Uhnoo et al., 1984). They are responsible for 5% to 20% cases of acute diarrhea in children (Uhnoo et al., 1984; Kotloff

et al., 1989; Uhnoo et al., 1990; Bon et al., 1999) and are found in clinical samples throughout the year with little seasonal variation (de Jong et al., 1983).

The enteric serotypes 40 and 41 have been designated as subgroup F adenoviruses. They share the adenovirus group antigen and are distinguished from each other and from other nonenteric serotypes by serology and DNA restriction patterns (Wadell, 1984). These two serotypes are shed in large numbers from the gut of infected patients and were originally described as being noncultivable or "fastidious" adenoviruses, because they could not be cultivated in cell cultures that generally supported the propagation of other adenovirus types. Later, however, it was discovered that enteric adenoviruses could be propagated in Graham 293 cells, a cell line of human embrionic kidney (HEK) cells transformed with adenovirus 5 early (E) region 1 (Graham et al., 1977; Takiff et al., 1981), although at lower levels than other serotypes. This suggests that E1 functions are poorly expressed in cells infected with adenovirus types 40 and 41, and therefore it was postulated that the inability of these serotypes to grow in cell lines normally supportive for other adenovirus types was due to the relative inability of the adenovirus 41 E1A gene to transactivate other adenovirus 41 genes (Takiff et al., 1984; van Loon et al., 1985a). The Graham 293 cell retains the E1A and E1B regions of the adenovirus genome covalently linked to the host DNA. The mechanism of facilitation of the growth of the EAd40 in 293 cells seems to be a function of the E1B-55 kd protein (Mautner et al., 1989). Efficient replication of adenovirus types 40 and 41 has also been achieved in other cell lines, including Hep-2 cells, Chang conjuntiva cells, CaCo-2 cells (Perron-Henry et al., 1988; Pintó et al., 1994), and PLC/PRF/5 cells (a primary liver carcinoma cell line) (Grabow et al., 1992).

The genome of adenovirus 40 has been sequenced (Davidson et al., 1993) (GenBank accession no. L19443) and described in detail (Mautner et al., 1995). The main difference between adenovirus 40 and the other human adenovirus serotypes is the presence of two distinct fiber genes, a single VA gene involved in late translation and a highly divergent E3 region. The growth restriction of adenovirus 41 in cell cultures seems to be less severe than that of serotype 40 because a number of cell lines have been found to support the propagation of serotype 41 but not of serotype 40 (de Jong et al., 1983; Uhnoo et al., 1983; van Loon et al., 1985b). The blockade in adenovirus 41 replication occurs within the early phase of the infectious cycle (Tiemessen et al., 1996). In a study on the ability of adenovirus 40 E1A encoded products (proteins 249R and 221R) for *trans*-activation (van Loon et al., 1987; Ishino et al., 1988), it was found that the adenovirus 40 E1A promoter does indeed contain transcription factor binding sites sufficient for *trans*-activation by the adenovirus 5 E1A 289R protein. It is possible that adenovirus 40 has evolved to use components of the RNA processing machinery that are unique to enterocytes (Stevenson and Mautner, 2003). Hence, a better understanding of the replication and pathogenicity of adenovirus 40 will require the development of intestinal cell cultures.

8.0. OTHER ENTERIC VIRUSES

Although rotaviruses, caliciviruses, astroviruses, and enteric adenoviruses are the most prominent viral pathogens of acute gastroenteritis, other candidates such as coronaviruses, toroviruses, and picobirnaviruses are also considered to have capability to cause diarrhea in humans.

8.1. Coronaviruses

Coronavirus is a genus of the Coronaviridae family in the order Nidovirales (Enjuanes et al., 2000). The first reports of coronavirus-like particles in stools of patients with diarrhea were documented by Caul and Clarke (1975), who reported cultivation of these viral particles in human embryo intestinal organ culture and human embryo kidney monolayers (Caul and Clarke, 1975). Since then, the existence of human enteric coronavirus (HEC) has been controversial (Mathan et al., 1975; Caul and Egglestone, 1977). Some investigators have considered these particles as viruses and as potential pathogens in human diarrhea, necrotizing enterocolitis, and malabsorption. Other authors cite their variable size, their presence in stools of normal subjects, and their failure to be cultivated *in vitro* as evidence that they might represent cell debris or even portions of other microorganisms (Macnaughton and Davies, 1981). However, isolation and propagation of enteric coronaviruses have been reported (Resta et al., 1985; Luby et al., 1999). The enveloped particles are 120–160 nm in diameter and possess a genome of linear, positive-sense, single-stranded RNA of approximately 30 kb in size. The genome is surrounded by the nucleocapsid protein (N) with helical symmetry, which is contained in an envelope, in which the spike (S), envelope (E), hemagglutinin esterase (HE), and membrane (M) proteins are embedded. Proteins are expressed from RNA molecules that are in most cases subgenomic, and all mRNAs carry a leader sequence derived from the 5′ end of the genome (Lai and Holmes, 2001).

8.2. Toroviruses

Toroviruses (family Coronaviridae, order Nidovirales) are enveloped, positive-sense RNA viruses that have been implicated in enteric disease in cattle and possibly in humans. Despite their potential veterinary and clinical relevance, little is known about the epidemiology and molecular genetics of toroviruses (Koopmans and Horzinek, 1994; Smits et al., 2003).

Toroviruses have not been propagated in cell cultures, with the sole exception so far of the equine Berne virus. Early seroepidemiological surveys based on Berne virus cross-neutralization assays and enzyme-linked immunosorbent assay (ELISA) indicated that toroviruses occur in a wide variety of ungulate hosts (cattle, sheep, goats, and swine) (Koopmans and Horzinek, 1994). Breda virus was detected in the stools of diarrheic calves but could not be isolated in cell cultures (Woode et al., 1982). Thereafter, two antigenically distinct serotypes of Breda virus have been identified, referred to as bovine toroviruses 1 and 2 (BoTV-1 and BoTV-2) (Woode et al., 1985).

Morphologically similar pleomorphic particles characterized by a well-defined fringe around the outer edges have also been found in human stools (Beards et al., 1984; Duckmanton et al., 1997). These viruses have a unique morphology with a helical nucleocapsid in a torus-shaped configuration within an envelope carrying large spikes. In samples from children and adults with diarrhea, torovirus-like particles cross-reactive with Berne virus–specific and Breda virus–specific antisera were detected by immunoelectron microscopy (Beards et al., 1984; Beards et al., 1986; Duckmanton et al., 1997), and torovirus antigens were detected by ELISA (Koopmans et al., 1997). Recently, the nucleotide sequence of the Berne virus genomic RNA has been completed (Smits et al., 2003).

With a genome length of 28 kb, toroviruses are among the largest RNA viruses, rivaled in size only by the coronaviruses. The 5′-most two-thirds of the genome is taken up by two huge overlapping open reading frames, 1a and 1b, encoding polyproteins from which various subunits of the viral replicase/transcriptase are derived (Snijder et al., 1990a). Downstream of ORF1b, there are four cistrons of 5 kb, 0.7 kb, 1.2 kb, and 0.5 kb (as ordered from 5′ to 3′). These encode the structural proteins, the spike (S), membrane (M), hemagglutinin-esterase (HE), and nucleocapsid (N) proteins, respectively (Snijder and Horzinek, 1993). The structural proteins are translated from a 3′-coterminal nested set of subgenomic mRNAs, produced by discontinuous and nondiscontinuous RNA synthesis (Snijder et al., 1990b; van Vliet et al., 2002). Four genotypes, displaying 30% to 40% sequence divergence, have been recently distinguished, exemplified by bovine torovirus (BToV) Breda, porcine torovirus (PToV), equine torovirus Berne, and the putative human torovirus (Smits et al., 2003). It remains unclear, however, whether the toroviruses are host specific and how the torovirus genotypes are geographically distributed.

8.3. Picobirnaviruses

Picobirnavirus is the tentative name for a new virus genus in the family Birnaviridae (Leong et al., 2000). Picobirnaviruses were first observed in 1988 in fecal samples of humans and rats (Pereira et al., 1988a, 1988b). The viruses have subsequently been found in many mammals and birds (Rosen, 2003) and have been frequently detected in cases of human gastroenteritis associated with human immunodeficiency virus infections (HIV) (Grohmann et al., 1993; Gonzalez et al., 1998; Liste et al., 2000). Association of picobirnavirus with nonbacterial gastroenteritis outbreaks has also been reported recently (Banyai et al., 2003).

Morphological, physicochemical, and genomic characteristics suggest that picobirnaviruses belong to a distinct group of viruses. The small size of the virion and the nature of the viral genome prompted the name picobirnaviruses, referring to *pico* (small) and *birna* (two RNA segments). The virion is 25–41 nm in diameter, nonenveloped, and has a buoyant density of 1.38 to 1.42 g/cm^3 (Pereira et al., 1988b; Ludert et al., 1995). However, virions with a diameter of around 35 nm have been found most often (Ludert et al.,

1991; Gallimore et al., 1995). The genome consists of two segments of double-stranded RNA with lengths estimated around 2.3–2.6 Kpb and 1.5–1.9 Kpb, respectively (Gallimore et al., 1995; Chandra, 1997). These viruses are non-cultivable. They are detected by EM and polyacrylamide gel electrophoresis (PAGE) (Ludert and Liprandi, 1993; Gallimore et al., 1995; Cascio et al., 1996). Picobirnavirus genomic fragments migrate in PAGE within the migration range of the genomic segments of group A rotaviruses (Pereira et al., 1988a; Ludert et al., 1991; Rosen et al., 2000). This fact led to the discovery of these viruses when fecal samples containing both rotaviruses and picobirnaviruses were subjected to PAGE (Pereira et al., 1988a, 1988b). Currently, RT-PCR is used to detect human picobirnaviruses in fecal samples (Rosen et al., 2000).

Limited information is available on the genetic sequence of picobirnaviruses. To date, genomic segment 1 of a rabbit and a human picobirnavirus and segment 2 of two human picobirnaviruses have been cloned (Green et al., 1999; Rosen et al., 2000). Human picobirnavirus sequences have been determined mostly for the smaller genomic segment that encodes the RNA-dependent RNA polymerase (Rosen et al., 2000). The genomic segment 1 has been postulated to encode for the capsid protein (Green et al., 1999; Rosen et al., 2000). Based on the sequence data of the RNA polymerase, there are two major genogroups within human picobirnaviruses, for which prototype strains are 1-CHN-97 and 4-GA-91 (Rosen et al., 2000).

9.0. SUMMARY

Food-borne and waterborne viruses mainly cause gastroenteritis (caliciviruses, rotaviruses, astroviruses, and enteric adenoviruses), hepatitis (hepatitis A virus, hepatitis E virus), and other diseases (enteroviruses). Other enteric viruses like kobuviruses, coronaviruses, toroviruses, and picornaviruses also cause diarrhea, although the causative role for some of these viruses in humans is still controversial.

Human caliciviruses have been recognized as the leading cause of acute gastroenteritis outbreaks and sporadic cases in children and adults worldwide. Enteric caliciviruses belonging to the Norovirus and Sapovirus genera remain refractory to cell culture propagation. This limitation has hampered our ability to investigate their biology, pathogenesis, and host immunity, although molecular approaches are providing new insights into these areas. The morphology, composition, and structure of several enteric viruses have been elucidated in recent years. Cryo-electron microscopy and x-ray crystallography have been crucial for this purpose. Biochemical and structural studies of virus-like particles produced by recombinant baculoviruses are contributing to better understand the structure-function relationships of the capsid proteins. Viral genome organization is being clarified for all these viruses, as well as their replication and gene expression strategies. Most of the proteins encoded by the viral genomes have been characterized and their

functions identified. Sequence analysis of viral genes is currently being applied in molecular epidemiological studies. However, many questions still remain to be answered. The development of efficient reverse genetics systems would be extremely useful in the analysis of the mechanisms of viral replication and of gene expression of the different enteric viruses.

Biochemical characterization of viral interactions with cells and analysis of the functional properties of the viral proteins are providing a better understanding of the pathogenesis of enteric viruses. Rotavirus NSP4 is the first viral enterotoxin to be characterized. Several cell membrane molecules have been identified recently as being receptors for different enteric viruses (i.e., integrins and hsc70 for rotaviruses, ABH histo-blood group carbohydrates for noroviruses). Studies on human susceptibility to norovirus infections have characterized some resistant nonsecretor (Se-) individuals in the population, which is a breakthrough in our knowledge of norovirus-host interactions. Similarly, molecular analyses of orally transmitted viruses causing hepatitis are clarifying the phylogenetic relationships between these viruses and other viral genera, as well as their pathophysiological mechanisms.

10.0. REFERENCES

Adah, M. I., Wade, A., and Taniguchi, K., 2001, Molecular epidemiology of rotaviruses in Nigeria: detection of unusual strains with G2P[6] and G8P[1] specificities. *J. Virol.* 39:3969–3975.

Ali, I. K., McKendrick, L., Morley, S. J., and Jackson, R. J., 2001, Activity of the hepatitis A virus IRES requires association between the cap-binding translation initiation factor (eIF4E) and eIF4G. *J. Virol.* 75:7854–7863.

Amano, J., and Oshima, M., 1999, Expression of the H type 1 blood group antigen during enterocyte differentiation of Caco-2 cells. *J. Biol. Chem.* 274:21209–21216.

