Reviews in
MEDICAL VIROLOGY VOL. 2: 97–106 (1992)

Astroviruses

M. M. Willcocks†, M. J. Carter†* and C. R. Madeley†‡
†*Division of Virology, School of Pathological Sciences, New Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, UK and* ‡*Department of Virology, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne NE1 4LP, UK*

INTRODUCTION

Astroviruses are one of a number of viruses discovered when electron microscopy (EM) was used to examine extracts from faeces in the mid 1970s. Most of the faeces were from infants with diarrhoea and these investigations identified rotaviruses, the (then) uncultivable adenoviruses, caliciviruses, small round viruses, small round structured viruses (including Norwalk), coronaviruses and astroviruses. All were associated with diarrhoea and vomiting, some with outbreaks of food poisoning, but diligent searches found all to be present in some stools from apparently healthy individuals.[1]

Astroviruses were characterised as 28 nm particles with a smooth margin. In some preparations they appear to have surface projections but this has yet to be confirmed. The viruses were named from the unusual surface motif of a five- or six-pointed star seen on approximately 10% of particles (*astra*, a star).[2,3] This motif covers the presenting surface of the particle, but the detailed structure of the virion is not yet understood. Typical astroviruses are illustrated in Figure 1, and the EM appearance of the particles is described in more detail below.

Since the discovery of astroviruses, particles of similar appearance have been recognised in the stools of a variety of animals including lambs,[4] calves,[5] pigs,[6] cats[7] and ducklings.[8] In humans, and in all but one of the animal viruses investigated, the virus has been associated with outbreaks of diarrhoea. In the duckling, however, astrovirus infection is associated with hepatitis and has a mortality rate of approximately 25% in 3–6 week old ducklings, adult ducks being spared.[8]

Despite the association of these viruses with disease in man, few details about them are established. There are conflicting reports of the number and size of the proteins they contain, and their replication strategy and even their taxonomic status are unclear. To a large extent this is due to difficulties in growing these viruses *in vitro*. Recently these have been overcome, and the application of recombinant DNA technology in this area has opened new fields of study. This review will consider the available information and attempt to summarise the current position of these agents as human pathogens and as members of the virus world.

*Author to whom correspondence should be addressed.

ISSN 1052–9276/92/020097–10 $10.00

PATHOGENESIS

Human astroviruses most frequently cause disease in young children, and by 5 years of age more than 80% of children show serological evidence of previous infection.[9] Illness rates can rise in the elderly; in an outbreak in a convalescent hospital in Marin County, USA, 51% of the elderly patients developed symptoms.[10] The virus responsible for this outbreak has since been classified as an astrovirus.[11,12] Astrovirus infection in infants can lead to a mild diarrhoea, but there is rarely fever or vomiting. The diarrhoea is normally short-lived and is usually treated conservatively. Short term monosaccharide intolerance has been reported.[13] In older children (up to 5 years of age) the clinical features are similar and include diarrhoea (generally without vomiting), headache, malaise and mild fever. Infection is again generally short-lived (24–48 h) and complications are rare.

The relative contribution of astroviruses to the total incidence of virus-associated diarrhoea is not precisely known. They have been generally assumed to be relatively minor contributors to this condition. However, since illness is mild and of short duration, figures for virus incidence based on hospitalised patients will underestimate the frequency of astrovirus-associated illness. Furthermore, difficulties in positively identifying astrovirus particles may compound this, and some reports now indicate that astroviruses may be the second commonest cause of viral gastroenteritis in non-hospitalised patients (see below).

Duodenal biopsy during infection has shown the presence of astrovirus particles within the epithelial cells of the villus.[14] Similar results have been found in animal studies. Ovine astrovirus has been shown to infect the upper epithelial cells of the small intestinal villi.[15,16] Growth of virus *in vitro* suggests crypt cells may be susceptible although they are relatively spared *in vivo* and haemorrhagic diarrhoea has not been reported.

ASTROVIRUS GROWTH AND ISOLATION

Culture

Until recently astroviruses could not be propagated directly in continuous cell lines. Lee and Kurtz[17] developed a method for the growth of human astrovirus in primary

