Isolation and Characterization of Feline Rotavirus

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SUMMARY

Feline rotavirus was detected by electron microscopy in the faecal samples of a cat, and was propagated in an established cell line of foetal rhesus monkey kidney, MA104, cell cultures. Morphologically, feline rotavirus was indistinguishable from known rotaviruses. Complete particles showed a characteristic ‘spoke-like’ arrangement of inner capsomeres surrounded by an outer layer. Intracytoplasmic inclusion bodies in different sizes and shapes were produced in infected MA104 cells. Reproducible clear-cut plaques were produced by feline rotavirus in MA104 cells under the overlay of carboxymethylcellulose in the presence of trypsin. Feline rotavirus was distinct from human, canine, bovine, porcine and simian rotaviruses by the plaque reduction neutralization test. Feline rotavirus, like canine and simian rotaviruses, was found to be less dependent upon trypsin than human, bovine, porcine, chicken and turkey rotaviruses. A seroepidemiological survey (September 1979 to August 1980) showed that 20 out of 61 (32.8%) randomly sampled hospitalized cats at the Cornell Veterinary Teaching Hospital in Ithaca, New York had antibody titres against feline rotavirus. Oral inoculation of cats with feline rotavirus did not produce any clinical disease, but most cats did mount an immune response to the virus following inoculation.

INTRODUCTION

Rotavirus is a common cause of gastroenteritis in the young of many, if not all, mammalian and avian species (Bergeland et al., 1977; Flewett & Woode, 1978; McNulty, 1978; McNulty et al., 1978a, b, 1979; Wyatt et al., 1978; Holmes, 1979; Jones et al., 1979; Eugster & Sneed, 1980). Rotaviruses have been detected in man, cattle, mouse, guinea-pig, sheep, goat, pig, monkey, horse, antelope, bison, deer, rabbit, dog, chicken, turkey, duck and parrot. Recently, in England rotaviruses have been visualized by electron microscopy in a faecal sample of a cat with diarrhoea (Chrystie et al., 1979). However, clear demonstration that they were distinct feline rotaviruses and not rotaviruses of other animal species, was not reported. Here we report the isolation, propagation and characterization of feline rotavirus. We also report on attempts, albeit unsuccessful, to produce disease in three different age groups of specific pathogen-free (SPF) colony cats.

METHODS

Source of isolate. As part of a routine screening procedure for enteric viruses, faecal samples were collected from a cat with chronic arthritis which was submitted to the Cornell Veterinary Teaching Hospital.

Electron microscopy. Electron microscopical examination of faecal samples was performed as previously described (Hoshino & Scott, 1980). Approx. 20% faecal suspensions for virus isolation were prepared in Eagle’s minimum essential medium (MEM) with antibiotics and no
Table 1. Rotaviruses used in this study

<table>
<thead>
<tr>
<th>Virus/strain</th>
<th>Host</th>
<th>Country</th>
<th>Year</th>
<th>Source</th>
<th>Titre*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feline/Taka</td>
<td>Cat</td>
<td>U.S.A.</td>
<td>1980</td>
<td>MA104 cell culture fluid</td>
<td>4 x 10^7</td>
</tr>
<tr>
<td>Canine/CU-1</td>
<td>Dog</td>
<td>U.S.A.</td>
<td>1980</td>
<td>MA104 cell culture fluid</td>
<td>2 x 10^7</td>
</tr>
<tr>
<td>Bovine/NCDV</td>
<td>Cattle</td>
<td>U.S.A.</td>
<td>1967</td>
<td>MA104 cell culture fluid</td>
<td>5 x 10^5</td>
</tr>
<tr>
<td>Porcine/OSU</td>
<td>Pig</td>
<td>U.S.A.</td>
<td>1976</td>
<td>MA104 cell culture fluid</td>
<td>4 x 10^5</td>
</tr>
<tr>
<td>Simian/SA-11</td>
<td>Monkey</td>
<td>South Africa</td>
<td>1958</td>
<td>MA104 cell culture fluid</td>
<td>2 x 10^7</td>
</tr>
</tbody>
</table>

* Titre was expressed in p.f.u./ml.

serum, centrifuged at 1200 g for 10 min (crude faecal suspension) and the supernatants were ultracentrifuged at 10000 g for 10 min (clarified faecal suspension). One to four drops of crude faecal suspension were mixed with 20 drops of distilled water, one drop of 0.1% bovine serum albumin and three to four drops of 4% phosphotungstic acid adjusted to pH 7. This mixture was applied to a carbon–parlodion-coated 200 mesh copper grid with an all-glass nebulizer and examined in a Philips 201 electron microscope at 80 kV. Infected cell culture suspensions, after three cycles of quick freezing and thawing, were examined as above.