Ando, T., Monroe, S. S., Gentsch, J. R., Jin, Q., Lewis, D. C., and Glass, R. I., 1995, Detection and differentiation of antigenically distinct small round-structured viruses (Norwalk-like viruses) by reverse transcription-PCR and southern hybridization. *J. Clin. Microbiol.* 33:64–71.

Ando, T., Noel, J. S., and Fankhauser, R. L., 2000, Genetic classification of "Norwalk-like viruses." *J. Infect. Dis.* 181(Suppl 2):S336–S348.

Appleton, H., and Higgins, P. G., 1975, Viruses and gastroenteritis in infants. *Lancet* 1:1297.

Arankalle, V. A., Chadha, M. S., and Chobe, L. P., 1999, Long-term serological follow up and cross-challenge studies in rhesus monkeys experimentally infected with hepatitis E virus. *J. Hepatol.* 30:199–204.

Arankalle, V. A., Joshi, Y. K., Kulkarni, A. M., Gandhe, S. S., Chobe, L. P., Rautmare, S. S., Mishra, A. C., and Padbhidri, S. P., 2001, Prevalence of anti-hepatitis E virus antibodies in different Indian animal species. *J. Viral Hepatol.* 8:223–227.

Armah, G. E., Steele, A. D., Binka, F. N., Esona, M. D., Asmah, R. H., Anto, F., Brown, D., Green, J., Cutts, F., and Hall, A., 2003, Changing patterns of rotavirus genotypes in Ghana: emergence of human rotavirus G9 as a major cause of diarrhea in children. *J. Clin. Microbiol.* 41:2317–2322.

Ashley, C. R., and Caul, E. O., 1982, Potassium tartrate-glycerol as a density gradient substrate for separation of small, round viruses from human feces. *J. Clin. Microbiol.* 16:377–381.

Atmar, R. L., and Estes, M. K., 2001, Diagnosis of noncultivatable gastroenteritis viruses, the human caliciviruses. *Clin. Microbiol. Rev.* 14:15–37.

Balayan, M. S., 1997, Epidemiology of hepatitis E virus infection. *J. Viral Hepatol.* 4:155–165.

Ball, J. M., Tian, P., Zeng, C. Q.-Y., Morris, A. P., and Estes, M. K., 1996, Age-dependent diarrhea induced by a rotaviral nonstructural glycoprotein. *Science* 272:101–103.

Banyai, K., Jakab, F., Reuter, G., Bene, J., Uj, M., Melegh, B., and Szucs, G., 2003, Sequence heterogeneity among human picobirnaviruses detected in a gastroenteritis outbreak. *Arch. Virol.* 148:2281–2291.

Bass, D. M., and Qiu, S., 2000, Proteolytic processing of the astrovirus capsid. *J. Virol.* 74:1810–1814.

Beards, G. M., Hall, C., Green, J., Flewett, T. H., Lamouliatte, F., and Du Pasquier, P., 1984, An enveloped virus in stools of children and adults with gastroenteritis that resembles the Breda virus of calves. *Lancet* 1:1050–1052.

Beards, G. M., Brown, D. W., Green, J., and Flewett, T. H., 1986, Preliminary characterisation of torovirus-like particles of humans: comparison with Berne virus of horses and Breda virus of calves. *J. Med. Virol.* 20:67–78.

Belliot, G., Laveran, H., and Monroe, S. S., 1997, Detection and genetic differentiation of human astroviruses: phylogenetic grouping varies by coding region. *Arch. Virol.* 142:1323–1334.

Benkö, M., Harrach, B., and Russell, W. C., 2000, Family *Adenoviridae*, in: *Virus Taxonomy. Classification and Nomenclature of Viruses. Seventh Report of the International Committe on Taxonomy of Viruses* (M. H. V. van Regenmortel, C. M. Fauquet, D. H. L. Bishop, E. B. Carstens, M. K. Estes, S. M. Lemon, J. Maniloff, M. A. Mayo, D. J. McGeoch, C. R. Pringle, and R. B. Wickner, eds.), Academic Press, San Diego, pp. 227–238.

Berke, T., and Matson, D. O., 2000, Reclassification of the Caliciviridae into distinct genera and exclusion of hepatitis E virus from the family on the basis of comparative phylogenetic analysis. *Arch. Virol.* 145:1421–1436.

Berke, T., Golding, B., Jiang, X., Cubitt, D. W., Wolfaardt, M., Smith, A. W., and Matson, D. O., 1997, Phylogenetic analysis of the Caliciviruses. *J. Med. Virol.* 52:419–424.

Bertolotti-Ciarlet, A., White, L. J., Chen, R., Prasad, B. V., and Estes, M. K., 2002, Structural requirements for the assembly of Norwalk virus-like particles. *J. Virol.* 76:4044–4055.

Bertolotti-Ciarlet, A., Chen, R., Estes, M. K., and Prasad, B. V., 2003, Structure of Norwalk virus: the prototype human calicivirus, in: *Viral Gastroenteritis* (U. Desselberger and J. Gray, eds.), Elsevier, Amsterdam, pp. 455–466.

Bishop, R. F., 1996, Natural history of human rotavirus infections. *Arch. Virol*. Suppl. 12:119–128.

Black, D. N., Burroughs, J. N., Harris, T. J. R., and Brown, F., 1978, The structure and replication of calicivirus RNA. *Nature* 274:614–615.

Blackhall, J., Fuentes, A., and Magnusson, G., 1996, Genetic stability of a porcine rotavirus RNA segment during repeated plaque isolation. *Virology* 225:181–190.

Blacklow, N. R., Dolin, R., Fedson, D. S., DuPont, H. L., Northrup, R. S., Hornick, R. B., and Chanock, R. M., 1972, Acute infectious nonbaterial gastroenteritis: etiology and pathogenesis. *Ann. Intern. Med.* 76:993–1008.

Blutt, S. E., Kirkwood, C. D., Parreño, V., Warfield, K. L., Ciarlet, M., Estes, M. K., Bok, K., Bishop, R. F., and Conner, M. E., 2003, Rotavirus antigenaemia and viraemia: a common event? *Lancet* 362:1445–1449.

Blutt, S. E., Crawfird, S. E., Warfield, K. L., Lewis, D. E., Estes, M. K., and Conner, M. E., 2004, The VP7 outer capsid protein of rotavirus induces polyclonal B-cell activation. *J. Virol.* 78:6974–6981.

Bon, F., Fascia, P., Dauvergne, M., Tenenbaum, D., Planson, H., Petion, A. M., Pothier, P., and Kohli, E., 1999, Prevalence of group A rotavirus, human calicivirus, astrovirus, and adenovirus type 40 and 41 infections among children with acute gastroenteritis in Dijon, France. *J. Clin. Microbiol.* 37:3055–3058.

Borman, A. M., Michel, Y. M., and Kean, K. M., 2001, Detailed analysis of the requirements of hepatitis A virus internal ribosome entry segment for the eukaryotic initiation factor complex eIF4F. *J. Virol.* 75:7864–7871.

Borovec, S. V., and Andersen, D. A., 1993, Synthesis and assembly of hepatitis A virus-specific proteins in BS-C-1 cells. *J. Virol.* 67:3095–3102.

Brown, E. A., Zajac, A. J., and Lemon, S. M., 1994, In vitro characterization of an internal ribosomal entry site (IRES) present within the 5′ nontranslated region of hepatitis A virus RNA—comparison with the IRES of encephalomyocarditis virus. *J. Virol.* 68:1066–1074.

Browne, E. P., Bellamy, R., and Taylor, J. A., 2000, Membrane-destabilizing activity of rotavirus NSP4 is mediated by a membrane-proximal amphipatic domain. *J. Gen. Virol.* 81:1955–1959.

Brunet, J.-P., Cotte-Laffitte, J., Linxe, C., Quero, A.-M., Géniteau-Legendre, M., and Servin, A., 2000, Rotavirus infection induces an increase in intracellular calcium concentration in human intestinal epithelial cells: role in microvillar actin alteration. *J. Virol.* 74:2323–2332.

Bryan, J., Tsarev, S., Iqbal, M., Ticehurst, J., Emerson, S., Ahmed, A., Duncan, J., Rafiqui, A. R., Malik, I. A., and Purcell, R. H., 1994, Epidemic hepatitis E in Pakistan: patterns of serologic response and evidence that antibody to hepatitis E virus protects against disease. *J. Infect. Dis.* 170:517–521.

Buesa, J., de Souza, C. O., Asensi, M., Martínez, C., Prat, J., Gil, M. T., 2000, VP7 and VP4 genotypes among rotavirus strains recovered from children with gastroenteritis over a 3-year period in Valencia, Spain. *Eur. J. Epidemiol.* 16:501–506.

Buesa, J., Collado, B., López-Andújar, P., Abu-Mallouh, R., Rodríguez Díaz, J., García Díaz, A., Prat, J., Guix, S., Llovet, T., Prats, G., and Bosch, A., 2002, Molecular epidemiology of caliciviruses causing outbreaks and sporadic cases of acute gastroenteritis in Spain. *J. Clin. Microbiol.* 40:2854–2859.

Burke, B., and Desselberger, U., 1996, Rotavirus pathogenicity. *Virology* 218:299–305.

Caballero, S., Guix, S., El-Senousy, W. M., Calicó, I., Pintó, R. M., and Bosch, A., 2003, Persistent gastroenteritis in children infected with astrovirus: association with serotype-3 strains. *J. Med. Virol.* 71:245–250.

Carter, M. J., 1990, Transcription of feline calicivirus RNA. *Arch. Virol.* 114:143–152.

Cascio, A., Bosco, M., Vizzi, E., Giammanco, A., Ferraro, D., and Arista, S., 1996, Identification of picobirnavirus from faeces of Italian children suffering from acute diarrhea. *Eur. J. Epidemiol.* 12:545–547.

Castello, A. A., Argüelles, M. H., Villegas, G. A., López, N., Ghiringhelli, D. P., Semorile, L., and Glikmann, G., 2000, Characterization of human group C rotavirus in Argentina. *J. Med. Virol.* 62:199–207.

Caul, E. O., and Appleton, H., 1982, The electron microscopical and physical characteristics of small round human fecal viruses: an interim scheme for classification. *J. Med. Virol.* 9:257–265.

Caul, E. O., and Clarke, S. K. R., 1975, Coronavirus propagated from patient with nonbacterial gastroenteritis. *Lancet* 2:953–954.

Caul, E. O., and Egglestone, S. I., 1977, Further studies on human enteric coronaviruses. *Arch. Virol.* 54:107–117.

Chandra, R., 1997, Picobirnavirus, a novel group of undescribed viruses of mammals and birds: a minireview. *Acta Virol.* 41:59–62.

Charpilienne, A., Lepault, J., Rey, F., and Cohen, J., 2002, Identification of rotavirus VP6 residues located at the interface with VP2 that are essential for capsid assembly and transcriptase activity. *J. Virol.* 76:7822–7831.

Chen, D., and Patton, J. T., 1998, Rotavirus RNA replication requires a single-stranded 3′ end for efficient minus-strand synthesis. *J. Virol.* 72:7387–7396.

Chen, D., Estes, M. K., and Ramig, R. F., 1992, Specific interactions between rotavirus outer capsid proteins VP4 and VP7 determine expression of a cross-reactive, neutralizing VP4-specific epitope. *J. Virol.* 66:432–439.

Chen, D. Y., Zeng, C. Q.-Y., Wentz, M. J., Gorziglia, M., Estes, M. K., and Ramig, R. F., 1994, Template-dependent, *in vitro* replication of rotavirus RNA, *J. Virol.* 68:7030–7039.

Chen, D., Barros, M., Spence, E., and Patton, J. T., 2001, Features of the 3′-consensus sequence of rotavirus mRNAs critical to minus strand synthesis. *Virology* 281:221–229.

Chen, R., Neill, J. D., Noel, J. S., Hutson, A. M., Glass, R. I., Estes, M. K., and Prasad, B. V., 2004, Inter- and intragenus structural variations in caliciviruses and their functional implications. *J. Virol.* 78:6469–6479.

Chiba, S., Sakuma, Y., Kogasaka, R., Akihara, M., Horino, K., Nakao, T., and Fukui, S., 1979, An outbreak of gastroenteritis associated with calicivirus in an infant home. *J. Med. Virol.* 4:249–254.

Ciarlet, M., and Estes, M. K., 1999, Human and most animal rotavirus strains do not require the presence of sialic acid on the cell surface for efficient infectivity. *J. Gen. Virol.* 80:943–948.

Ciarlet, M., and Estes, M., 2001, Interactions between rotavirus and gastrointestinal cells. *Curr. Opin. Microbiol.* 4:435–441.

Ciarlet, M., Crawford, S. E., and Estes, M., 2001, Differential infection of polarized epithelial cell lines by sialic acid-dependent and sialic acid-independent rotavirus strains. *J. Virol.* 75:11834–11850.

Ciarlet, M., Crawford, S. E., Cheng, E., Blutt, S. E., Rice, D. A., Bergelson, J. M., and Estes, M. K., 2002a, VLA-2 ($\alpha 2\beta 1$) integrin promotes rotavirus entry into cells but is not necessary for rotavirus attachment. *J. Virol.* 76:1109–1123.