Accepted 28 February 1992

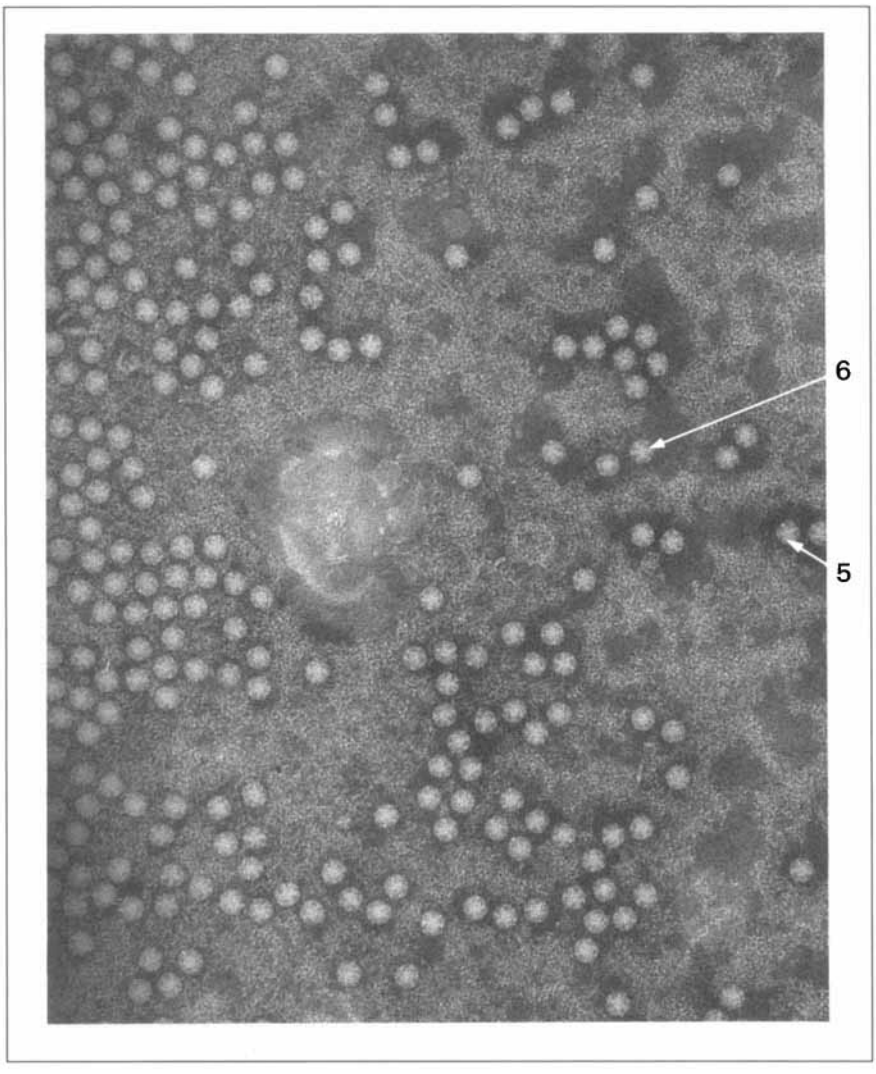

Figure 1. Electron micrograph of astrovirus particles. Typical human astrovirus particles from stool. Negative contrasted image prepared with 3% potassium phosphotungstate pH 7.0. Virus particles bearing five- and six-pointed surface stars are indicated. (Magnification × 200 000.)

human embryo kidney (HEK) cells. In common with other enteric viruses growth was dependent on the addition of trypsin to the culture medium. Virus isolates passaged six times in HEK cells became adapted to cell culture and could be passaged in the continuous rhesus monkey cell line LLC-MK2, although trypsin was still required.

Irregular supplies of primary human embryo cells limits this approach, and so the number of isolates made remains small. More recently, Willcocks and co-workers[18] circumvented this problem by growing astroviruses directly from stool in CaCo-2 cells; a continuous line with crypt cell-like properties (see below).

Serotypic variation

Utilizing HEK-adapted virus isolates, Lee and Kurtz[19] were able to produce sufficient virus to raise antisera against isolates of human astroviruses. These sera were tested by immune EM (IEM) for their ability to aggregate astroviruses from other stool samples. This technique quickly identified two distinct serotypes of astroviruses, and a subsequent survey in the Oxford, UK, area increased this to five. Of these, serotype 1 was[20] by far the most prevalent (77%). These authors found that none of the human serotypes cross-reacted with bovine or ovine astrovirus.

Since then, monoclonal antibodies to human astro-viruses have been produced and Herrmann and co-workers[12] have developed an enzyme immunoassay (EIA) for the detection of astroviruses. They found that when antisera were used in this test a high degree of cross-reactivity was observed between the five serotypes, even though the sera could distinguish well between serotypes by other techniques such as immunofluorescence or IEM. This result indicated the possible presence of a group antigen which was subsequently confirmed by the selection of a monoclonal antibody which reacted with all serotypes.

There is no corresponding information on serotype variation among animal astroviruses, but these have not been examined thoroughly due to difficulties in cultivation. Both feline and porcine astroviruses have been passaged serially in embryo cells from the appropriate animal. The feline virus grew in primary cultures and the porcine in an established line derived from pig-embryo cells. However, both viruses also required trypsin for growth.[21,22] No cross-reaction was found between feline and human astrovirus when tested by reciprocal IEM.

