An Antigenic Subunit Present in Rotavirus Infected Faeces

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SUMMARY

It has been found by immune electron microscopy that rotavirus-infected faeces, calf or human, contain an antigenic subunit associated with the inner of the two virus capsids. This internal component represents the group specific antigen for the rotavirus group and the subunit reacts with both homologous and heterologous antiserum. It can therefore be used in diagnostic tests and in this paper its use as a reagent for immunodiffusion is described.

INTRODUCTION

Since the discovery in 1972 of a reovirus-like particle in the stools of scouring calves (Fernelius et al. 1972), particles of identical morphology have been found in the faeces of numerous species, invariably associated with enteric infection (Lancet, 1975). Superficially, the particles resemble reovirus, and have an inner capsid of 55 nm diam. and an outer capsid of 70 nm diam. (Flewett et al. 1974a). However, the particles are morphologically distinct from reoviruses, and since their most outstanding structural feature is the spoke-like arrangement of the subunits on the outer of their two capsids, the term rotavirus (Flewett et al. 1974a) has been generally adopted to describe this group of enteric organisms (Almeida & Zuckerman, 1976). Very soon after their discovery it was shown that there are serological relationships between the rotaviruses associated with different species (Flewett et al. 1974b; Kapikian et al. 1974; Davidson et al. 1975). Kapikian et al. (1975) showed that calf virus can be used as a diagnostic antigen for studying human rotavirus infection. More recently it has been shown that serological crossing between members of the rotavirus group is, for the most part, mediated by the antigens of the inner capsid (Woode et al. 1976). We have investigated this phenomenon further and here present the finding that rotavirus-infected faeces contain considerable amounts of a subunit of the internal component. These subunits can be seen by immune electron microscopy, and the extracts containing them make a suitable antigen for the immunodiffusion studies.

METHODS

Viruses and antisera. A naturally occurring outbreak of rotavirus infection in a dairy herd provided calf faecal material. Human stools containing rotavirus were kindly supplied by Dr J. Banatvala, St Thomas's Hospital Medical School. All specimens of stools were stored at -20°C until use.

Bovine antibody to rotavirus was provided by serum from adult animals in the dairy herd. Human antibody to rotavirus was found by screening serum from normal individuals.
Preparation of faecal extracts. Suspensions (20\%) of stools were made with phosphate buffered saline and held at 37\°C in a water bath for 30 min. During this period the suspensions were mixed thoroughly at least three times using a mechanical mixer (Rotamix, Hook and Tucker Ltd.). The mixture was then clarified by centrifuging for 15 min at 8000 g. The supernatant fluid was passed through a Millipore prefilter together with a 1.2 \mu m filter in a Swinnex assembly. The resultant clear fluid was used for electron microscopy. For immunodiffusion it was concentrated 25 times in a Minicon B15 (Amicon Corp.). Using this method, 1 ml of faeces yields 0.2 ml of final concentrate.

Electron microscopy. Both direct negative staining and immune electron microscopy (IEM) were carried out as follows. Samples of 0.5 ml filtered faecal suspension were placed in tubes and 0.1 ml rotavirus antiserum (bovine or human) was added to the tube to be used for IEM and 0.1 ml saline was added to a control tube. The tubes were left at room temperature for 1 h and then centrifuged for 1 h at 12000 g. The supernatant fluid was discarded and the pellets used for negative staining in the usual manner.

Immunodiffusion. Agarose (0.9\%) in tris/EDTA buffer (Prince, 1968) was used for double immunodiffusion. Two ml was used to cover a glass slide and a template of 3 mm holes at 3 mm spacing was employed. After filling, the gels were left overnight at room temperature. Lines were recorded the next day using dark ground illumination in a photographic enlarger (Almeida et al. 1965).
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RESULTS

Electron microscopy

Examination by direct electron microscopy of both calf and human faecal material, prepared by the method described, revealed the presence of numerous rotavirus particles. Both complete double-shelled particles and incomplete inner capsids could be seen.

As previously described (Kapikian et al. 1974), IEM showed that homologous antibody formed two separate types of aggregate containing either the internal capsids or the complete virus. However, IEM additionally revealed that, while aggregates of the complete virus contained particles and antibody alone (Fig. 1a), those of the internal component were built up of internal capsids, fragments of internal capsid and considerable amounts of a small structure that appeared to be a subunit of the internal capsid (Fig. 1b).

