Astrovirus-associated gastroenteritis in children

C. R. ASHLEY, E. O. CAUL, AND W. K. PAVER¹

From the Public Health Laboratory, Myrtle Road, Bristol BS2 8EL, UK

SUMMARY In a small astrovirus-associated outbreak of gastroenteritis in a ward of a local children's hospital two out of five children with symptoms excreted astrovirus particles. No astrovirus particles were found in faeces from the remaining asymptomatic child, and no other viral or bacterial pathogens were found in any of the children. Virus excretion persisted for only a few days. Rising antibody titres to the astrovirus particles were demonstrated in one child, and IgM was also demonstrated in this patient's serum.

In recent years one of the most successful techniques in the search for viral agents, which might cause gastroenteritis in man, has been the direct examination of faecal emulsions by electron microscopy (Kapikian *et al.*, 1972; Flewett *et al.*, 1973; Paver *et al.*, 1973; Bishop *et al.*, 1974; Caul *et al.*, 1975; Caul and Egglestone, 1977; Appleton and Higgins, 1975; Madeley and Cosgrove, 1975, 1976). The detection of virus particles in faeces alone is insufficient evidence to allow a causal role to be assigned to the virus particles. A causal role may be partially established by the demonstration of a rise in antibody titre to the particles at the time of illness using paired sera from cases of the disease. This has been demonstrated for the Norwalk agent and rotaviruses by immune electron microscopy and other immunological techniques; but similar rises in antibody were not demonstrated with the 22 nm parvovirus-like particles in those paired sera that were tested (Paver *et al.*, 1973). Recently, some serological work has been carried out with astroviruses (Kurtz *et al.*, 1977).

This paper describes the occurrence and morphology of astroviruses found by examination of faecal emulsions from children aged 1 to 7 years in a ward outbreak of gastroenteritis in a local children's hospital in April 1975. In addition, the results of immune electron microscopy are described using paired sera from one child with particular reference to the detection of specific immunoglobulin M. This child with gastroenteritis was excreting astrovirus particles at the time of symptoms.

Material and methods

Two faecal specimens were collected from a child (patient T) who had been admitted to hospital with nephrotic syndrome and who subsequently developed severe gastroenteritis. These specimens were taken one day and three days after the child had diarrhoea.

Routine 10% faecal emulsions were prepared in phosphate-buffered saline (PBS), clarified by centrifugation for 30 minutes in a bench centrifuge, and concentrated for examination in the electron microscope by centrifugation for two hours at 50 000 *g* in a Sorvall RC2-B ultracentrifuge. Two further specimens were collected from this child eight days and 14 days after the onset of symptoms. Twenty-five per cent faecal emulsions were prepared from these two specimens, clarified, and concentrated by centrifugation for four hours at 110 000 *g* in a Spinco Model 'L' ultracentrifuge.

Single specimens of faeces were also collected from five other children in the same ward. One of these (patient M) had been discharged from hospital three days after patient T had diarrhoea but was readmitted three days later with an attack of vomiting. This lasted for two days and the child then developed diarrhoea which persisted for two days. A faecal specimen was collected the day before the onset of diarrhoea. Three other children also had gastrointestinal symptoms at some stage during their stay in hospital; two of these had vomiting without diarrhoea and the other (patient P) had diarrhoea and vomiting. The fifth child was asymptomatic during his stay in hospital. Twenty-five per cent emulsions were prepared from these five faecal samples and processed as described above.

¹ Present address: Public Health Laboratory, Withington Hospital, Manchester.

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All faecal specimens were examined for the presence of known bacterial pathogens.

Paired serum samples were obtained from patient T eight days and 14 days respectively after the onset of symptoms. Figure 1 summarises these data.

Key:

- days in hospital
- incidence of vomiting
- incidence of diarrhoea
- × astroviruses in faeces
- ↓ negative faecal sample

Fig. 1 Course of ward outbreak of astrovirus-associated gastroenteritis.