Ciarlet, M., Ludert, J. E., Iturriza-Gómara, M., Liprandi, F., Gray, J. J., Desselberger, U., and Estes, M. K., 2002b, Initial interaction of rotavirus strains with N-acetylneuraminic (sialic) acid residues on the cell surface correlates with VP4 genotype, not species of origin. *J. Virol.* 76:4087–4095.

Clarke, I. N., and Lambden, P. R., 1997, Viral zoonoses and food of animal origin: caliciviruses and human disease. *Arch. Virol.* Suppl 13:141–152.

Clarke, I. N., and Lambden, P. R., 2000, Organization and expression of calicivirus genes. *J. Infect. Dis.* 181(Suppl 2):S309–316.

Clarke, I. N., and Lambden, P. R., 2001, The molecular biology of human caliciviruses. *Novartis Found. Symp.* 238:180–191; discussion 191–196.

Cohen, J. I., Ticehurst, J. R., Feinstone, S. M., Rosenblum, B., and Purcell, R. H., 1987a, Hepatitis A virus cDNA and its RNA transcripts are infectious in cell culture. *J. Virol.* 61:3035–3039.

Cohen, J. I., Ticehurst, J. R., Purcell, R. H., Buckler-White, A., and Baroudy, B. M., 1987b, Complete nucleotide sequence of wild-type hepatitis A virus: comparison with different strains of hepatitis A virus and other picornaviruses. *J. Virol.* 61:50–59.

Coppo, P., Scieux, C., Ferchal, F., Clauvel, J., and Lassoued, K., 2000, Astrovirus enteritis in a chronic lymphocytic leukemia patient treated with fludarabine monophosphate. *Ann. Hematol.* 79:43–45.

Costa-Mattioli, M., Ferre, V., Monpoeho, S., Garcia, L., Colina, R., Billaudel, S., Vega, I., Perez-Bercoff, R., and Cristina, J., 2001, Genetic variability of hepatitis A virus in South America reveals heterogeneity and co-circulation during epidemic outbreaks. *J. Gen. Virol.* 82:2647–2652.

Coulepis, A. G., Locarnini, S. A., Westaway, E. G., Tannock, G. A., and Gust, I. D., 1982, Biophysical and biochemical characterization of hepatitis A virus. *Intervirology* 18:107–127.

Coulson, B. S., Londrigan, S. L., and Lee, D. J., 1997, Rotavirus contains integrin ligand sequences and a disintegrin-like domain that are implicated in virus entry into cells. *Proc. Natl. Acad. Sci. U.S.A.* 94:5389–5394.

Crawford, S. E., Mukherjee, S. K., Estes, M. K., Lawton, J. A., Shaw, A. L., Ramig, R. F., and Prasad, V. V., 2001, Trypsin cleavage stabilizes the rotavirus VP4 spike. *J. Virol.* 75:6052–6061.

Cubitt, W. D., Mitchell, D. K., Carter, M. J., Willcocks, M. M., and Holzel, H., 1999, Application of electronmicroscopy, enzyme immunoassay, and RT-PCR to monitor an outbreak of astrovirus type 1 in a paediatric bone marrow transplant unit. *J. Med. Virol.* 57:313–321.

Cunliffe, N. A., Woods, P. A., Leite, J. P. G., Das, B. K., Ramachandran, M., Bhan, M. K., Hart, C. A., Glass, R. I., and Gentsch, J. R., 1997, Sequence analysis of NSP4 gene of human rotavirus allows classification into two main genetic groups. *J. Med. Virol.* 53:41–50.

Cunliffe, N. A., Gondwe, J. S., Broadhead, R. L., Molyneaux, M. E., Woods, P. A., Bresee, J. S., Glass, R. I., Gentsch, J. R., and Hart, C. A., 1999, Rotavirus G and P types in children with acute diarrhea in Blantyre, Malawi, from 1997 to 1998: predominance of novel P[6]G8 strains. *J. Med. Virol.* 57:308–312.

Dalton, R. M., Pastrana, E. P., and Sanchez-Fauquier, A., 2003, Vaccinia virus recombinant expressing an 87-kilodalton polyprotein that is sufficient to form astrovirus-like particles. *J. Virol.* 77:9094–9098.

Dastjerdi, A. M., Green, J., Gallimore, C. I., Brown, D. W., and Bridger, J. C., 1999, The bovine Newbury agent-2 is genetically more closely related to human SRSVs than to animal caliciviruses. *Virology* 254:1–5.

Daughenbaugh, K. F., Fraser, C. S., Hershey, J. W., and Hardy, M. E., 2003, The genome-linked protein VPg of the Norwalk virus binds eIF3, suggesting its role in translation initiation complex recruitment. *EMBO J.* 22:2852–2859.

Davidson, A. J., Telford, E. A. R., Watson, M. S., McBride, K., and Mautner, V., 1993, The DNA sequence of adenovirus type 40. *J. Mol. Biol.* 234:1308–1316.

de Chastonay, J., and Siegl, G., 1987, Replicative events in hepatitis A virus-infected MRC-5 cells. *Virology* 157:268–275.

de Jong, J. C., Wigand, R., Kidd, A. H., Wadell, G., Kapsenberg, J. G., Muzerie, C. J., Wermenbol, A. G., and Firtzlaff, R. G., 1983, Candidate adenoviruses 40 and 41: fastidious adenoviruses from human infant stool. *J. Med. Virol.* 11:215–231.

De Serres, G., Cromeans, T. L., Levesque, B., Brassard, N., Barthe, C., Dionne, M., Prud'homme, H., Paradis, D., Shapiro, C. N., Nainan, O. V., and Margolis, H. S., 1999, Molecular confirmation of hepatitis A virus from well water: epidemiology and public health implications. *J. Infect. Dis.* 179:37–43.

Denisova, E., Dowling, W., LaMonica, R., Shaw, R., Scarlata, S., Ruggeri, F., and Mackow, E. R., 1999, Rotavirus capsid protein VP5* permeabilizes membranes. *J. Virol.* 73:3147–3153.

Desselberger, U., 1996, Genome rearrangements of rotaviruses. *Adv. Virus Res.* 46:69–95.

Dolin, R., Levy, A. G., Wyatt, T. S., Thornhill, T. S., and Gardner, J. D., 1975, Viral gastroenteritis induced by the Hawaii agent. Jejunal histopathology and serologic response. *Am. J. Med.* 59:761–768.

Dormitzer, P. R., Greenberg, H. B., and Harrison, S. C., 2001, Proteolysis of monomeric recombinant rotavirus VP4 yields an oligomeric VP5* core. *J. Virol.* 75:7339–7350.

Dowdle, W. R., Cochi, S. L., Oberste, S., and Sutter, R., 2001, Preventing polio from becoming a reemerging disease. *Emerg. Infect. Dis.* 7:549–550.

Duckmanton, L., Luan, B., Devenish, J., Tellier, R., and Petric, M., 1997, Characterization of torovirus from human fecal specimens. *Virology* 239:158–168.

Duizer, E., Schwab, K. J., Neill, F. H., Atmar, R. L., Koopmans, M. P., and Estes, M. K., 2004, Laboratory efforts to cultivate noroviruses. *J. Gen. Virol.* 85:79–87.

Dunham, D. M., Jiang, X., Berke, T., Smith, A. W., and Matson, D. O., 1998, Genomic mapping of a calicivirus VPg. *Arch. Virol.* 143:2421–2430.

Enjuanes, L., Brian, D., Cavanagh, D., Holmes, K., Lai, M. M. C., Laude, H., Masters, P., Rottier, P. J. M., Siddell, S. G., Spaan, W. J. M., Taguchi, F., and Talbot, P., 2000, *Coronaviridae*, in: *Virus Taxonomy*: *The Classification and Nomenclature of Viruses. The Seventh Report of the International Committee on Taxonomy of Viruses*, Academic Press, San Diego, pp. 835–849.

Emerson, S. U., and Purcell, R. H., 2003, Hepatitis E virus. *Rev. Med. Virol.* 13:145–154.

Emerson, S. U., Zhang, M., Meng, X. J., Nguyen, H., St Claire, M., Govindarajan, S., Huang, Y. K., and Purcell, R. H., 2001, Recombinant hepatitis E virus genomes infectious for primates: importance of capping and discovery of a cis-reactive element. *Proc. Natl. Acad. Sci. U.S.A.* 98:15270–15275.

Estes, M. K., 2001, Rotaviruses and their replication, in: *Fields Virology* (D. M. Knipe, P. M. Howley et al., eds.), Lippincott Williams & Wilkins, Philadelphia, pp. 1747–1785.

Estes, M., and Cohen, J., 1989, Rotavirus gene structure and function. *Microbiol. Rev.* 53:410–449.

Ettayebi, K., and Hardy, M. E., 2003, Norwalk virus nonstructural protein p48 forms a complex with the SNARE regulator VAP-A and prevents cell surface expression of vesicular stomatitis virus G protein. *J. Virol.* 77:11790–11797.

Fankhauser, R. L., Monroe, S. S., Noel, J. S., Humphrey, C. D., Bresee, J. S., Parashar, U. D., Ando, T., and Glass, R. I., 2002, Epidemiologic and molecular trends of "Norwalk-like viruses" associated with outbreaks of gastroenteritis in the United States. *J. Infect. Dis.* 186:1–7.

Farkas, T., Zhong, W. M., Jing, Y., Huang, P. W., Espinosa, S. M., Martinez, N., Morrow, A. L., Ruiz-Palacios, G. M., Pickering, L. K., and Jiang, X., 2004, Genetic diversity among sapoviruses. *Arch. Virol.* 149:1309–1323.

Fiore, A. E., 2004, Hepatitis A transmitted by food. *Clin. Infect. Dis.* 38:705–715.

Fiore, L., Greenberg, H. B., and Mackow, E. R., 1991, The VP8 fragment of VP4 is the rhesus rotavirus hemagglutinin. *Virology* 181:553–563.

Flewett, T. H., Bryden, A. S., Davies, H. A., and Morris, C. A., 1973, Epidemic viral enteritis in a long-stay childrens ward. *Lancet* 1:4–5.

Flynn, W. T., and Saif, L. J., 1988, Serial propagation of porcine enteric calicivirus-like virus in porcine kindney cells. *J. Clin. Microbiol.* 26:203–212.

Fujiwara, K., Yokosuka, O., Imazeki, F., Saisho, H., Saotome, N., Suzuki, K., Okita, K., Tanaka, E., and Omata, M., 2003, Analysis of the genotype-determining region of hepatitis A viral RNA in relation to disease severities. *Hepatol. Res.* 25:124–134.

Gaggero, A., O'Ryan, M., Noel, J. S., Glass, R. I., Monroe, S. S., Mamani, N., Prado, V., and Avendano, L. F., 1998, Prevalence of astrovirus infection among Chilean children with acute gastroenteritis. *J. Clin. Microbiol.* 36:3691–3693.

Gallimore, C. I., Appleton, H., Lewis, D., Green, J., and Brown, D. W., 1995, Detection and characterisation of bisegmented double-stranded RNA viruses (picobirnaviruses) in human faecal specimens. *J. Med. Virol.* 45:135–140.

Garkavenko, O., Obriadina, A., Meng, J., Anderson, D. A., Benard, H. J., Schroeder, B. A., Khudyakov, Y. E., Fields, H. A., and Croxson, M. C., 2001, Detection and characterisation of swine hepatitis E virus in New Zealand. *J. Med. Virol.* 65: 525–529.

Geigenmuller, U., Ginzton, N. H., and Matsui, S. M., 1997, Construction of a genome-length cDNA clone for human astrovirus serotype 1 and synthesis of infectious RNA transcripts. *J. Virol.* 71:1713–1717.

Geigenmuller, U., Chew, T., Ginzton, N., and Matsui, S. M., 2002a, Processing of nonstructural protein 1a of human astrovirus. *J. Virol.* 76:2003–2008.

Geigenmuller, U., Ginzton, N. H., and Matsui, S. M., 2002b, Studies on intracellular processing of the capsid protein of human astrovirus serotype 1 in infected cells. *J. Gen. Virol.* 83:1691–1695.

Gilbert, J. M., and Greenberg, H. B., 1998, Cleavage of rhesus rotavirus VP4 after arginine 247 is essential for rotavirus like particle-induced fusion from without. *J. Virol.* 72:5323–5327.

Glass, M. J., Jia, X. Y., and Summers, D. F., 1993, Identification of the hepatitis A virus internal ribosome entry site: in vivo and in vitro analysis of bicistronic RNAs containing the HAV 5′ noncoding region. *Virology* 193:842–852.

Glass, P. J., White, L. J., Ball, J. M., Leparc-Goffart, I., Hardy, M. E., and Estes, M. K., 2000a, Norwalk virus open reading frame 3 encodes a minor structural protein. *J. Virol.* 74:6581–6591.

Glass, R. I., Noel, J., Ando, T., Fankhauser, R., Belliot, G., Mounts, A., Parashar, U. D., Bresee, J. S., and Monroe, S. S., 2000b, The epidemiology of enteric caliciviruses from humans: a reassessment using new diagnostics. *J. Infect. Dis.* 181(Suppl 2): S254–S261.

Gonzalez, G. G., Pujol, F. H., Liprandi, F., Deibis, L., and Ludert, J. E., 1998, Prevalence of enteric viruses in human immunodeficiency virus seropositive patients in Venezuela. *J. Med. Virol.* 55:288–292.