Route of cell infection

Human astroviruses can be propagated directly from stool samples in CaCo-2 cells.[18] Although trypsin is still required for serial passage of the virus, it is not necessary for the initial infection. CaCo-2 cells are relatively resistant to protease and monolayers remain intact in the presence of this enzyme. Consequently cell destruction due to virus replication is clearly visible following infection with human astroviruses. In contrast, LLC-MK2 cell monolayers are destroyed by the action of the trypsin and further cell destruction caused by the virus is not easily discernible.

CaCo-2 cells are a continuous line of human colonic carcinoma cells originally derived from a primary colonic tumour[23] and retain many properties characteristic of gut cells. The cells differentiate in culture and thus mimic the situation in the small intestine where cells pass through various stages of maturity[24] and have been used as a model for small intestinal transport systems.[25] They possess the mucosal chloride transport characteristic of crypt cells.[26] However, animal studies have shown that both ovine and bovine astroviruses mainly infect epithelial cells towards the top of the intestinal villi, crypt cells appear to be relatively spared.[16] Consequently CaCo-2 cells may not exactly mimic the natural host cell infected *in vivo*.

CaCo-2 cells grow in culture to form monolayers of polarised cells with tight junctions.[27] Chloride secretion and corresponding fluid movement leads to the net transport of water across the monolayer. In common with other such epithelia, functional asymmetry is reflected in differences in the distribution of some membrane proteins between the basolateral and apical membranes of the cells. This may include the receptors recognised by viruses and which enable them to bind to their target cells. This would induce a polarity in the initial stages of virus infection with cells being susceptible to virus on one surface but not the other. For instance, rotavirus infection of CaCo-2 cells can occur from either surface, whereas that by Semliki Forest virus is clearly polar, virus adsorbing mainly via the basolateral surface.[28] Human astrovirus also infects CaCo-2 cells in a polar manner, infecting preferentially at the basolateral surface (Figure 2). This is surprising because astrovirus would be expected to infect the apical cell surface facing into the gut lumen, the side accessible to virus *in vivo*. However, this apparent polarity could be a consequence of the growth of the virus *in vitro*, and suitable receptors which are limited to the basolateral surfaces of CaCo-2 cells, might be present on the apical surfaces of differentiated intestinal villus epithelial cells.

Other viruses which infect the gastrointestinal tract are known to display a similar pattern. Reovirus serotype 1 is also known to bind to the basolateral surface of crypt cells.[29] In this case it was suggested that reovirus could enter the host via the M-cells, which overlie the Peyer's patches in the gut and function to present luminal antigens to cells of the immune system located in the patches. The virus could then infect adjacent crypt cells by binding to their basolateral and not to their apical surfaces. This mechanism could be used by other enteric viruses, such as astrovirus, to gain entry to the cells lining the gut. However, this model would predict that astroviruses would infect crypt cells *in vivo*. Animal studies do not support this, and human studies are very limited. Nonetheless, infection may not be restricted to the villus tips, and duodenal biopsy during human infection has shown that human astrovirus can infect cells on the proximal part of the villus.[14] Furthermore, unlike astrovirus, reovirus infection is limited to the crypts *in vivo* and is unable to spread up the villus, even though isolated intestinal epithelial cells can also bind the virus at their basolateral surfaces. Consequently, if this route is used by astroviruses, the process must differ in some respects.

ASTROVIRUS PARTICLE STRUCTURE

Biophysical characteristics

Since human astroviruses could not be cultivated easily *in vitro* most information on particle structure came initially from work on the ovine astrovirus propagated in gnotobiotic lambs, but later studies using cultivated human astrovirus yielded similar results. Both ovine and human astroviruses formed two discrete bands on caesium chloride density gradients. In ovine astrovirus these were of densities 1·365 and 1·39 g/ml and RNA was predominantly associated with the higher density band although the protein composition of the other band was not examined.[30] Human astrovirus serotype 1 yielded two bands of densities 1·32 and 1·35 g/ml and identical polypeptides were found in each.[18] It seems probable that the particles of lower density represent empty capsids similar to those formed in picornavirus infections.

Protein composition of the particles

The protein compositions of astroviruses from several different hosts have now been examined. Table 1 compares the virion-associated polypeptides of ovine astrovirus,[30] porcine astrovirus[22] and human astrovirus serotypes: 4[31]; 1[32,18] and 2[33]. Considerable variation in the protein compositions have been reported for the different astroviruses but some general features are apparent.

