DETECTION OF ASTROVIRUS-LIKE IN DIARRHOEIC STOOL AND ITS COEXISTENCE WITH ROTAVIRUS

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SUMMARY

A clinical case is described in this paper in that a 5-month old baby girl severely malnourished and dehydrated presented a prolonged acute diarrhoea. No enteropathogenic bacteria or parasites were demonstrated. Virological study by electron microscopy (EM) showed that the patient shed both astrovirus-like and rotavirus in the watery stool as long as 12 days after the onset. Immune electron microscopy (IEM) performed with the patient serum revealed clumps of both viruses. It is suggest that this may be a case of mixed infection due to astrovirus-like and rotavirus.

INTRODUCTION

Astrovirus is one of the viruses associated to acute diarrhoea in childhood. It was first described in 1975 in faeces of newborn babies with diarrhoea, although it was also found in symptomless babies. Astrovirus has since then been found associated to diarrhoea episodes. A strong evidence that the virus is a human enteric pathogen came from the findings that lamb astrovirus induced diarrhoea in young lambs.

The identification of astrovirus rests upon its morphologic features by electron microscopic (EM) observation for the time being. The 28-nm virus particles resemble five-or six-pointed star.

Likely others intestinal viruses, e.g. rotavirus, astrovirus seems to have a worldwide distribution.

In Brazil astrovirus was recently described in a study of viral diarrhoea. In our laboratory we detected astrovirus-like in diarrhoeic stool coexisting with rotavirus. The importance of astrovirus as an aetiologic agent in infant diarrhoea and the procedure for EM visualization are discussed in this paper.

MATERIAL AND METHODS

Diarrhoeic stool was collected from L.C.B. a 5-month old female baby twelve days after the onset of the diarrhoea. The child had fever, vomiting and evacuated five to ten times a day. Watery stool had either no blood or mucous. L.C.B. was severely malnourished and dehydrated. Virological, bacteriological and parasitological studies were carried out. Paired blood samples were also collected.

For the virological study electron microscopy (EM) was carried out and the watery stool sample was treated as following: a small volume of the material was diluted with a drop of distilled water in a glass slide. A drop of the suspension was deposited on a Formvar-precoated EM grid and negatively stained with 2% PTA, pH=6.5 and washed twice with distilled water after the staining. This procedure is referred to super direct electron microscopy (SD) in the present work. An additional volume of faeces was diluted at 10-20% (v/v) with PBS and shaken for 5 minutes with glass beads. 0.5 ml of the clarified supernatant (4 000 g, 15 minutes at 4°C) was added to 0.2 ml of 10% human immune globulin (HIG), U.S.P., Gammar,
Armour Pharm. Co., Arizona, USA and incubated at 37°C for 1 hour. The mixture was spun down at 12000 g for 1 hour. The pellet was resuspended with three drops of distilled water and negatively stained for EM. The latter procedure was also performed with the undiluted patient's second serum sample instead of HIG. The observation was carried out in a Philips 301 electron microscope.

RESULTS

The study of the patient material by the super direct electron microscopy (SD) showed a large number of rotavirus particles averaging 0.5 particles per mesh. Although the grids were thoroughly screened no other viral particles were observed. The immune electron microscopy (IEM) performed with HIG revealed several clumps of astrovirus-like (Fig. 1) and rotavirus. The IEM carried out with the second serum also showed clumps of the viruses. Either IEM or SD showed no other virus. Most rotavirus particles observed in these preparations were complete but electron dense empty forms were also found (not shown). Astrovirus-like presented the peculiar structure of 5-pointed star in about 10% of the total number of particles (Fig. 1). Some particles seemed to be empty but nevertheless the star points could be seen (a) and others had a smooth contour with structure that may correspond to the star points (b). Furthermore a few particles (c) showed a peculiar contour in that tips of the star points are beyond the particle edge.