Gosert, R., Cassinotti, P., Siegl, G., and Weitz, M., 1996, Identification of hepatitis A virus non-structural protein 2B and its release by the major virus protease 3C. *J. Gen. Virol.* 77:247–255.

Grabow, W. O. K., Puttergill, D. L., and Bosch, A., 1992, Propagation of adenoviruses types 40 and 41 in the PLC/PRF/5 primary liver carcinoma cell line. *J. Virol. Methods* 37:201–208.

Graham, F. L., Smiley, J., Russell, W. C., and Nairn, R., 1977, Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. Gen. Virol.* 36: 59–72.

Graham, K. L., Halasz, P., Tan, Y., Hewish, M. J., Takada, Y., Mackow, E., Robinson, M. K., and Coulson, B. S., 2003, Integrin-using rotaviruses bind α2β1 integrin α2 I domain via VP4 DGE sequence and recognize αXβ2 and αVβ3 by using VP7 during cell entry. *J. Virol.* 77:9969–9978.

Green, K. Y., Kapikian, A. Z., Valdesuso, J., Sosnovtsev, S., Treanor, J. J., and Lew, J. F., 1997, Expression and self-assembly of recombinant capsid protein from the antigenically distinct Hawaii human calicivirus. *J. Clin. Microbiol.* 35:1909–1914.

Green, J., Gallimore, C. I., Clewley, J. P., and Brown, D. W., 1999, Genomic characterisation of the large segment of a rabbit picobirnavirus and comparison with the atypical picobirnavirus of Cryptosporidium parvum. *Arch. Virol.* 144:2457–2465.

Green, J., Vinje, J., Gallimore, C. I., Koopmans, M., Hale, A., Brown, D. W., Clegg, J. C., and Chamberlain, J., 2000a, Capsid protein diversity among Norwalk-like viruses. *Virus Genes* 20:227–236.

Green, K. Y., Ando, T., Balayan, M. S., Clarke, I. N., Estes, M. K., Matson, D. O., Nakata, S., Neill, J. D., Studdert, M. J., and Thiel, H. J., 2000b, Taxonomy of the caliciviruses. *J. Infect. Dis.* 181:S322–S330.

Green, K. Y., Chanock, R. M., and Kapikian, A. Z., 2001, Human caliciviruses, in: *Fields Virology*, 4th ed. (D. M. Knipe, P. M. Howley et al., eds.), Lippincott Williams & Wilkins, Philadelphia, pp. 841–874.

Green, K. Y., Mory, A., Fogg, M. H., Weisberg, A., Belliot, G., Wagner, M., Mitra, T., Ehrenfeld, E., Cameron, C. E., and Sosnovtsev, S. V., 2002, Isolation of enzymatically active replication complexes from feline calicivirus-infected cells. *J. Virol.* 76:8582–8595.

Griffin, D. D., Fletcher, M., Levy, M. E., Ching-Lee, M., Nogami, R., Edwards, L., Peters, H., Montague, L., Gentsch, J. R., and Glass, R. I., 2002, Outbreaks of adult gastroenteritis traced to a single genotype of rotavirus. *J. Infect. Dis.* 185: 1502–1505.

Grohmann, G. S., Glass, R. I., Pereira, H. G., Monroe, S. S., Hightower, A. W., Weber, R., and Bryan, R. T., 1993, Enteric viruses and diarrhea in HIV-infected patients. Enteric Opportunistic Infections Working Group. *N. Engl. J. Med.* 329:14–20.

Guerrero, C. A., Zárate, S., Corkidi, G., López, S., and Arias, C., 2000, Biochemical characterization of rotavirus receptors in MA104 cells. *J. Virol.* 74:9362–9371.

Guerrero, C. A., Bouyssounade, D., Zárate, S., Isa, P., López, T., Espinosa, R., Romero, P., Méndez, E., López, S., and Arias, C. F., 2002, Heat shock cognate protein 70 is involved in rotavirus cell entry. *J. Virol.* 76:4096–4102.

Guix, S., Caballero, S., Villena, C., Bartolomé, R., Latorre, C., Rabella, N., Simó, M., Bosch, A., and Pintó, R. M., 2002, Molecular epidemiology of astrovirus infection in Barcelona, Spain. *J. Clin. Microbiol.* 40:133–139.

Guo, M., and Saif, L. J., 2003, Pathogenesis of enteric calicivirus infections, in: *Viral Gastroenteritis* (U. Desselberger and J. Gray, eds.), Elsevier, Amsterdam, pp. 489–503.

Guo, C., Nakagomi, M., Mochizuki, M., Ishida, H., Kiso, M., Ohta, Y., Suzuki, T., Miyamoto, D., Hidari, K., and Suzuki, Y., 1999a, Ganglioside GM(1a) on the cell surface is involved in the infection by human rotavirus KUN and MO strains. *J. Biochem. (Tokyo)* 126:683–688.

Guo, M., Chang, K. O., Hardy, M. E., Zhang, Q., Parwani, A. V., and Saif, L. J., 1999b, Molecular characterization of a porcine enteric calicivirus genetically related to Sapporo-like human caliciviruses. *J. Virol.* 73:9625–9631.

Guo, M., Hayes, J., Cho, K. O., Parwani, A. V., Lucas, L. M., and Saif, L. J., 2001, Comparative pathogenesis of tissue culture adapted and wild type Cowden porcine

enteric calicivirus (PEC) in gnotobiotic pigs and induction of diarrhea by intravenous inoculation of wild type PEC. *J. Virol.* 75:9239–9251.

Halaihel, N., Liévin, V., Ball, J., Estes, M. K., Alvarado, F., and Vasseur, M., 2000, Direct inhibitory effect of rotavirus NSP4(114-135) peptide on the Na+-D-glucose symporter of rabbit intestinal brush border membrane. *J. Virol.* 74:9464–9470.

Hale, A. D., Crawford, S. E., Ciarlet, M., Green, J., Gallimore, C., Brown, D. W., Jiang, X., and Estes, M. K., 1999, Expression and self-assembly of Grimsby virus: antigenic distinction from Norwalk and Mexico viruses. *Clin. Diagn. Lab. Immunol.* 6:142–145.

Hardy, M. E., Tanaka, T. N., Kitamoto, N., White, L. J., Ball, J. M., Jiang, X., and Estes, M. K., 1996, Antigenic mapping of the recombinant Norwalk virus capsid protein using monoclonal antibodies. *Virology* 217:252–261.

Hardy, M. E., Kramer, S. F., Treanor, J. J., and Estes, M. K., 1997, Human calicivirus genogroup II capsid sequence diversity revealed by analyses of the prototype Snow Mountain agent. *Arch. Virol.* 142:1469–1479.

Herbert, T. P., Brierley, I., and Brown, T. D., 1996, Detection of the ORF3 polypeptide of feline calicivirus in infected cells and evidence for its expression from a single, functionally bicistronic, subgenomic mRNA. *J. Gen. Virol.* 77:123–127.

Herbert, T. P., Brierley, I., and Brown, T. D., 1997, Identification of a protein linked to the genomic and subgenomic mRNAs of feline calicivirus and its role in translation. *J. Gen. Virol.* 78:1033–1040.

Hewish, M. J., Takada, Y., and Coulson, B. S., 2000, Integrins alfa2beta1 and alfa4beta1 can mediate SA11 rotavirus attachment and entry into cells. *J. Virol.* 74:228–236.

Hollinger, F. B., and Emerson, S. U., 2001, Hepatitis A virus, in: *Fields Virology* (D. M. Knipe, P. M. Howley et al., eds.), Lippincott Williams & Wilkins, Philadelphia, pp. 779–840.

Horie, Y., Nakagomi, O., Koshimura, Y., Nakagomi, Y., Suzuki, Y., Oka, T., Sasaki, S., Matsuda, Y., and Watanabe, S., 1999, Diarrhea induction by rotavirus NSP4 in the homologous mouse model system. *Virology* 398–407.

Horwitz, M. S., 2001, Adenoviruses, in: *Fields Virology*, 4th ed. (D. M. Knipe, P. M. Howley et al., eds.), Lippincott Williams & Wilkins, Philadelphia, pp. 2301–2326.

Hoshino, Y., Nishikawa, K., Benfield, D. A., and Gorziglia, M., 1994, Mapping of antigenic sites involved in serotype-cross-reactive neutralization on group A rotavirus outercapsid glycoprotein. *Virology* 199:233–237.

Hsieh, S. Y., Meng, X. J., Wu, Y. H., Liu, S. T., Tam, A. W., Lin, D. Y., and Liaw, Y. F., 1999, Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. *J. Clin. Microbiol.* 37: 3828–3834.

Hughes, A. L., 2004, Phylogeny of the *Picornaviridae* and differential evolutionary divergence of picornavirus proteins. *Infect. Genet. Evol.* 4:143–152.

Hughes, P. J., and Stanway, G., 2000, The 2A proteins of three diverse picornaviruses are related to each other and to the H-rev107 family of proteins involved in the control of cell proliferation. *J. Gen. Virol.* 81:201–207.

Hutin, Y. J., Pool, V., Cramer, E. H., Nainan, O. V., Weth, J., Williams, I. T., Goldstein, S. T., Gensheimer, K. F., Bell, B. P., Shapiro, C. N., Alter, M. J., and Margolis, H. S., 1999, A multistate, foodborne oubreak of hepatitis A. National Hepatitis A Investigation Team. *N. Engl. J. Med.* 340:595–602.

Hutson, A. M., Atmar, R. L., Graham, D. Y., and Estes, M. K., 2002, Norwalk virus infection and disease is associated with ABO histo-blood group type. *J. Infect. Dis.* 185:1335–1337.

Hutson, A. M., Atmar, R. L., and Estes, M. K., 2004, Norovirus disease: changing epidemiology and host susceptibility factors. *Trends Microbiol.* 12:279–287.

Isa, P., Realpe, M., Romero, P., S., L., and Arias, C. F., 2004, Rotavirus RRV associates with lipid membrane microdomains during cell entry. *Virology* 322:370–381.

Ishino, M., Ohashi, Y., Emoto, T., Sawada, Y., and Fujinaga, K., 1988, Characterisation of adenovirus type 40 E1 region. *Virology* 165:95–102.

Iturriza-Gomara, M., Green, J., Brown, D. W. G., Ramsay, M., Desselberger, U., and Gray, J., 2000, Molecular epidemiology of human group A rotavirus infections in the United Kingdom between 1995 and 1998. *J. Clin. Microbiol.* 38:4394–4401.

Iturriza-Gómara, M., Wong, C., Blome, S., Desselberger, U., and Gray, J., 2002, Molecular characterization of VP6 genes of human rotavirus isolates: correlation of genogroups with subgroups and evidence of independent segregation. *J. Virol.* 76:6596–6601.

Jayaram, H., Estes, M. K., and Prasad, B. V. V., 2004, Emerging themes in rotavirus cell entry, genome organization, transcription and replication. *Virus. Res.* 101: 67–81.

Jiang, X., Graham, D. Y., Wang, K., and Estes, M. K., 1990, Norwalk virus genome cloning and characterization. *Science* 250:1580–1583.

Jiang, X., Wang, M., Graham, D. Y., and Estes, M. K., 1992, Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. *J. Virol.* 66:6527–6532.

Jiang, B., Monroe, S. S., Koonin, E. V., Stine, S. E., and Glass, R. I., 1993a, RNA sequence of astrovirus: distinctive genomic organization and a putative retrovirus-like ribosomal frameshifting signal that directs the viral replicase synthesis. *Proc. Natl. Acad. Sci. U. S. A.* 90:10539–10543.

Jiang, X., Wang, M., Wang, K., and Estes, M. K., 1993b, Sequence and genomic organization of Norwalk virus. *Virology* 195:51–61.

Jiang, X., Turf, E., Hu, J., Barrett, E., Dai, X. M., Monroe, S., Humphrey, C., Pickering, L. K., and Matson, D. O., 1996, Outbreaks of gastroenteritis in elderly nursing homes and retirement facilities associated with human caliciviruses. *J. Med. Virol.* 50:335–341.

Jiang, X., Espul, C., Zhong, W. M., Cuello, H., and Matson, D. O., 1999a, Characterization of a novel human calicivirus that may be a naturally occurring recombinant. *Arch. Virol.* 144:2377–2387.

Jiang, X., Huang, P. W., Zhong, W. M., Farkas, T., Cubitt, D. W., and Matson, D. O., 1999b, Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-like caliciviruses by RT-PCR. *J. Virol. Methods* 83:145–154.

Johansen, K., Hinkula, J., Espinoza, F., Levi, M., Zeng, C., Rudén, U., Vesikari, T., Estes, M., and Svensson, L., 1999, Humoral and cell-mediated immune responses in humans to the NSP4 enterotoxin of rotavirus. *J. Med. Virol.* 59:369–377.

Jourdan, N., Brunet, J. P., Sapin, C., Blais, A., Cotte-Laffitte, J., Forestier, F., Quero, A.-M., Trugnan, G., and Servin, A. L., 1998, Rotavirus infection reduces sucrase-isomaltase expression in human intestinal epithelial cells by perturbing protein targeting and organization of microvillar cytoskeleton. *J. Virol.* 72: 7228–7236.

Kabrane-Lazizi, Y., Fine, J. B., Elm, J., Glass, G. E., Higa, H., Diwan, A., Gibbs, C. J., Jr., Meng, X. J., Emerson, S. U., and Purcell, R. H., 1999a, Evidence for widespread infection of wild rats with hepatitis E virus in the United States. *Am. J. Trop. Med. Hyg.* 61:331–335.