All the astrovirus isolates contain at least two major polypeptides with M_rs between 29 000 and 33 500 which often comigrate with an M_r of 33 000. The porcine virus has been reported to contain larger proteins (36 000–39 000) which could represent intracellular precursors since this preparation was only semi-purified. The larger molecules occasionally observed in human astrovirus have been similarly attributed.[31]

Three of the four human isolates also contained a slightly smaller protein (M_r between 20 000 and 26 500) and a smaller 13 000 protein was found in the porcine virus. This protein was seen unequivocally in those isolates in which it was detected, so its absence from some of the earlier reports for both ovine and human astrovirus is surprising. Herring and co-workers[30] reported only two polypeptides (both approximately 33 000) in ovine astrovirus purified using sodium dodecyl sulphate. We have shown that similar treatment of the human astrovirus serotype 1 removes the 24 000 protein from the virion almost completely and thus could account for its absence in earlier reports.[18] There is a single report of a small

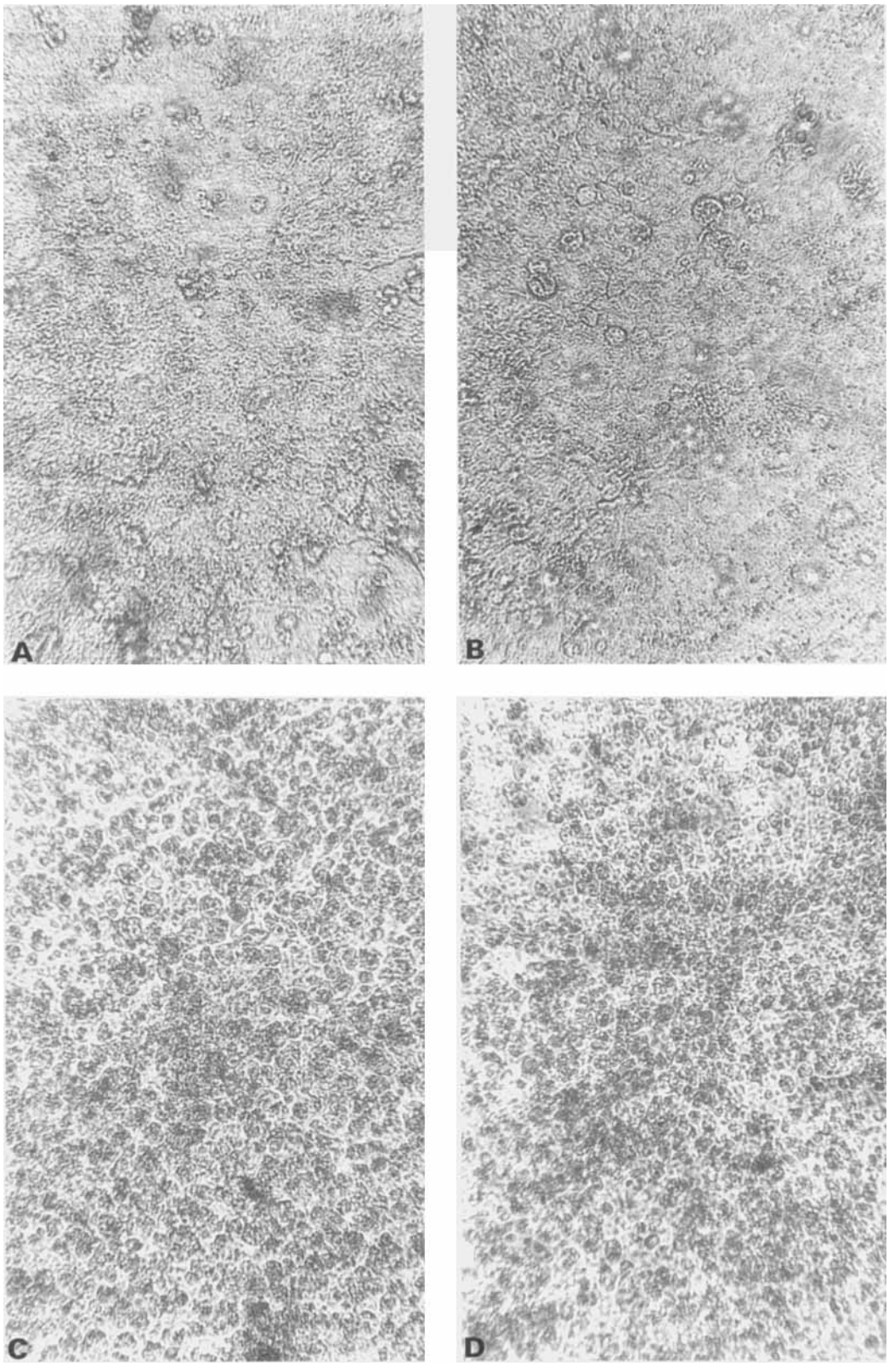

Figure 2. Polarity of infection of CaCo-2 cell monolayers by astrovirus. CaCo-2 cell monolayers were grown on porous (300 nm) membranes which provide support for the monolayer (Costar Transwell). The confluent cell monolayer then divides the culture well into two compartments separating fluid above and below the membrane. Virus inocula can then be added to medium in contact with either the apical or basolateral surfaces. Cells were then infected with human astrovirus or poliovirus (without the addition of trypsin) at either the apical or the basolateral surface. The cells were incubated at 37°C and examined daily for the development of CPE. The monolayers were photographed through a light microscope using a × 40 objective. (A) Uninfected CaCo-2 monolayer (6 days). (B) CaCo-2 cell monolayer infected at the apical surface with human astrovirus and photographed at 6 days p.i. Cell appearance is unaltered from panel A, above. (C) CaCo-2 cell monolayer infected at the basolateral surface with human astrovirus, shown at 6 days p.i. Degenerating cells are visible as dark, granular structures. (D) CaCo-2 cell monolayer infected at the apical surface with poliovirus photographed at 3 days p.i. Cellular degeneration is visible as dark granulation of the cells.

Table 1. Relative molecular masses of polypeptides associated with different astrovirus preparations

Human serotypes				Ovine	Porcine[a]	Consensus
1	1	2	4			
					39 000	
			36 500		36 000	
33 000			33 000	33 000		
33 000	33 500		33 000	33 000		two larger proteins
	31 500		32 000			
		31 000			31 000	
		29 000			30 000	
26 500						
	24 000					one smaller protein
		20 000				
			?	?	13 000	
5200						

[a]Proteins observed in immunoprecipitates from infected cell lysates, rather than purified virus preparations.
? Indicates that a protein might have been lost during purification (see text).
Data taken from references 18, 22, 30–33.

(M_r 5200) polypeptide in the virion,[32] but in many of the examinations listed in Table 1 the polyacrylamide gel systems used would not have retained a protein of this size so the existence of this molecule has still to be confirmed.