Immunodiffusion

Only one major line formed on the immunodiffusion plates between antigens and antisera when a template incorporating both calf and human virus and bovine and human antiserum was used. Identity could be shown between the line formed by the calf antigen and serum and that formed by the human virus and antiserum. There was also identity between these lines and those formed by the heterologous reaction of each virus antigen with antiserum from the other species (Fig. 2a). In order to establish that the line in agar gel was indeed a specific one, the precipitin line was cut from the agar, lightly homogenized in distilled water and examined in the electron microscope. Only one variety of complex was present in the gel line and that was of the type associated with the internal component of the virus, consisting of internal capsids, fragments and subunit material.

By means of suitably placed reference antigen or antiserum, the immunodiffusion test was successfully used to screen serum samples for the presence of antibody, and faecal extracts for virus antigen (Fig. 2b).
DISCUSSION

The electron microscope technique of negative staining is one of the few means by which immune complexes can be examined directly. If the technique is carried out correctly, false positive results do not occur. Visualized complexes contain only components that are antigenically related (Almeida, 1976). However, antigenic relatedness does not necessarily imply morphological similarity, and there are several instances of a visualized immune complex containing more than one type of morphological structure. For example, in the hepatitis B system an immune complex of the surface antigen contains no less than three structural forms (Almeida & Waterson, 1975). In the present study, IEM revealed complexes of two different types; first, simple aggregates that consisted of intact rotaviruses and antibody alone and second, aggregates associated with the internal capsid of the virus. Unlike the complexes of intact virus, the latter exhibited a heterogeneous appearance. The constituents of the aggregates ranged from seemingly intact particles, through internal capsids, to fragments of these same capsids and finally to particulate material that formed the bulk of the complex. Since all stages in the disintegration of the inner capsid could be visualized it seems reasonable to conclude that the particulate material represents the structural subunits of this component. In support of this view, Martin et al. (1975) describe a subunit associated with the internal component of a rotavirus, and the presence of fragmented forms of the inner capsid when the virus was purified by sucrose density gradient. The presence of the occasional seemingly intact rotavirus particle in the internal structure complexes is explained by the fact that even a small break in the outer component will expose internal antigen so that the particle is trapped in what would appear to be the wrong aggregate.

Two explanations can be made for our finding of a subunit in association with only the internal component of rotaviruses. First, when the complete particle starts to degrade, subunits may also be released from the outer capsid but these retain no antigenic activity. Alternatively, the subunits and fragmented internal components may result from incomplete virus synthesis and may never have been integrated into a virus capsid.

From the work of Woode et al. (1976), it appears that the serological relationships that occur between different viruses of the rotavirus group are mediated by the antigen, or antigens, of the internal capsid. This means that the subunit which we describe is one of the group specific antigens of rotaviruses and hence of value as a diagnostic reagent in studying both homologous and heterologous systems. Since double diffusion in agar gel can be used to study the antigens of rotaviruses (Woode et al. 1976) we used this technique to evaluate the subunit. In immunodiffusion tests, preparations containing subunits, whether derived from calf or human stool material, gave rise to one major line when reacted with either homologous or heterologous antiserum and it could be shown that this line was common to all reagents. The lack of a second type-specific line with the homologous system is almost certainly due to the fact that while the subunit can move rapidly through the agar gel, complete virus particles would have only poor penetrating ability.

Although immunodiffusion is not a particularly sensitive technique when compared to some other serological tests it has a considerable advantage in the present context. Rotavirus extracts from faeces, no matter how carefully prepared, are likely to have extraneous material present and this can lead to anti-complementary or other non-specific effects. Using gel diffusion, subsidiary lines occasionally appear but these can be identified by means of a suitably placed reference antigen or antibody. In initial studies we have used the antigenic subunit in immunodiffusion tests to screen for the occurrence of rotavirus antigen
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and antibody. In tests on normal human serum from a small number of young adults antibody was detected in 80% of the specimens.

To summarize, it would appear that rotavirus infected faeces contain an antigenic subunit associated with the inner capsid of the virus. Since this inner capsid represents the group specific antigens of the rotavirus group the subunit has potential as a serological reagent. Here we describe its use in an immunodiffusion test but it should also be possible to exploit it in other diagnostic tests.

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REFERENCES


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