Kabrane-Lazizi, Y., Meng, X. J., Purcell, R. H., and Emerson, S. U., 1999b, Evidence that the genomic RNA of hepatitis E virus is capped. *J. Virol.* 73:8848–8850.

Kamer, G., and Argos, P., 1984, Primary structural comparison of RNA-dependent polymerases from plant, animal and bacterial viruses. *Nucleic. Acids. Res.* 12:7269–7282.

Kang, J. A., and Funkhouser, A. W., 2002, A proposed vestigial translation initiation motif in VP1 of hepatitis A virus. *Virus. Res.* 87:11–19.

Kapikian, A. Z., Wyatt, R. G., Dolin, R., Thornhill, T. S., Kalica, A. R., and Chanock, R. M., 1972, Visualization by immune electron microscopy of a 27 nm particle associated with acute infectious nonbacterial gastroenteritis. *J. Virol.* 10:1075–1081.

Kapikian, A. Z., Hoshino, Y., and Chanock, R. M., 2001, Rotaviruses, in: *Fields. Virology* (D. M. Knipe, P. M. Howley et al., eds.), Lippincott Williams & Wilkins, Philadelphia, pp. 1787–1833.

Karst, S. M., Wobus, C. E., Lay, M., Davidson, J., and Virgin, H. W., 2003, STAT1-dependent innate immunity to a Norwalk-like virus. *Science* 299:1575–1578.

Khudyakov, Y. E., Lopareva, E. N., Jue, D. L., Crews, T. K., Thyagarajan, S. P., and Fields, H. A., 1999, Antigenic domains of the open reading frame 2-encoded protein of hepatitis E virus. *J. Clin. Microbiol.* 37:2863–2871.

Kiang, D., and Matsui, S. M., 2002, Proteolytic processing of a human astrovirus non-structural protein. *J. Gen. Virol.* 83:25–34.

King, A. M. Q., Brown, F., Christian, P., Hovi, T., Hyypia, T., Knowles, N. J., Lemon, S. M., Minor, P. D., Palmenberg, A. C., Skern, T., and Stanway, G., 1999, Picornavirus taxonomy: a modified species definition and proposal for three new genera. XIth International Congress of Virology, Sydney, Australia.

King, A. M. Q., Brown, F., Christian, P., Hovi, T., Hyypiä, T., Knowles, N. J., Lemon, S. M., Minor, P. D., Palmenburg, A. C., Skern, T., and Stanway, G., 2000, Family *Picornaviridae*, in: *Virus Taxonomy. The Classification and Nomenclature of Viruses. The Seventh Report of the International Committee on Taxonomy of Viruses*. Academic Press, San Diego, pp. 657–678.

Kobayashi, S., Sakae, K., Suzuki, Y., Ishiko, H., Kamata, K., Suzuki, K., Natori, K., Miyamura, T., and Takeda, N., 2000, Expression of recombinant capsid proteins of chitta virus, a genogroup II Norwalk virus, and development of an ELISA to detect the viral antigen. *Microbiol. Immunol.* 44:687–693.

Konno, T., Suzuki, H., Ishida, N., Chiba, R., Mochizuki, K., and Tsunoda, A., 1982, Astrovirus-associated epidemic gastroenteritis in Japan. *J. Med. Virol.* 9:11–17.

Koonin, E. V., Gorbalenya, A. E., Purdy, M. A., Rozanov, M. N., Reyes, G. R., and Bradley, D. W., 1992, Computer-assisted assignment of functional domains in the nonstructural polyprotein of hepatitis E virus: delineation of an additional group of positive-strand RNA plant and animal viruses. *Proc. Natl. Acad. Sci. U. S. A.* 89:8259–8263.

Koopmans, M., and Horzinek, M. C., 1994, Toroviruses of animals and humans: a review. *Adv. Virus Res.* 43:233–273.

Koopmans, M. P., Goosen, E. S., Lima, A. A., McAuliffe, I. T., Nataro, J. P., Barrett, L. J., Glass, R. I., and Guerrant, R. L., 1997, Association of torovirus with acute and persistent diarrhea in children. *Pediatr. Infect. Dis. J.* 16:504–507.

Koopmans, M., Vinjé, J., de Wit, M., Leenen, I., van der Poel, W., and van Duynhoven, Y., 2000, Molecular epidemiology of human enteric caliciviruses in The Netherlands. *J. Infect. Dis.* 181(Suppl 2):S262–S269.

Koopmans, M., Vinje, J., Duizer, E., de Wit, M., and van Duijnhoven, Y., 2001, Molecular epidemiology of human enteric caliciviruses in The Netherlands. *Novartis Found. Symp.* 238:197–214; discussion 214–218.

Koopmans, M., von Bonsdorff, C.-H., Vinjé, J., de Medici, D., and Monroe, S., 2002, Foodborne viruses. *FEMS Microbiol. Rev.* 26:187–205.

Koopmans, M., van Strien, E., and Vennema, H., 2003, Molecular epidemiology of human caliciviruses, in: *Viral Gastroenteritis* (U. Desselberger and J. Gray, eds.), Elsevier, Amsterdam, pp. 523–554.

Korkaya, H., Jameel, S., Gupta, D., Tyagi, S., Kumar, R., Zafrullah, M., Mazumdar, M., Lal, S. K., Xiaofang, L., Sehgal, D., Das, S. R., and Sahal, D., 2001, The ORF3 protein of hepatitis E virus binds to Src homology 3 domains and activates MAPK. *J. Biol. Chem.* 276:42389–42400.

Kotloff, K. L., Losonsky, G. A., Morris, J. G., Jr., Wasserman, S. S., Singh-Naz, N., and Levine, M. M., 1989, Enteric adenovirus infection and childhood diarrhea: an epidemiologic study in three clinical settings. *Pediatrics* 84:219–225.

Kusov, Y. Y., Weitz, M., Dollenmaier, G., et al., 1996, RNA-protein interactions at the 3′-end of the hepatitis A virus RNA. *J. Virol.* 70:1890–1897.

Kuyumcu-Martinez, M., Belliot, G., Sosnovtsev, S. V., Chang, K. O., Green, K. Y., and Lloyd, R. E., 2004, Calicivirus 3C-Like proteinase inhibits cellular translation by cleavage of poly(A)-binding protein. *J. Virol.* 78:8172–8182.

Labbé, M., Charpilienne, A., Crawford, S. E., Estes, M. K., and Cohen, J., 1991, Expression of rotavirus VP2 produces empty corelike particles. *J. Virol.* 65:2946–2952.

Lai, M. M. C., and Holmes, K. V., 2001, *Coronaviridae*: The viruses and their replication, in: *Fields Virology*, 4th ed. (D. M. Knipe, P. M. Howley et al., eds.), Lippincott Williams & Wilkins, Philadelphia, pp. 1163–1185.

Lambden, P. R., Caul, E. O., Ashley, C. R., and Clarke, I. N., 1993, Sequence and genome organization of a human small round-structured (Norwalk-like) virus. *Science* 259:516–519.

Lambden, P. R., Caul, E. O., Ashley, C. R., and Clarke, I. N., 1994, Human enteric caliciviruses are genetically distinct from small round structured viruses. *Lancet* 343:666–667.

Lawton, J. A., Estes, M. K., and Prasad, B. V., 1997, Three-dimensional visualization of mRNA release from actively transcribing rotavirus particles. *Nat. Struct. Biol.* 4:118–121.

Lawton, J. A., Estes, M. K., and Prasad, B. V., 2000, Mechanisms of genome transcription in segmented dsRNA viruses. *Adv. Virus Res.* 55:185–229.

Lazdins, I., Coulson, B., Kirkwood, C., Dyall-Smith, M., Masendycz, P. J., Sonza, S., and Holmes, I. H., 1995, Rotavirus antigenicity is affected by the genetic context and glycosylation of VP7. *Virology* 209:80–89.

Lee, T. W., and Kurtz, J. B., 1982, Human astrovirus serotypes. *J. Hyg. (Lond.)* 89:539–540.

Lee, T. W., and Kurtz, J. B., 1994, Prevalence of human astrovirus serotypes in the Oxford region 1976–92, with evidence for two new serotypes. *Epidemiol. Infect.* 112:187–193.

Lemon, S. M., and Binn, L. N., 1983, Antigenic relatedness of two strains of hepatitis A virus determinant by cross-neutralization. *Infect. Immunol.* 42:418–420.

Lemon, S. M., and Robertson, B. H., 1993, Current perspectives in the virology and molecular biology of hepatitis A virus. *Semin. Virol.* 4:285–295.

Lemon, S. M., Jansen, R. W., and Newbold, J. E., 1985, Infectious hepatitis A virus particles produced in cell culture consist of three distinct types with different buoyant densities in CsCl. *J. Virol.* 54:78–85.

Leong, J. C., Brown, D., Dobos, P., Kibenge, F. S. B., Lufert, J. E., Muller, H., Mundt, E., and Nicholson, B., 2000, Family *Birnaviridae*, in: *Virus Taxonomy. Classifica-*

tion and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, pp. 481–490.

Lewis, D. C., Lightfoot, N. F., Cubitt, W. D., and Wilson, S. A., 1989, Outbreaks of astrovirus type 1 and rotavirus gastroenteritis in a geriatric in-patient population. *J. Hosp. Infect.* 14:9–14.

Lewis, T. L., Greenberg, H. B., Herrmann, J. E., Smith, L. S., and Matsui, S. M., 1994, Analysis of astrovirus serotype 1 RNA, identification of the viral RNA-dependent RNA polymerase motif, and expression of a viral structural protein. *J. Virol.* 68:77–83.

Li, F., Riddell, M. A., Seow, H. F., Takeda, N., Miyamura, T., and Anderson, D. A., 2000, Recombinant subunit ORF2.1 antigen and induction of antibody against immunodominant epitopes in the hepatitis E virus capsid protein. *J. Med. Virol.* 60: 379–386.

Lindesmith, L., Moe, C., Marionneau, S., Ruvoen, N., Jiang, X., Lindblad, L., Stewart, P., LePendu, J., and Baric, R., 2003, Human susceptibility and resistance to Norwalk virus infection. *Nat. Med.* 9:548–553.

Liste, M. B., Natera, I., Suarez, J. A., Pujol, F. H., Liprandi, F., and Ludert, J. E., 2000, Enteric virus infections and diarrhea in healthy and human immunodeficiency virus-infected children. *J. Clin. Microbiol.* 38:2873–2877.

Liu, M., Mattion, N. M., and Estes, M. K., 1992, Rotavirus VP3 expressed in insect cells possesses guanylyltransferase activity. *Virology* 188:77–84.

Liu, B. L., Clarke, I. N., Caul, E. O., and Lambden, P. R., 1995, Human enteric caliciviruses have a unique genome structure and are distinct from the Norwalk-like viruses. *Arch. Virol.* 140:1345–1356.

Liu, B., Clarke, I. N., and Lambden, P. R., 1996, Polyprotein processing in Southampton virus: identification of 3C-like protease cleavage sites by in vitro mutagenesis. *J. Virol.* 70:2605–2610.

Liu, B. L., Lambden, P. R., Gunther, H., Otto, P., Elschner, M., and Clarke, I. N., 1999a, Molecular characterization of a bovine enteric calicivirus: relationship to the Norwalk-like viruses. *J. Virol.* 73:819–825.

Liu, B. L., Viljoen, G. J., Clarke, I. N., and Lambden, P. R., 1999b, Identification of further proteolytic cleavage sites in the Southampton calicivirus polyprotein by expression of the viral protease in *E. coli*. *J. Gen. Virol.* 80:291–296.

López, S., and Arias, C. F., 2004, Multistep entry of rotavirus into cells: a Versaillesque dance. *Trends Microbiol.* 12:271–278.

López-Vazquez, A., Martin Alonso, J. M., Casais, R., Boga, J. A., and Parra, F., 1998, Expression of enzymatically active rabbit hemorrhagic disease virus RNA-dependent RNA polymerase in *Escherichia coli*. *J. Virol.* 72:2999–3004.

Lopman, B. A., Brown, D. W., and Koopmans, M., 2002, Human caliciviruses in Europe. *J. Clin. Virol.* 24:137–160.

Lopman, B. A., Reacher, M. H., Van Duijnhoven, Y., Hanon, F. X., Brown, D., and Koopmans, M., 2003, Viral gastroenteritis outbreaks in Europe, 1995–2000. *Emerg. Infect. Dis.* 9:90–96.

Lopman, B., Vennema, H., Kohli, E., Pothier, P., Sanchez, A., Negredo, A., Buesa, J., Schreier, E., Reacher, M., Brown, D., Gray, J., Iturriza, M., Gallimore, C., Bottiger, B., Hedlund, K. O., Torven, M., von Bonsdorff, C. H., Maunula, L., Poljsak-Prijatelj, M., Zimsek, J., Reuter, G., Szucs, G., Melegh, B., Svennson, L., van Duijnhoven, Y., and Koopmans, M., 2004, Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. *Lancet* 363:682–688.

Love, D. N., and Sabine, M., 1975, Electron microscopic observation of feline kidney cells infected with a feline calicivirus. *Arch. Virol.* 48:213–228.