Thus, the consensus which is emerging is that the viruses may contain at least three major species of protein; two of M_r approximately 29 000–33 000 and a smaller polypeptide which is more variable between strains (M_r 13 000–26 500).

Astrovirus RNA

Ovine astrovirus contains a single-stranded RNA of M_r $2{\cdot}7 \times 10^6$, equivalent to approximately 7800 nucleotides. This was assumed to be positive in sense since it possessed a poly A tract of approximately 14 nucleotides at the 3′ end.[30] Likewise the genome of human astrovirus is between 7200 and 7600 nucleotides in length, and consists of a single-stranded polyadenylated RNA.[31,33–35] Two groups have reported the cloning of parts of the genome and, although the complete sequence has not yet been determined, the sequence of the 3′ terminal 1034 nucleotides has been presented.[34,35] Sequence analysis of this region has implications for virus taxonomy and the clones have also been used as probes to examine intracellular RNA synthesis (see below).

ASTROVIRUS REPLICATION

Molecular cloning of astrovirus RNA has been carried out on two isolates to date, a serotype 2 astrovirus, adapted to serial growth by passage in human embryo cells and subsequently grown in LLC-MK2 cells;[33] and a serotype 1 astrovirus isolated in CaCo-2 cells and cloned from cells infected in the first virus passage from stool.[35] Two reports concern the intracellular synthesis of virus RNA. In the first Monroe and colleagues[33] reported two species of RNA, one presumed to be genomic (7800 nucleotides) and a smaller molecule (referred to as subgenomic) of approximately 2800 nucleotides which were synthesised in LLC-MK2 cells infected with human astrovirus serotype 2. The second report[35] studied a serotype 1 human astrovirus grown in CaCo-2 cells. In this case only the genomic size RNA was detected.

These observations have taxonomic implications (see below) and this disparity must be resolved. It is possible that the synthesis of subgenomic astrovirus RNA is variable and occurs at too low a level to be clearly observed in the CaCo-2 cells. Alternatively this species may have little sequence similarity to the 3′ terminus of the virus used as probe. Although seen in large amount by Monroe and co-workers, detection was primarily based on the incorporation of labelled uridine into the RNA. Detection by hybridisation with 3′-specific probes was reported, but no details were provided. However, such a subgenomic RNA, lacking extensive 3′ sequence similarity to the genome, would be unique among RNA viruses.

Intracellular protein synthesis has yet to be investigated in detail. Monroe and co-workers[33] reported a precursor protein of M_r 90 000 in LLC-MK2 cells infected with astrovirus serotype 2. This could be cleaved *in vitro* by trypsin to yield the three presumed virus structural polypeptides of M_r 31 000, 29 000 and 20 000.

TAXONOMIC STATUS OF ASTROVIRUSES

Comparison with other virus groups

It is unclear whether the astroviruses are a distinct family or whether they should be classified in an already established

virus group. The only candidate families would be *Picornaviridae* or *Caliciviridae* since all other positively stranded animal RNA viruses are enveloped. Both picornaviruses and caliciviruses possess genomes of similar size to the astroviruses, but these two groups differ in the construction of their particles, genome organisation and replication strategy.