DIRECT ELECTRON MICROSCOPY

The ultracentrifuged deposits were resuspended in a drop of distilled water. A formvar-carbon-coated 250 mesh grid was floated on a drop of the suspension for a few minutes, blotted, washed on a drop of water, blotted again, and floated on a drop of 1.5% phosphotungstic acid at pH 6.5 for 20 seconds. Grids were blotted, air-dried, and examined at $\times 40\,000$ magnification in an AEI 801 electron microscope.

IMMUNE ELECTRON MICROSCOPY

The astrovirus antigen for immune electron microscopy was an ultracentrifuged deposit of a 25% emulsion of faeces from patient T, resuspended in distilled water. Antisera were diluted 1/10 to 1/640 in PBS. Antigen/antibody mixtures were incubated at room temperature for one hour and then centrifuged at $35\,000\,g$ for two hours. The deposits were resuspended in distilled water, and grids were prepared and stained with 1.5% phosphotungstic acid at pH 6.5. Grids were examined under code. Clumps containing three or more particles in conjunction with visible antibody and with regular spacing between the particles were considered to be evidence

of the presence of antibody in the serum.

EXAMINATION OF SERUM FOR THE PRESENCE OF SPECIFIC IMMUNOGLOBULIN M

Serum samples were fractionated in a sucrose density gradient (Caul *et al.*, 1974). Eleven fractions were collected, and each fraction was divided into two equal parts. One part of each fraction was incubated for one hour at room temperature with one-tenth of its volume of 0.5 M 2-mercaptoethanol; the other half was incubated with PBS. After this treatment antigen was added to each fraction, and the resulting mixtures were incubated at room temperature for one hour and then centrifuged at $35\,000\,g$ for two hours. A control consisting of antigen mixed with PBS was included. Grids were prepared and stained as before and examined under code.

Results

No bacterial pathogens were isolated from any of the faecal specimens examined.

DIRECT ELECTRON MICROSCOPY

Astrovirus particles were detected in the first two faecal samples from patient T but not in the two subsequent specimens (Fig. 2). The micrograph

Fig. 2 Astrovirus particles in faeces showing characteristic star-like morphology. Bar represents 100 nm.

shows particles with a diameter of approximately 28 nm, having a distinctive morphological appearance. The impression of a five- or six-pointed star, as described by Madeley and Cosgrove (1975), can clearly be seen. Identical particles were also detected in the faeces of patient M but no virus particles were detected in the faeces of patient P. This latter specimen was collected before the onset of symptoms, but unfortunately a second specimen was not obtained at the time of symptoms.

IMMUNE ELECTRON MICROSCOPY

The particles in the faeces of patient T were clumped by both serum samples. From the results of titration of the sera it was clear that there was at least a four-fold rise in the titre of antibody to the astrovirus particles over the period of six days between collection of the two samples (Table 1). Although a few aggregates were seen in the preparation treated with the earlier serum sample at a dilution of 1/640, these aggregates were small and there was no visible antibody between the particles. A few small aggregates were also seen in the control preparation treated with phosphate-buffered saline instead of serum.

SEPARATION OF IMMUNOGLOBULINS BY SUCROSE DENSITY GRADIENT CENTRIFUGATION

Two peaks of antibody were seen in the untreated fractions. These were in fractions 2 and 3 and in fractions 6, 7, and 8. The antibody in fractions 2 and 3

was specific immunoglobulin M, as shown by its removal by treatment with 2-mercaptoethanol. The antibody in fractions 6, 7, and 8 was specific immunoglobulin G and/or A, and this was unaffected by 2-mercaptoethanol treatment. These results are shown in Table 2. There was some aggregation of particles in the preparations treated with fractions 10 and 11. This was possibly caused by beta-lipoproteins in human serum, although the presence of small amounts of immunoglobulin G cannot be ruled out.