Luby, J. P., Clinton, R., and Kurtz, S., 1999, Adaptation of human enteric coronavirus to growth in cell lines. *J. Clin. Virol.* 12:43–51.

Ludert, J. E., and Liprandi, F., 1993, Identification of viruses with bi- and trisegmented double-stranded RNA genome in faeces of children with gastroenteritis. *Res. Virol.* 144:219–224.

Ludert, J. E., Hidalgo, M., Gil, F., and Liprandi, F., 1991, Identification in porcine faeces of a novel virus with a bisegmented double stranded RNA genome. *Arch. Virol.* 117:97–107.

Ludert, J. E., Abdul-Latiff, L., Liprandi, A., and Liprandi, F., 1995, Identification of picobirnavirus, viruses with bisegmented double stranded RNA, in rabbit faeces. *Res. Vet. Sci.* 59:222–225.

Macnaughton, M. R., and Davies, H. A., 1981, Human enteric coronaviruses. Brief review. *Arch. Virol.* 70:301–313.

Madeley, C. R., 1979, Comparison of the features of astroviruses and caliciviruses seen in samples of feces by electron microscopy. *J. Infect. Dis.* 139:519–523.

Madore, H. P., Treanor, J. J., and Dolin, R., 1986, Characterization of the Snow Mountain agent of viral gastroenteritis. *J. Virol.* 58:487–492.

Maguire, A. J., Green, J., Brown, D. W., Desselberger, U., and Gray, J. J., 1999, Molecular epidemiology of outbreaks of gastroenteritis associated with small round-structured viruses in East Anglia, United Kingdom, during the 1996–1997 season. *J. Clin. Microbiol.* 37:81–89.

Malcolm, B. A., Chin, S. M., Jewell, D. A., Stratton-Thomas, J. R., Thudium, K. B., Ralston, R., and Rosenberg, S., 1992, Expression and characterization of recombinant hepatitis A virus 3C proteinase. *Biochemistry* 31:3358–3363.

Manes, S., del Real, G., and Martinez, A. C., 2003, Pathogens: raft hijackers. *Nat. Rev. Immunol.* 3:557–568.

Marin, M. S., Casais, R., Alonso, J. M., and Parra, F., 2000, ATP binding and ATPase activities associated with recombinant rabbit hemorrhagic disease virus 2C-like polypeptide. *J. Virol.* 74:10846–10851.

Marionneau, S., Cailleau-Thomas, A., Rocher, J., Le Moullac-Vaidye, B., Ruvoen, N., Clément, M., and Le Pendu, J., 2001, ABH and Lewis histo-blood group antigens, a model for the meaning of oligosaccharide diversity in the face of a changing world. *Biochimie* 83:565–573.

Marionneau, S., Ruvoen, N., Le Moullac-Vaidye, B., Clement, M., Cailleau-Thomas, A., Ruiz-Palacios, G., Huang, P., Jiang, X., and Le Pendu, J., 2002, Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals. *Gastroenterology* 122:1967–1977.

Martin, A., Escriou, N., Chao, S. F., Girard, M., Lemon, S. M., and Wychowski, C., 1995, Identification and site-directed mutagenesis of the primary (2A/2B) cleavage site of the hepatitis A virus polyprotein: functional impact on the infectivity of HAV RNA transcripts. *Virology* 213:213–222.

Martin, A., Bénichou, D., Chao, S.-F., Cohen, L. M., and Lemon, S. M., 1999, Maturation of the hepatitis A virus capsid protein VP1 is not dependent on processing by the $3C^{pro}$ proteinase. *J. Virol.* 73:6220–6227.

Mast, E. E., Alter, M. J., Holland, P. V., and Purcell, R. H., 1998, Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E Virus Antibody Serum Panel Evaluation Group. *Hepatology* 27:857–861.

Mathan, M., Mathan, V. I., Swaminathan, S. P., Yesudoss, S., and Baker, S. J., 1975, Pleomorphic virus-like particles in human faeces. *Lancet* 1:1068–1069.

Mathieu, M., Petitpas, I., Navaza, J., Lepault, J., Kohli, E., Pothier, P., Prasad, B. V., Cohen, J., and Rey, F. A., 2001, Atomic structure of the major capsid protein of rotavirus: implications for the architecture of the virion. *EMBO J.* 20:1485–1497.

Matsui, M., 1997, Astrovirus, in: *Clinical Virology* (D. D. Richman, R. J. Whitley, and F. G. Hayden, eds.), Churchill Livingstone, New York, pp. 1111–1121.

Matsui, S. M., and Greenberg, H. B., 2000, Immunity to calicivirus infection. *J. Infect. Dis.* 181(Suppl 2):S331–S335.

Matsui, M., and Greenberg, H. B., 2001, Astroviruses, in: *Fields Virology* (D. M. Knipe, P. M. Howley et al., eds.), Lippincott Williams & Wilkins, Philadelphia, pp. 875–893.

Maunula, L., and von Bonsdorf, C.-H., 1998, Short sequences define genetic lineages: phylogenetic analysis of group A rotaviruses based on partial sequences of genome segments 4 and 9. *J. Gen. Virol.* 79:321–332.

Mautner, V., MacKay, N., and Steinthorsdottir, V., 1989, Complementation of enteric adenovirus type 40 for lytic growth in tissue culture by E1B 55K function of adenovirus types 5 and 12. *Virology* 171:619–622.

Mautner, V., Steinthorsdottir, V., and Bailey, A., 1995, Enteric adenoviruses. *Curr. Top. Microbiol. Immunol.* 179:229–282.

Mayo, M. A., 2002, Virus taxonomy—Houston 2002. *Arch. Virol.* 147:1071–1076.

Melnick, J. L., 1996, Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses, in: *Fields Virology* (B. N. Fields, D. M. Knipe, P. M. Howley et al., eds.), Lippincott-Raven, Philadelphia, pp. 655–712.

Méndez, E., Fernández-Luna, T., López, S., Mendez-Toss, M., and Arias, C. F., 2002, Proteolytic processing of a serotype 8 human astrovirus ORF2 polyprotein. *J. Virol.* 76:7996–8002.

Meng, X. J., 2000, Novel strains of hepatitis E virus identified from humans and other animal species: is hepatitis E a zoonosis? *J. Hepatol.* 33:842–845.

Meng, X. J., Purcell, R. H., Halbur, P. G., Lehman, J. R., Webb, D. M., Tsareva, T. S., Haynes, J. S., Thacker, B. J., and Emerson, S. U., 1997, A novel virus in swine is closely related to the human hepatitis E virus. *Proc. Natl. Acad. Sci. U. S. A.* 94:9860–9865.

Meng, X. J., Halbur, P. G., Haynes, J. S., Tsareva, T. S., Bruna, J. D., Royer, R. L., Purcell, R. H., and Emerson, S. U., 1998a, Experimental infection of pigs with the newly identified swine hepatitis E virus (swine HEV), but not with human strains of HEV. *Arch. Virol.* 143:1405–1415.

Meng, X. J., Halbur, P. G., Shapiro, M. S., Govindarajan, S., Bruna, J. D., Mushahwar, I. K., Purcell, R. H., and Emerson, S. U., 1998b, Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *J. Virol.* 72:9714–9721.

Meng, J., Dai, X., Chang, J. C., Lopareva, E., Pillot, J., Fields, H. A., and Khudyakov, Y. E., 2001, Identification and characterization of the neutralization epitope(s) of the hepatitis E virus. *Virology* 288:203–211.

Midthun, K., Greenberg, H. B., Kurtz, J. B., Gary, G. W., Lin, F. Y., and Kapikian, A. Z., 1993, Characterization and seroepidemiology of a type 5 astrovirus associated with an outbreak of gastroenteritis in Marin County, California. *J. Clin. Microbiol.* 31:955–962.

Mikami, T., Nakagomi, T., Tsutsui, R., Ishikawa, K., Onodera, Y., Arisawa, K., and Nakagomi, O., 2004, An outbreak of gastroenteritis during school trip caused by serotype G2 group A rotavirus. *J. Med. Virol.* 73:460–464.

Minor, P., 1991, *Picornaviridae*, in: *Classification and nomenclature of viruses* (R. I. B. Francki, C. M. Fauquet, and D. L. Knudson, eds.), Springer-Verlag, Wien, pp. 320–326.

Mirazimi, A., Magnusson, K.-E., and Svensson, L., 2003, A citoplasmic region of the NSP4 enterotoxin of rotavirus is involved in retention in the endoplasmic reticulum. *J. Gen. Virol.* 84:875–883.

Monroe, S. S., Stine, S. E., Gorelkin, L., Herrmann, J. E., Blacklow, N. R., and Glass, R. I., 1991, Temporal synthesis of proteins and RNAs during human astrovirus infection of cultured cells. *J. Virol.* 65:641–648.

Monroe, S. S., Holmes, J. L., and Belliot, G. M., 2001, Molecular epidemiology of human astroviruses. *Novartis Found. Symp.* 238:237–245; discussion 245–249.

Morales, M., Barcena, J., Ramirez, M. A., Boga, J. A., Parra, F., and Torres, J. M., 2004, Synthesis in vitro of rabbit hemorrhagic disease virus subgenomic RNA by internal initiation on (-)sense genomic RNA: mapping of a subgenomic promoter. *J. Biol. Chem.* 279:17013–17018.

Morris, A. P., and Estes, M. K., 2001, Microbes and microbial toxins: Paradigms for microbial-mucosal interactions. VIII. Pathological consequences of rotavirus infection and its enterotoxin. *Am. J. Physiol. Gastrointest. Liver Physiol.* 281: G303–G310.

Morris, A. P., Scott, J. K., Ball, J. M., Zeng, C. Q.-Y., O'Neal, W. K., and Estes, M. K., 1999, NSP4 elicits age-dependent diarrhea and Ca^{2+}-mediated I^- influx into intestinal crypts of CF mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 277: G431–G444.

Mossel, E. C., and Ramig, R. F., 2003, A lymphatic mechanism of rotavirus extraintestinal spread in the neonatal mouse. *J. Virol.* 77:12352–12356.

Mustafa, H., Palombo, E. A., and Bishop, R. F., 2000, Epidemiology of astrovirus infection in young children hospitalized with acute gastroenteritis in Melbourne, Australia, over a period of four consecutive years, 1995 to 1998. *J. Clin. Microbiol.* 38:1058–1062.

Nakata, S., Kogawa, K., Numata, K., Ukae, S., Adachi, N., Matson, D. O., Estes, M. K., and Chiba, S., 1996, The epidemiology of human calicivirus/Sapporo/82/Japan. *Arch. Virol.* Suppl 12:263–270.

Neill, J. D., 1990, Nucleotide sequence of a region of the feline calicivirus genome which encodes picornavirus-like RNA-dependent RNA polymerase, cysteine protease and 2C polypeptides. *Virus Res.* 17:145–160.

Nilsson, M., Hedlund, K. O., Thorhagen, M., Larson, G., Johansen, K., Ekspong, A., and Svensson, L., 2003, Evolution of human calicivirus RNA in vivo: accumulation of mutations in the protruding P2 domain of the capsid leads to structural changes and possibly a new phenotype. *J. Virol.* 77:13117–13124.

Nishikawa, K., Hoshino, Y., Taniguchi, K., Green, J., Greenberg, H. B., Kapikian, A. Z., Chanock, R. M., and Gorziglia, M., 1989, Rotavirus VP7 neutralization epitopes of serotype 3 strains. *Virology* 171:503–515.

Noel, J., and Cubitt, D., 1994, Identification of astrovirus serotypes from children treated at the Hospitals for Sick Children, London 1981–93. *Epidemiol. Infect.* 113:153–159.

Noel, J. S., Lee, T. W., Kurtz, J. B., Glass, R. I., and Monroe, S. S., 1995, Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing. *J. Clin. Microbiol.* 33:797–801.

Noel, J. S., Liu, B. L., Humphrey, C. D., Rodriguez, E. M., Lambden, P. R., Clarke, I. N., Dwyer, D. M., Ando, T., Glass, R. I., and Monroe, S. S., 1997, Parkville virus: a

novel genetic variant of human calicivirus in the Sapporo virus clade, associated with an outbreak of gastroenteritis in adults. *J. Med. Virol.* 52:173–178.

Numata, K., Hardy, M. E., Nakata, S., Chiba, S., and Estes, M. K., 1997, Molecular characterization of morphologically typical human calicivirus Sapporo. *Arch. Virol.* 142:1537–1552.

Oberste, M. S., Maher, K., Kilpatrick, D. R., and Pallansch, M. A., 1999, Molecular evolution of the human enteroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification. *J. Virol.* 73:1941–1948.

Obert, G., Peiffer, I., and Servin, A., 2000, Rotavirus-induced structural and functional alterations in tight junctions of polarized intestinal Caco-2 cell monolayers. *J. Virol.* 74:4645–4651.

O'Brien, J. A., Taylor, J. A., and Bellamy, A. R., 2000, Probing the structure of rotavirus NSP4: a short sequence at the extreme C terminus mediates binding to the inner capsid particle. *J. Virol.* 74:5388–5394.

Oliver, S. L., Dastjerdi, A. M., Wong, S., El-Attar, L., Gallimore, C., Brown, D. W., Green, J., and Bridger, J. C., 2003, Molecular characterization of bovine enteric caliciviruses: a distinct third genogroup of noroviruses (Norwalk-like viruses) unlikely to be of risk to humans. *J. Virol.* 77:2789–2798.