Structural proteins

Resemblance between astroviruses and members of the *Picornaviridae* (particularly the enteroviruses) has been noted. This is particularly marked in their stability to acid or heat and their resistance to ether, chloroform or alcohols.[31,32] Astroviruses also show similarities with the picornaviruses in their buoyant density in caesium chloride and their pattern of polypeptides. The picornaviruses typically contain four polypeptides (in poliovirus[36] these have M_r's of 33 500, 30 000, 26 400 and 7400) but not all the members of the picornavirus family show the typical pattern of four polypeptides as clearly as this. It has been reported that the smallest picornavirus protein (VP4) is easily lost during the purification of some picornaviruses and thus may be overlooked.[37] This general pattern is very similar to the consensus derived from Table 1 for astrovirus structural proteins, although a fourth astrovirus protein has not been confirmed.

In contrast, the caliciviruses, and the related small round structured viruses, such as Norwalk virus, contain only one major polypeptide of approximately 60 000.[38,39]

Genome organisation

The picornavirus genome contains a single large open reading frame which is translated directly from the full-length RNA to form a single polyprotein (reviewed by Rueckert[37]). In contrast, the calicivirus genome contains separate open reading frames although some may be fused in certain viruses. However, both rabbit and feline caliciviruses possess a small conserved open reading frame at the 3′ end of their RNAs which could specify a highly basic protein.[40,41] This may not be a universal feature of caliciviruses, since it is not present in hepatitis E virus, a candidate member of this group.[42]

The sequence of the 3′ terminus of the human astrovirus genome[35] does not contain the small open reading frame found in the caliciviruses. Instead a single open reading frame extends throughout the clone and terminates 83 residues from the 3′ end of the virus. Although the complete genome organisation has not been determined, this structure is suggestive of more similarity to the picornaviruses than to the caliciviruses.

In the picornaviruses the non-coding region (3′-NCR), between the end of the open reading frame and the poly A tail of the virus RNA, is thought to encode signals required for polymerase recognition and there is noticeable sequence conservation in this region between various picornaviruses.[37] Similarities in these functional regions of the genomes of different viruses could thus also provide evidence for potential relatedness between virus groups. Therefore, the 3′-NCR of the human astrovirus genome (serotype 1) was compared by computer analysis with the corresponding regions of several picornaviruses and caliciviruses. The resulting dendrogram is shown in Figure 3.

Astrovirus was grouped among the picornaviruses, rather than the calicivirus sequences by this procedure. The picornaviruses formed two main groups and the astrovirus 3′-NCR was grouped with those of poliovirus types 1–3, Coxsackie virus A21 and enterovirus 70 (group A). All these viruses fell within a larger cluster (groups A+B) containing all the enterovirus subgroup sequences compared, but excluding those of the rhinovirus and cardiovirus subgroups, hepatitis A and foot-and-mouth-disease virus. These formed a heterogeneous third group with the calicivirus sequences (group C).

The picornaviruses can also be grouped on the basis of their predicted RNA folding patterns at the 3′ terminus. The RNA may form single, double or treble loop structures.[43] All the viruses grouped with the astrovirus in this analysis are of the 'two loop' family. Possible folding of the astrovirus 3′ terminus was investigated by computer modelling and the theoretical fold derived consisted of a double loop and very closely resembled that of the polioviruses with which it was grouped.[35] However, sequence identity between astro- and picornavirus 3′-NCR is small, and such similarity as there is need not imply a relationship between the viruses. The 3′ termini of these viruses are presumably subject to similar selection pressures and similarity in sequence and structure could therefore arise by convergent rather than divergent processes.

Replication strategy

The principal difference between picornaviruses and caliciviruses arises from the production of multiple intracellular RNAs during calicivirus replication. The calicivirus genome is transcribed to form at least one subgenomic RNA from which the internal open reading frame specifying the capsid protein can be expressed. Each RNA species is then translated independently in the cell. This subgenomic RNA is derived from the 3′ terminus of the virus RNA and is readily detected in the infected cell. The possibility of other such molecules has been considered but these could be artefacts resulting from degradation or associations between forms of the two definite intracellular RNAs.[41,42,44–46]

Unfortunately the data concerning the number of intracellular RNAs synthesised by astroviruses are contradictory. One report identifies a subgenomic molecule and one does not.[33,35] These two observations need to be reconciled. Whilst the synthesis of a subgenomic RNA may be variable between host cell or virus, it could also arise from the presence of a defective interfering RNA. However, extensive steps were taken to eliminate this possibility and such an origin is unlikely.

Unique features of the astroviruses

Whilst the above discussion would imply a closer relationship between astroviruses and the picornaviruses than between the astroviruses and caliciviruses, there are other factors unique to the astroviruses which may have a bearing on their taxonomic status.