Discussion

Small, round virus-like particles are not uncommon in human faecal preparations. The majority of these particles have no distinguishing morphological characteristics. However, caliciviruses and astroviruses, as described by Madeley and Cosgrove (1975), are usually sufficiently distinctive to allow recognition. The particles that we have described are indistinguishable from astroviruses, as demonstrated by the characteristic five- or six-pointed star-like appearance on the surface of the particles. This star-like morphology is not always apparent on all particles, and where particles are present in small numbers, in aggregates, recognition as astroviruses may not always be possible. Astrovirus particles were found only in association with symptoms of diarrhoea or diarrhoea and vomiting. In one child (patient M) large numbers of virus particles were present in the faeces before the onset of diarrhoea;

Table 1 *Clumping of astrovirus particles with sera from patient T*

Dilution of serum	Serum taken eight days after onset of symptoms				Serum taken 14 days after onset of symptoms			
	No. of particles		No. of clumps	Visible antibody	No. of particles		No. of clumps	Visible antibody
	Unclumped	Clumped			Unclumped	Clumped		
1/10	27	264	37	+	34	249	36	+
1/40	5	686	28	+	0	577	26	+
1/160	15	109	6	+	22	469	16	+
1/640	15	17	5	-	53	270	8	+

Table 2 *Clumping of astrovirus particles with serum collected from patient T 14 days after onset of symptoms and fractionated in a sucrose density gradient*

Fraction	Fractions untreated				Fractions treated with 2-mercaptoethanol			
	No. of particles		No. of clumps	Visible antibody	No. of particles		No. of clumps	Visible antibody
	Unclumped	Clumped			Unclumped	Clumped		
1	51	78	13	-	105	65	15	-
2	20	151	24	+	61	39	10	-
3	3	634	29	+	58	60	8	-
4	18	404	45	+	4	590	25	+
5	8	284	25	+	8	475	11	+
6	5	1048	29	+	8	700	26	+
7	6	450	28	+	20	407	26	+
8	6	196	11	+	3	289	19	+

*Antibody excess

however, he had vomited on the day of examination and also the day previously. In a further patient (P) vomiting and diarrhoea developed three days after his faecal sample had been shown to be negative by electron microscopy. We suggest that this specimen had been examined too early for the detection of virus particles. A further specimen could not be obtained from this patient at the time of symptoms. Faecal specimens from the remaining three children, who had no diarrhoea, were all negative on examination by electron microscopy; thus we were unable to comment on the significance of the vomiting in two of these children.

Patient T excreted astrovirus particles for at least three days after the onset of diarrhoea, but a further sample taken eight days after onset of diarrhoea was negative. Therefore, as judged by electron microscopy, virus did not persist in the faeces. Only one sample of faeces was collected from patient M. From our limited information there appeared to be a close association between the onset of symptoms, particularly diarrhoea, and excretion of astrovirus in the faeces. However, asymptomatic excretion of astrovirus particles has been described in neonates

(Madeley and Cosgrove, 1975) and we also have observed a similar asymptomatic excretion in neonates (unpublished observations). A similar situation also occurs with rotavirus infections (Chrystie *et al.*, 1975).

The addition of antibody to the astrovirus particles obscured their distinctive star-like morphology (Figs 3 and 4). Thus in situations where these particles might be found aggregated in small numbers in faeces, the typical morphology might not be seen if the aggregation is the result of an immune response.

As can be seen from Tables 1 and 2, the addition of patients' sera greatly increased the number of particles in aggregates. The demonstration of a rising titre of antibody in paired sera from patient T at the time of her illness accompanied by the excretion of virus particles is indicative of a current astrovirus infection. This was further supported by the demonstration of specific IgM in the serum of this patient, indicating a primary type infection. The specificity of sucrose fractionation of whole serum was controlled by the addition of 2-mercaptoethanol and clearly demonstrated two antibody peaks.

Fig. 3 Astrovirus particles aggregated by sucrose density gradient separated IgM from the patient's convalescent serum. Note close and regular spacing of the particles. Star-like morphology only partially obscured. Bar represents 100 nm.

Fig. 4 Astrovirus particles aggregated by sucrose density gradient separated IgG from the patient's convalescent serum. Star-like morphology completely obscured by excess antibody. Bar represents 100 nm.

Our findings suggest a causal role for astroviruses in gastroenteritis, although their role in symptomless neonatal infections remains to be clarified.

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Requests for reprints to: C. R. Ashley, Public Health Laboratory, Myrtle Road, Kingsdown, Bristol, BS2 8EL