Palombo, E. A., Bishop, R. F., and Cotton, R. G. H., 1993, Sequence conservation within neutralization epitope regions of VP7 and VP4 proteins of human serotype G4 rotavirus isolates. *Arch. Virol.* 133:323–334.

Panda, S. K., Ansari, I. H., Durgapal, H., Agrawal, S., and Jameel, S., 2000, The in vitro-synthesized RNA from a cDNA clone of hepatitis E virus is infectious. *J. Virol.* 74:2430–2437.

Parashar, U. D., Hummelman, E. G., Bresee, J. S., Miller, M. A., and Glass, R. I., 2003, Global illness and deaths caused by rotavirus disease in children. *Emerg. Infect. Dis.* 9:565–572.

Parwani, A. V., Flynn, W. T., Gadfield, K. L., and Saif, L. J., 1991, Serial propagation of porcine enteric calicivirus: effects of medium supplementation with intestinal contents or enzymes. *Arch. Virol.* 120:115–122.

Patton, J. T., and Gallegos, C. O., 1990, Rotavirus RNA replication: single-strand RNA extends from the replicase particle. *J. Gen. Virol.* 71:1087–1094.

Patton, J. T., Kearney, K., and Taraporewala, Z., 2003, Rotavirus genome replication: role of the RNA-binding proteins, in: *Viral Gastroenteritis* (U. Desselberger and J. Gray, eds.), Elsevier, Amsterdam, pp. 165–183.

Pereira, H. G., Fialho, A. M., Flewett, T. H., Candeias, J. A. N., and Andrade, Z. P., 1988a, Novel viruses in human faeces. *Lancet* 2:103–104.

Pereira, H. G., Flewett, T. H., Candeias, J. A., and Barth, O. M., 1988b, A virus with bisegmented double-stranded RNA genome in rat (*Oryzomys nigripes*). *J. Gen. Virol.* 69:2749–2754.

Perron-Henry, D. M., Herrmann, J. E., and Blacklow, N. R., 1988, Isolation and propagation of enteric adenoviruses in HEp-2 cells. *J. Clin. Microbiol.* 26:1445–1447.

Pesavento, J. B., Lawton, J. A., Estes, M. K., and Prasad, B. V. V., 2001, The reversible condensation and expansion of the rotavirus genome. *Proc. Natl. Acad. Sci. U. S. A.* 98:1381–1386.

Pesavento, J. B., Estes, M. K., and Prasad, B. V. V., 2003, Structural organization of the genome in rotavirus, in: *Viral Gastroenteritis* (U. Desselberger and J. Gray, eds.), Elsevier, Amsterdam.

Pfister, T., and Wimmer, E., 2001, Polypeptide p41 of a Norwalk-like virus is a nucleic acid-independent nucleoside triphosphatase. *J. Virol.* 75:1611–1619.

Pintó, R. M., Díez, J. M., and Bosch, A., 1994, Use of the colonic carcinoma cell line CaCo-2 for in vivo amplification and detection of enteric viruses. *J. Med. Virol.* 44:310–315.

Pintó, R. M., Abad, F. X., Gajardo, R., and Bosch, A., 1996, Detection of infectious astroviruses in water. *Appl. Environ. Microbiol.* 62:1811–1813.

Pintó, R. M., Villena, C., Le Guyader, F., Guix, S., Caballero, S., Pommepuy, M., and Bosch, A., 2001, Astrovirus detection in wastewater samples. *Water Sci. Technol.* 43:73–76.

Piron, M., Vende, P., Cohen, J., and Poncet, D., 1998, Rotavirus RNA-binding protein NSP3 interacts with eIF4GI and evicts the poly(A) binding protein from eIF4F. *EMBO J.* 17:5811–5821.

Poncet, D., Laurent, S., and Cohen, J., 1994, Four nucleotides are the minimal requirement for RNA recognition by rotavirus non-structural protein NSP3. *EMBO J.* 13:4165–4173.

Prasad, B. V., Wang, G. J., Clerx, J. P. M., and Chiu, W., 1988, Three-dimensional structure of rotavirus. *J. Mol. Biol.* 199:269–275.

Prasad, B. V., Rothnagel, R., Jiang, X., and Estes, M. K., 1994, Three-dimensional structure of baculovirus-expressed Norwalk virus capsids. *J. Virol.* 68:5117–5125.

Prasad, B. V., Hardy, M. E., Jiang, X., and Estes, M. K., 1996a, Structure of Norwalk virus. *Arch. Virol.* Suppl 12:237–242.

Prasad, B. V., Rothnagel, R., Zeng, C. Q., Jakana, J., Lawton, J. A., Chiu, W., and Estes, M. K., 1996b, Visualization of ordered genomic RNA and localization of transcriptional complexes in rotavirus. *Nature* 382:471–473.

Prasad, B. V., Hardy, M. E., Dokland, T., Bella, J., Rossmann, M. G., and Estes, M. K., 1999, X-ray crystallographic structure of the Norwalk virus capsid. *Science* 286:287–290.

Probst, C., Jecht, M., and Gauss-Mueller, V., 1998, Processing of proteinase precursors and their effect on hepatitis A virus particle formation. *Virology* 72:8013–8020.

Purcell, R. H., and Emerson, S. U., 2001, Hepatitis E virus, in: *Fields Virology* (D. M. Knipe, P. M. Howley et al., eds.), Lippincott Williams & Wilkins, Philadelphia, pp. 3051–3061.

Racaniello, V. R., 2001, *Picornaviridae*: the viruses and their replication, in: *Fields Virology*, 4th ed. (D. M. Knipe, P. M. Howley et al., eds.), Lippincott Williams & Wilkins, Philadelphia, pp. 685–722.

Ramig, R., 2000, Mixed infections with rotaviruses: protocols for reassortment, complementation and other assays, in: *Rotaviruses: Methods and Protocols* (J. Gray and U. Desselberger, eds.), Humana Press, Totowa, NJ, pp. 79–100.

Ramig, R. F., and Estes, M. K., 2003, The dsRNA segments and proteins of simian rotavirus A / SA11 (genus *Rotavirus*: family *Reoviridae*), in: *The RNAs and Proteins of dsRNA Viruses* (P. C. Mertens and D. H. Bamford, eds.). Available at http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/Rotavirus.htm.

Ray, P., Malik, J., Singh, R. K., Bhatnagar, S., Kumar, R., and Bhan, M. K., 2003, Rotavirus nonstructural protein NSP4 induces heterotypic antibody responses during natural infection in children. *J. Infect. Dis.* 187:1786–1793.

Resta, S., Luby, J. P., Rosenfeld, C. R., and Siegel, J. D., 1985, Isolation and propagation of a human enteric coronavirus. *Science* 229:978–981.

Riddell, M. A., Li, F., and Anderson, D. A., 2000, Identification of immunodominant and conformational epitopes in the capsid protein of hepatitis E virus by using monoclonal antibodies. *J. Virol.* 74:8011–8017.

Risco, C., Carrascosa, J. L., Pedregosa, A. M., Humphrey, C. D., and Sanchez-Fauquier, A., 1995, Ultrastructure of human astrovirus serotype 2. *J. Gen. Virol.* 76:2075–2080.

Robertson, B. H., Jansen, R. W., Khanna, B., Totsuka, A., Nainan, O. V., Siegl, G., Widell, A., Margolis, H. S., Isomura, S., Ito, K., Ishizu, T., Moritsugu, Y., and Lemon, S. M., 1992, Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. *J. Gen. Virol.* 73:1365–1377.

Robinson, R. A., Burgess, W. H., Emerson, S. U., Leibowitz, R. S., Sosnovtseva, S. A., Tsarev, S., and Purcell, R. H., 1998, Structural characterization of recombinant hepatitis E virus ORF2 proteins in baculovirus-infected insect cells. *Protein Expr. Purif.* 12:75–84.

Rolsma, M. D., Kuhlenschmidt, T. B., Gelberg, H. B., and Kuhlenschmidt, M. S., 1998, Structure and function of a ganglioside receptor for porcine rotavirus. *J. Virol.* 72:9079–9091.

Rosen, B., 2003, Molecular characterization and epidemiology of picobirnaviruses, in: *Viral Gastroenteritis* (U. Desselberger and J. Gray, eds.), Elsevier, Amsterdam, pp. 633–644.

Rosen, B. I., Fang, Z. Y., Glass, R. I., and Monroe, S. S., 2000, Cloning of human picobirnavirus genomic segments and development of an RT-PCR detection assay. *Virology* 277:316–329.

Rossmann, M. G., and Johnson, J. E., 1989, Icosahedral RNA virus structure. *Annu. Rev. Biochem.* 58:533–573.

Ruvöen-Clouet, N., Ganière, J. P., André-Fontain, G., Blanchard, D., and Le Pendu, J., 2000, Binding of rabbit haemorrhagic disease virus to antigens of the ABH blood group family. *J. Virol.* 74:11950–11954.

Sánchez, G., Bosch, A., and Pintó, R. M., 2003, Genome variability and capsid structural constraints of hepatitis A virus. *J. Virol.* 77:452–459.

Sanekata, T., Ahmed, M. U., Kader, A., Taniguchi, K., and Kobayashi, N., 2003, Human group B rotavirus infections cause severe diarrhea in children and adults in Bangladesh. *J. Clin. Microbiol.* 41:2187–2190.

Sasaki, J., Kusuhara, Y., Maeno, Y., Kobayashi, N., Yamashita, T., Sakae, K., Takeda, N., and Taniguchi, K., 2001, Construction of an infectious cDNA clone of Aichi virus (a new member of the family *Picornaviridae*) and mutational analysis of a stem-loop structure at the 5′ end of the genome. *J. Virol*. 75:8021–8030.

Schlauder, G. G., Dawson, G. J., Erker, J. C., Kwo, P. Y., Knigge, M. F., Smalley, D. L., Rosenblatt, J. E., Desai, S. M., and Mushahwar, I. K., 1998, The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. *J. Gen. Virol.* 79:447–456.

Schlauder, G. G., Desai, S. M., Zanetti, A. R., Tassopoulos, N. C., and Mushahwar, I. K., 1999, Novel hepatitis E virus (HEV) isolates from Europe: evidence for additional genotypes of HEV. *J. Med. Virol.* 57:243–251.

Schuffenecker, I., Ando, T., Thouvenot, D., Lina, B., and Aymard, M., 2001, Genetic classification of "Sapporo-like viruses." *Arch. Virol.* 146:2115–2132.

Servin, A. L., 2003, Effects of rotavirus infection on the structure and functions of intestinal cells, in: *Viral Gastroenteritis* (U. Desselberger and J. Gray, eds.), Elsevier, Amsterdam, pp. 237–254.

Shenk, E. S., 2001, Adenoviridae: the viruses and their replication, in: *Fields Virology*, 4th ed. (D. M. Knipe and P. M. Howley, eds.), Lippincott Williams & Wilkins, Philadelphia, pp. 2265–2300.

Siegl, G., de Chastonay, J., and Kronauer, G., 1984, Propagation and assay of hepatitis A virus in vitro. *J. Virol. Methods* 9:53–67.

Smiley, J. R., Hoet, A. E., Traven, M., Tsunemitsu, H., and Saif, L. J., 2003, Reverse transcription-PCR assays for detection of bovine enteric caliciviruses (BEC) and analysis of the genetic relationships among BEC and human caliciviruses. *J. Clin. Microbiol.* 41:3089–3099.

Smith, A. W., Akers, T. G., Madin, S. H., and Vedros, N. A., 1973, San Miguel sea lion virus isolation, preliminary characterization and relationship to vesicular exanthema of swine virus. *Nature* 244:108–110.

Smith, A. W., Skilling, D. E., Ensley, P. K., Benirschke, K., and Lester, T. L., 1983, Calicivirus isolation and persistence in a pygmy chimpanzee (*Pan paniscus*). *Science* 221:79–81.

Smits, S. L., Lavazza, A., Matiz, K., Horzinek, M. C., Koopmans, M. P., and de Groot, R. J., 2003, Phylogenetic and evolutionary relationships among torovirus field variants: evidence for multiple intertypic recombination events. *J. Virol.* 77: 9567–9577.

Smyth, M., Pettitt, T., Symonds, A., and Martin, J., 2003, Identification of the pocket factors in a picornavirus. *Arch. Virol.* 148:1225–1233.

Snijder, E. J., and Horzinek, M. C., 1993, Toroviruses: replication, evolution and comparison with other members of the coronavirus-like superfamily. *J. Gen. Virol.* 74:2305–2316.

Snijder, E. J., den Boon, J. A., Bredenbeek, P. J., Horzinek, M. C., Rijnbrand, R., and Spaan, W. J., 1990a, The carboxyl-terminal part of the putative Berne virus polymerase is expressed by ribosomal frameshifting and contains sequence motifs which indicate that toro- and coronaviruses are evolutionarily related. *Nucleic Acids Res.* 18:4535–4542.

Snijder, E. J., Horzinek, M. C., and Spaan, W. J., 1990b, A 3′-coterminal nested set of independently transcribed mRNAs is generated during Berne virus replication. *J. Virol.* 64:331–338.

Sosnovtsev, S., and Green, K. Y., 2003, Feline calicivirus as a model for the study of calicivirus replication, in: *Viral Gastroenteritis* (U. Desselberger and J. Gray, eds.), Elsevier, Amsterdam, pp. 467–503.