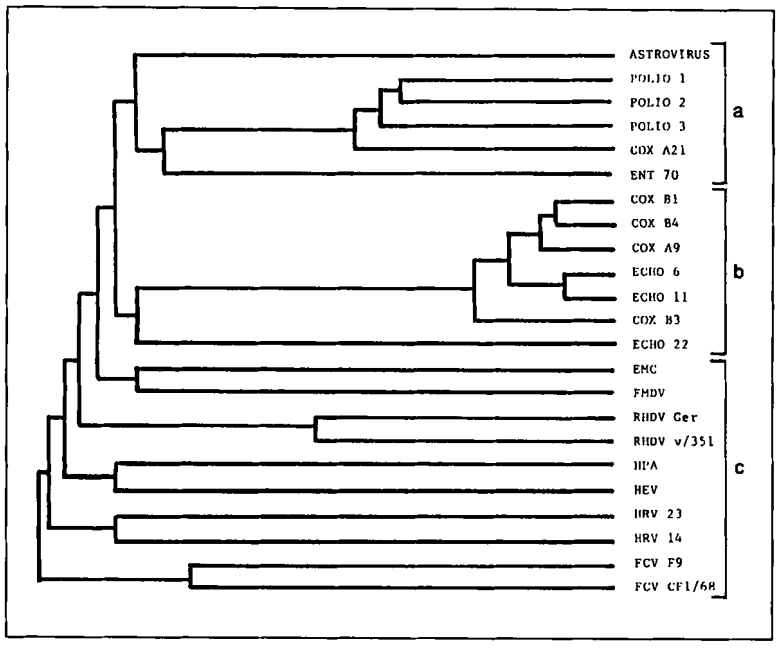

Figure 3. CLUSTAL analysis of 3′ non-coding regions between astrovirus, picornaviruses and caliciviruses. Nucleotide sequence of the 3′-NCRs of human astrovirus serotype 1 (A88/2 Newcastle), feline calicivirus, rabbit haemorrhagic disease virus, hepatitis E virus and representative members of the *Picornaviridae* were compared by the CLUSTAL program of PC-Gene (Intelligenetics, Geel, Belgium) using a gap penalty of 5 and a window size of 10. The computed relationships between sequences are presented as a dendrogram. The three groups referred to in the text are indicated. POLIO, poliovirus; COX, coxsackievirus; ENT, enterovirus; ECHO, echovirus; EMC, encephalomyocarditis virus; FMDV, foot-and-mouth disease virus; RHDV, rabbit haemorrhagic disease virus; HPA, hepatitis A virus; HEV, hepatitis E virus; HRV, human rhinovirus; FCV, feline calicivirus.

First, and perhaps the most obvious difference between astroviruses, caliciviruses and picornaviruses is visible by negative contrast electron microscopy. Picornaviruses are similar to astroviruses in size. However, they are invariably plain featureless spheres with some ring-like (empty) particles which have been penetrated by stain; no substructure is visible. In contrast about 10% of astroviruses bear the characteristic surface stars from which the virus derives its name. Some of these stars have five points and others six. These structures are unique and there is no satisfactory model for how they are achieved. Possession of a six-pointed star is superficially similar to the 'star of David' configuration seen on some caliciviruses, but the astrovirus star lacks a central hollow, and this is a fundamental difference.[47] Picornavirus (reviewed by Rueckert[37]) and calicivirus structures[48] are clearly based on an icosahedron. However, the centres of both the five and six pointed astrovirus stars appears to be an apex rather than a face. In structures based on an icosahedron an axis of 3-fold symmetry must pass through the centre of a face.[47,49] This requirement is fulfilled by caliciviruses but not, apparently, by astroviruses, and is consequently a fundamental point of difference.

Second, the picornaviruses encode the viral RNA polymerase (3D protein) at the 3′ terminus of the genome. There is amino acid similarity between picornaviruses in this region, and the functional motif believed typical of RNA polymerases (Tyr Gly Asp Asp)[50] is located within the last 150 amino acids in the polyprotein. The 3′ terminal protein predicted from the astrovirus sequence has no significant homology with picornavirus 3D, and the functional motif is not present within the 300 amino acids determined. This suggests that gene order could be different between the two viruses. Furthermore the astrovirus protein showed no significant homology with any protein in the *Swiss Prot 18* database.

Third, several workers have reported a movement of virus antigen into the cell nucleus early in replication and detected by immunofluorescence. This has been observed in both human (W. D. Cubitt, personal communication) and bovine astrovirus infections. Two isolates of calf astrovirus gave rise to a limited, discrete fluorescence in the nucleus of infected calf kidney cells, and a higher level of a more diffuse fluorescence was observed in the cytoplasm.[5,51] We have determined the sequence of an internal region of the human astrovirus genome which encodes a potential nuclear targeting motif,[52] although whether this is functional remains to be demonstrated. No nuclear involvement is seen following picornavirus[53,37] or calicivirus[46] infection, and this therefore represents a potential and significant difference in replication strategy between the astroviruses and both picornaviruses and caliciviruses.