DISCUSSION

The role played by rotavirus in infant diarrhoea is presently well settled down. In Brazil the virus was first described in 1976 and since then it has been found in outbreaks of diarrhoea in schools, in an isolated Indian community, and in sporadic cases. The ca...
se described in this study is an isolated one in
which the child (L.C.B.) eliminated large num­
ber of rotavirus particles even after twelve days
of the onset when the stool sample was collect­
ed. This might be an unusual feature in rotavi­ral infection for most cases virus particles are
shed in large number in the first four days,
moreover clinically it lasted longer than usual.
It is unlikely that enteropathogenic bacteria or
parasite were involved before the viral infection
for none of these agents were detected seven
days after the onset of the diarrhoea. The find­
ings that many rotavirus particles were clump­
ed by IEM using patients second serum sample
reveals antibody response to the virus. The
first serum sample was not tested. However
the possibility of the antibody rise in the past
can not be ruled out. On the other hand it is
likely that maternal antibody may not last
longer than two-three months after birth.

Concerning the astrovirus as an enteropa­
thogenic agent, evidences showed that adult
volunteers developed vomiting and diarrhoea,
and shed large number of astrovirus particles
after experimental ingestion of faecal filtrate
containing astrovirus. Furthermore virus-spe­
cific antibody rises were detected in volun­
teers. We have also found that L.C.B. shed
large number of astrovirus-like particles and
patient’s serum sample also revealed to con­
tain virus-specific antibody shown by viral
clumping in IEM. It was found that astroviral
infection like rotaviral infection is self-limited
and the former lasted in average 12 hours.
This study shows that viral shedding may last
far longer which may also be an unusual feature.
The long lasting clinical manifestation and the
viral shedding in both cases may well be due
to the patient’s severe state of malnutrition.
A study on this aspects would be rather im­
portant.

On the diagnosis point of view EM techni­ques are unique for astroviral detection for the
time being. We have found that SD technique
is very useful for viruses sized 50-nm and over
for diarrhoeic faeces. Our experience has shown
that rotavirus and adenovirus are fairly easy
to be detected in spite of faecal debris. It is
necessary however that grids be carefully wash­
ed with a few drops of distilled water in order
to avoid overstaining. On the other hand small­
er viruses sized 28-30nm may not be detected
by SD mostly due to small round impurities or
debris, which make difficult to differentiate
them unless they are found in an exceptional
number. Yet by IEM technique it is easier to
find small viruses for the faecal material is di­
luted and clarified by centrifugation therefore
the preparation is much clearer. However the
loss of virus in faecal suspension due to sedi­
mentation either by standing or centrifugation
must be considered, especially if the material
is poor in virus content and if particles are pre­
clumped. It has been shown that large and me­
dium-sized viruses even as a single particles
may sediment at low gravitational force, e.g.
2.100g. One mean to overcome this drawback
is by visualizing a crude preparation such as
by SD technique. Our experience in terms of
the comparison of SD and IEM has revealed
that the former technique in spite of the pre­
viously described limitation is more reliable
than the latter. However an extensive study
has to be carried out before any conclusion
be drawn on the efficiency of SD technique in
comparison to IEM. The use of commercial
HIG in IEM is rather important for it allows
clumping of other viruses especially in stool
specimens, however a testing for the best di­
lution of HIG has to be carried out for the
efficiency of the method.

RESUMO
Detecção de vírus semelhante a Astrovírus em
fezes diarréicas e sua coexistência com
Rotavírus

É apresentado neste trabalho um caso cli­
nico de diarreia aguda prolongada em uma
criança subnutrida e desidratada de 5 meses
de idade. O estudo virológico por microscopia
eletrônica mostrou que a criança eliminava, pe­
elas fezes aquosas, vírus semelhante, morfológi­
camente, ao astrovírus e rotavírus. A presença
destes vírus foi observada 12 dias após o início
da diarreia. A imunomicroscopia eletrônica rea­
lizada com o soro da criança mostrou agrega­
dos de ambos os vírus. Não foram detectadas
bactérias ou parasitas enteropatogênicos. Os
Autores sugerem que este pode ser um caso
de infecção mista de vírus semelhante ao as­
rovírus e rotavírus.

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