Stevenson, F., and Mautner, V., 2003, Aspects of the molecular biology of enteric adenoviruses, in: *Viral Gastroenteritis* (U. Desselberger and J. Gray, eds.), Elsevier, Amsterdam, pp. 389–406.

Sugieda, M., and Nakajima, S., 2002, Viruses detected in the caecum contents of healthy pigs representing a new genetic cluster in genogroup II of the genus "Norwalk-like viruses." *Virus Res.* 87:165–172.

Tafazoli, F., Zeng, C. Q., Estes, M., Magnusson, K.-E., and Svensson, L., 2001, NSP4 enterotoxin of rotavirus induces paracellular leakage in polarized epithelial cells. *J. Virol.* 75:1540–1546.

Takahashi, K., Iwata, K., Watanabe, N., Hatahara, T., Ohta, Y., Baba, K., and Mishiro, S., 2001, Full-genome nucleotide sequence of a hepatitis E virus strain that may be indigenous to Japan. *Virology* 287:9–12.

Takiff, H. E., Strauss, S. E., and Garon, C. F., 1981, Propagation and in vitro studies of previously non-cultivable enteral adenovirus in 293 cells. *Lancet* 2:832–834.

Takiff, H. E., Reinhold, W., Garon, C. F., and Straus, S. E., 1984, Cloning and physical mapping of enteric adenoviruses (candidate types 40 and 41). *J. Virol.* 51:131–136.

Tam, A. W., Smith, M. M., Guerra, M. E., Huang, C. C., Bradley, D. W., Fry, K. E., and Reyes, G. R., 1991, Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. *Virology* 185:120–131.

Tan, M., Huang, P., Meller, J., Zhong, W., Farkas, T., and Jiang, X., 2003, Mutations within the P2 domain of norovirus capsid affect binding to human histo-blood group antigens: evidence for a binding pocket. *J. Virol.* 77:12562–12571.

Tan, M., Hegde, R. S., and Jiang, X., 2004, The P domain of norovirus capsid protein forms dimer and binds to histo-blood group antigen receptors. *J. Virol.* 78:6233–6242.

Taylor, J. A., O'Brien, J. A., Lord, V. J., Meyer, J. C., and Bellamy, A. R., 1993, The RER-localized rotavirus intracellular receptor: a truncated purified soluble form is multivalent and bind virus particles. *Virology* 194:807–814.

Taylor, M. B., Walter, J., Berke, T., Cubitt, W. D., Mitchell, D. K., and Matson, D. O., 2001, Characterisation of a South African human astrovirus as type 8 by antigenic and genetic analyses. *J. Med. Virol.* 64:256–261.

Terashima, H., Chiba, S., Sakuma, Y., Kogasaka, R., Nakata, S., Minami, R., Horino, K., and Nakao, T., 1983, The polypeptide of a human calicivirus. *Arch. Virol.* 78:1–7.

Tian, P., Hu, Y., Schilling, W. P., Lindsay, D. A., Eiden, J., and Estes, M. K., 1994, The nonstructural glycoprotein of rotavirus affects intracellular calcium levels. *J. Virol.* 68:251–257.

Tian, P., Ball, J. M., Zeng, C. Q. Y., and Estes, M. K., 1996, The rotavirus nonstructural glycoprotein NSP4 possesses membrane destabilization activity. *J. Virol.* 70:6973–6981.

Tiemessen, C. T., Nel, M. J., and Kidd, A. H., 1996, Adenovirus 41 replication: cell-related differences in viral gene transcription. *Mol. Cell Probes* 10:279–287.

Tsarev, S., A., Tsareva, T. S., Emerson, S. U., Govindarajan, S., Shapiro, M., Gerin, J. L., and Purcell, R. H., 1994, Successful passive and active immunization of cynomolgus monkeys against hepatitis E. *Proc. Natl. Acad. Sci. U. S. A.* 91:10198–10202.

Tyagi, S., Korkaya, H., Zafrullah, M., Jameel, S., and Lal, S. K., 2002, The phosphorylated form of the ORF3 protein of hepatitis E virus interacts with its non-glycosylated form of the major capsid protein, ORF2. *J. Biol. Chem.* 277: 22759–22767.

Uhnoo, I., Wadell, G., Svensson, L., and Johansson, M., 1983, Two new serotypes of enteric adenovirus causing infantile diarrhoea. *Dev. Biol. Stand.* 53:311–318.

Uhnoo, I., Wadell, G., Svensson, L., and Johansson, M. E., 1984, Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. *J. Clin. Microbiol.* 20:365–372.

Uhnoo, I., Svensson, L., and Wadell, G., 1990, Enteric adenoviruses. *Baillieres Clin. Gastroenterol.* 4:627–642.

Unicomb, L. E., Banu, N. N., Azim, T., Islam, A., Bardhan, P. K., Faruque, A. S., Hall, A., Moe, C. L., Noel, J. S., Monroe, S. S., Albert, M. J., and Glass, R. I., 1998, Astrovirus infection in association with acute, persistent and nosocomial diarrhea in Bangladesh. *Pediatr. Infect. Dis. J.* 17:611–614.

van der Poel, W. H., Vinje, J., van Der Heide, R., Herrera, M. I., Vivo, A., and Koopmans, M. P., 2000, Norwalk-like calicivirus genes in farm animals. *Emerg. Infect. Dis.* 6:36–41.

van Loon, A. E., Maas, R., Vaessen, R. T. M., Reemst, A. M. C. B., Sussenbach, J. M., and Rozijn, T. H., 1985a, Cell transformation by the left terminal regions of the adenovirus 40 and 41 genomes. *Virology* 147:227–230.

van Loon, A. E., Rozijn, T. H., de Jong, J. C., and Sussenbach, J. S., 1985b, Physicochemical properties of the DNAs of the fastidious adenovirus species 40 and 41. *Virology* 140:197–200.

van Loon, A. E., Gilardi, P., Perricaudet, M., Rozijn, T. H., and Sussenbach, J. S., 1987, Transcriptional activation by the E1A regions of adenovirus types 40 and 41. *Virology* 160:305–307.

van Vliet, A. L., Smits, S. L., Rottier, P. J., and de Groot, R. J., 2002, Discontinuous and non-discontinuous subgenomic RNA transcription in a nidovirus. *EMBO J.* 21:6571–6580.

Vinje, J., and Koopmans, M. P., 1996, Molecular detection and epidemiology of small round-structured viruses in outbreaks of gastroenteritis in the Netherlands. *J. Infect. Dis.* 174:610–615.

Vinje, J., Green, J., Lewis, D. C., Gallimore, C. I., Brown, D. W., and Koopmans, M. P., 2000, Genetic polymorphism across regions of the three open reading frames of "Norwalk-like viruses." *Arch. Virol.* 145:223–241.

Vinje, J., Hamidjaja, R. A., and Sobsey, M. D., 2004, Development and application of a capsid VP1 (region D) based reverse transcription PCR assay for genotyping of genogroup I and II noroviruses. *J. Virol. Methods* 116:109–117.

Wadell, G., 1984, Molecular epidemiology of human adenoviruses. *Curr. Top. Microbiol. Immunol.* 110:191–220.

Weitz, M., Baroudy, B. M., Maloy, W. L., Ticehurst, J. R., and Purcell, R. H., 1986, Detection of a genome-linked protein (VPg) of hepatitis A virus and its comparison with other picornaviral VPgs. *J. Virol.* 60:124–130.

Weitz, M., and Siegl, G., 1998, Hepatitis A virus: structure and molecular virology, in: *Viral Hepatitis* (A. J. Zuckerman and H. C. Thomas, eds.), Churchill Livingstone, London, pp. 15–27.

White, L. J., Ball, J. M., Hardy, M. E., Tanaka, T. N., Kitamoto, N., and Estes, M. K., 1996, Attachment and entry of recombinant Norwalk virus capsids to cultured human and animal cell lines. *J. Virol.* 70:6589–6597.

White, L. J., Hardy, M. E., and Estes, M. K., 1997, Biochemical characterization of a smaller form of recombinant Norwalk virus capsids assembled in insect cells. *J. Virol.* 71:8066–8072.

Willcocks, M. M., Carter, M. J., Laidler, F. R., and Madeley, C. R., 1990, Growth and characterisation of human faecal astrovirus in a continuous cell line. *Arch. Virol.* 113:73–81.

Willcocks, M. M., Brown, T. D., Madeley, C. R., and Carter, M. J., 1994, The complete sequence of a human astrovirus. *J. Gen. Virol.* 75:1785–1788.

Willcocks, M. M., Boxall, A. S., and Carter, M. J., 1999, Processing and intracellular location of human astrovirus non-structural proteins. *J. Gen. Virol.* 80:2607–2611.

Wirblich, C., Thiel, H. J., and Meyers, G., 1996, Genetic map of the calicivirus rabbit hemorrhagic disease virus as deduced from the *in vitro* translation studies. *J. Virol.* 70:7974–7983.

Woode, G. N., Reed, D. E., Runnels, P. L., Herrig, M. A., and Hill, H. T., 1982, Studies with an unclassified virus isolated from diarrheic calves. *Vet. Microbiol.* 7:221–240.

Woode, G. N., Pohlenz, J. F., Gourley, N. E., and Fagerland, J. A., 1984, Astrovirus and Breda virus infections of dome cell epithelium of bovine ileum. *J. Clin. Microbiol.* 19:623–630.

Woode, G. N., Saif, L. J., Quesada, M., Winand, N. J., Pohlenz, J. F., and Gourley, N. K., 1985, Comparative studies on three isolates of Breda virus of calves. *Am. J. Vet. Res.* 46:1003–1010.

Yamashita, T., and Sakae, K., 2003, Molecular biology and epidemiology of Aichi virus and other diarrhoeogenic enteroviruses, in: *Viral Gastroenteritis* (U. Desselberger and J. Gray, eds.), Elsevier, Amsterdam, pp. 645–657.

Yamashita, T., Kobayashi, S., Sakae, K., Nakata, S., Chiba, S., Ishihara, Y., and Isomura, S., 1991, Isolation of cytopathic small round viruses with BS-C-1 cells from patients with gastroenteritis. *J. Infect. Dis.* 164:954–957.

Yamashita, T., Sakae, K., Kobayashi, S., Ishihara, Y., Miyake, T., Mubina, A., and Isomura, S., 1995, Isolation of cytopathic small round virus (Aichi virus) from Pakistani children and Japanese travelers from Southeast Asia. *Microbiol. Immunol.* 39:433–435.

Yamashita, T., Sakae, K., Tsuzuki, H., Suzuki, Y., Ishikawa, N., Takeda, N., Miyamura, T., and Yamazaki, S., 1998, Complete nucleotide sequence and genetic organization of Aichi virus, a distinct member of the Picornaviridae associated with acute gastroenteritis in humans. *J. Virol.* 72:8408–8412.

Yamashita, T., Ito, M., Kabashima, Y., Tsuzuki, H., Fujiura, A., and Sakae, K., 2003, Isolation and characterization of a new species of kobuvirus associated with cattle. *J. Gen. Virol.* 84:3069–3077.

Yeager, M., Dryden, K. A., Olson, N. H., Greenberg, H. B., and Baker, T. S., 1990, Three-dimensional structure of rhesus rotavirus by cryoelectron microscopy and image reconstruction. *J. Cell Biol.* 110:2133–2144.

Yokosuka, O., 2000, Molecular biology of hepatitis A virus: significance of various substitutions in the hepatitis A virus genome. *J. Gastroenterol. Hepatol.* 15(Suppl.):D91–D97.

Zafrullah, M., Ozdener, M. H., Panda, S. K., and Jameel, S., 1997, The ORF3 protein of hepatitis E virus is a phosphoprotein that associates with the cytoskeleton. *J. Virol.* 71:9045–9053.

Zárate, S., Espinosa, R., Romero, P., Guerrero, C. A., Arias, C. F., and López, S., 2000a, Integrin $\alpha 2\beta 1$ mediates the cell atachment of the rotavirus neuraminidase-resistant variant nar3. *Virology* 278:50–54.

Zárate, S., Espinosa, R., Romero, P., Méndez, E., Arias, C., and López, S., 2000b, The VP5 domain of VP4 can mediate attachment of rotaviruses to cells. *J. Virol.* 74:593–599.

Zhang, Y., McAtee, P., Yarbough, P. O., Tam, A. W., and Fuerst, T., 1997, Expression, characterization, and immunoreactivities of a soluble hepatitis E virus putative capsid protein species expressed in insect cells. *Clin. Diagn. Lab. Immunol.* 4:423–428.

Zhang, M., Zeng, C. Q.-Y., Morris, M. P., and Estes, M. K., 2000, A functional NSP4 enterotoxin peptide secreted from rotavirus-infected cells. *J. Virol.* 74:11663–11670.

Zhang, M., Purcell, R. H., and Emerson, S. U., 2001, Identification of the 5′ terminal sequence of the SAR-55 and MEX-14 strains of hepatitis E virus and confirmation that the genome is capped. *J. Med. Virol.* 65:293–295.

Zhou, Y., Li, L., Okitsu, S., Maneekarn, N., and Ushijima, H., 2003, Distribution of human rotaviruses, especially G9 strains, in Japan from 1996 to 2000. *Microbiol. Immunol.* 47:591–599.