IMPORTANCE OF ASTROVIRUSES IN DIARRHOEA

Astroviruses were discovered by electron microscopy, and EM remains the only diagnostic method with which all the viruses associated with diarrhoea can be detected. Other

techniques can only be applied to some viruses (polyacrylamide gel electrophoresis or antibody-based tests) and some prior selection has to be made. Most of these viruses are not easily propagated in cell cultures, and for some no culture methods have yet been found. This has limited the development of immunological assays. Some of the viruses commonly implicated in the causation of diarrhoea have readily apparent structures; the rotaviruses, adenoviruses, caliciviruses and astroviruses. Other particles observed lack clear identifying features. They may be small (22–30 nm) with a smooth margin (termed small round viruses; SRV), or slightly larger (30–38 nm) with an unclear (fuzzy) surface and periphery (the small round structured viruses; SRSV). Enzyme-immunoassays are only available commercially for the detection of the rotaviruses and the enteric adenoviruses, although an immunoassay for astrovirus has also recently been developed.[54]

It is generally believed that astroviruses are a relatively minor cause of viral gastroenteritis, with rotaviruses and adenoviruses being the main viruses involved.[55] However, it is likely that the incidence of viruses detected by EM may be biased by the relative ease with which these larger viruses can be identified in the electron microscope. They are often shed in large numbers and their characteristic size and shape make them easily recognisable. In contrast, astroviruses are more easily missed in the examination even when present in large numbers. Furthermore, there is evidence that the use of EM alone can result in the mis-classification of astroviruses. Particles showing the characteristic 'star-like' surface pattern can be identified without difficulty, but samples which contain viruses of indistinct morphology are not so readily identified. Lack of distinctive features could arise from poor preservation of structural features in the samples, or could be a fundamental property of some strains, as has been found in both rotaviruses and caliciviruses.[56,57]

Indistinct viruses can only be grouped on the basis of the particle size, the determination of which can vary between laboratories, or loosely divided into two groups on the basis of whether the particles exhibit a smooth or a rough surface margin (SRVs or SRSVs). This method would group unclear astroviruses with the SRVs, and unclear caliciviruses with the SRSVs. However, to complicate matters, astroviruses may not always show the typical smooth-edged appearance. Astroviruses released during early passages in CaCo-2 cells appeared larger and with a less distinct margin than did virions from passage five onwards. However, both types of released virions contained identical polypeptides and both were shown to be astrovirus serotype 1 by serology.[18] Consequently, even these systems are ambiguous; the Marin County agent was originally assigned by EM to the SRSV group[10] but was later re-classified as an astrovirus by serology.[11] Similarly the Harlow agent of gastroenteritis lacked typical astrovirus features but was identified as such by both IEM and immunofluorescence.[58] There is a requirement for a more widely applicable diagnostic method for all these viruses, one which relies less on the good morphological preservation of the sample and the skill of the operator than EM, but which could be developed for, and applied to, those viruses which remain uncultivable. Serological methods, especially those based on monoclonal antibodies have been successful in this regard.

Herrmann and co-workers[59] used a monoclonal antibody-based EIA test to survey stool samples from children in Thailand. The authors examined both children with diarrhoea and a cohort of normal contemporaries. Astrovirus was found in 8·6% of the stools from all children with gastroenteritis and in 2% from those without. Enteric adenoviruses were found[59] in 2·6% and rotavirus in 19%. Thus astroviruses were the second most prevalent agent associated with diarrhoea in this survey, and these data support the suggestion that previous diagnostic methods may have underestimated astrovirus infections. We have recently developed a dot-blot hybridisation test for the detection of astrovirus nucleic acid in stool samples,[60] and find a similar incidence in the Newcastle-upon-Tyne area of the UK. Astrovirus was also found here to be more common than adenoviruses 40 and 41 (Silcock *et al.*, in preparation).

Underestimation of astroviruses has also been indicated in other reports. For example, Oliver and Phillips[61] re-examined stools thought to contain small round viruses identified by EM and reported that 14 out of 53 samples actually contained astrovirus when examined by IEM. If such underdiagnosis is widespread it could imply an even greater incidence of astrovirus diarrhoea since some laboratories report SRVs as the second (after rotavirus) most common virus found in diarrhoeal faeces.[55]

CONCLUSION

The astroviruses are a group of undercharacterised agents associated with diarrhoea. The contribution of these viruses towards diarrhoeal disease may have been underestimated in the past. Whilst they show greater similarities to the picornaviruses than the caliciviruses, their replication strategy is still unclear. However, they also have features unique to themselves and this may eventually lead to the recognition of a new virus family.

ACKNOWLEDGEMENT

MMW is supported by the Medical Research Council.

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