**Cell cultures.** The initial isolation of feline rotavirus was done in first-transfer cells of primary feline kidney cells, an established cell line of African green monkey kidney (Vero) and an established foetal rhesus monkey kidney cell line (MA104) (Microbiological Associates, Bethesda, Md., U.S.A.). Growth medium for MA104 cell cultures was Eagle’s MEM supplemented with 5% newborn calf serum, 0.01% lactalbumin hydrolysate (LH), 0.075% sodium bicarbonate and antibiotics. Maintenance medium (MM) was Eagle’s MEM supplemented with 0.01% LH, 0.075% sodium bicarbonate, antibiotics and no serum.

**Isolation and propagation of feline rotavirus in cultured cells.** Confluent monolayer cultures (16 x 125 mm culture tubes) were washed once with phosphate-buffered saline (PBS) pH 7.2, treated for 10 min at 37 °C with PBS supplemented with 2.5 μg 2 x crystalline trypsin/ml (Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A.) and then were inoculated with 0.1 ml clarified faecal suspension. After 1 h adsorption at 37 °C, cell cultures were washed once with PBS, fed with 1 ml MM and incubated at 35 °C. When the cells showed 50 to 80% cytopathic change, the culture tubes were frozen and thawed quickly three times, centrifuged at 1200 g for 10 min and supernatants were inoculated into fresh cell cultures as described above.

**Examination for inclusion bodies.** The Leighton tube cultures of MA104 cells were prepared and inoculated with feline rotavirus as described above. When approx. 50% of cells showed cytopathic change, coverslips were removed, washed with PBS, fixed with methanol, stained with May–Grünewald–Giemsa stain and examined for inclusion bodies.

**Viruses.** Rotaviruses of different animal species employed in this study are summarized in Table 1. Feline and canine rotaviruses were isolated in our laboratory. Dr E. H. Bohl, Ohio Agricultural Research and Development Center, Wooster, Ohio, U.S.A., kindly supplied porcine rotavirus OSU strain. Simian rotavirus SA-11 strain was kindly made available by Dr M. K. Estes, Baylor College of Medicine, Houston, Texas, U.S.A. All virus stocks were prepared in MA104 cells.

**Preparation of hyperimmune sera.** Hyperimmune rabbit sera to feline and canine rotaviruses were prepared by intramuscular (i.m.) inoculation of 0.5 ml purified virus mixed with 0.5 ml Freund incomplete adjuvant followed by two i.m. re-inoculations of 1 ml purified virus.
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virus without adjuvant on days 21 and 28. Rabbits were bled 1 week after the last injection. Dr R. G. Wyatt, National Institute of Allergy and Infectious Diseases, Bethesda, Md., U.S.A., kindly supplied hyperimmune guinea-pig anti-human rotavirus 'WA' serum. Hyperimmune gnotobiotic pig anti-porcine rotavirus OSU serum and gnotobiotic calf anti-bovine rotavirus NCDV serum were kindly supplied by Dr E. H. Bohl. Hyperimmune rabbit anti-simian rotavirus SA-11 serum was kindly made available by Dr M. K. Estes.

Plaque assay. Plastic six-well plates (Costar, Cambridge Mass., U.S.A.) with confluent MA104 cell monolayers were washed twice with PBS and inoculated with 0.1 ml virus sample. The virus inoculum was allowed to adsorb for 1 h at 37 °C with rotational agitation every 20 min to distribute the sample. Excess inoculum was then removed, and 5 ml of the overlay medium was applied to each well. The overlay medium consisted of Eagle's basal medium, 0.75% carboxymethylcellulose (CMC; Hercules Incorporated, Wilmington, Del., U.S.A.), antibiotics, 50 μg DEAE-dextran/ml (Pharmacia) and 1 μg 2 × crystalline trypsin/ml. The cultures were incubated for 6 days in a humidified CO2 incubator and then stained with 0.1% crystal violet solution.

Plaque reduction neutralization test. Plaque reduction neutralization tests were performed by mixing equal volumes of twofold dilutions of sera (previously heat-inactivated at 56 °C for 30 min) and the virus suspension diluted to contain approx. 100 p.f.u./0.1 ml. After an incubation of 60 min at 37 °C in a water bath, 0.2 ml amounts of the virus–serum mixtures were inoculated on to the cell monolayers in six-well plates. Three wells were used for each dilution. After 60 min adsorption at 37 °C, an overlay medium was applied. The neutralizing antibody titres were expressed as reciprocals of the highest serum dilution causing a 50% reduction in plaque counts.

Immunofluorescent test. Monolayers of MA104 cells grown either on glass slides (Lab-Tek chamber slides, Lab-Tek Products, Napperville, Ill., U.S.A.) or on coverslips in Leighton tubes were infected with virus, washed with PBS, fixed with acetone at −20 °C for 15 min, air-dried at room temperature and then stored at −20 °C until used. The fixed cell cultures were stained by the indirect immunofluorescent method, using rabbit and feline sera and the corresponding fluorescein isothiocyanate (FITC)-conjugated anti-γ-globulin sera or protein A–FITC conjugate (Pharmacia). The slides were then counterstained with a 1 : 500 dilution of 1% Evans Blue in PBS for 10 min. Slides were then washed twice with PBS. After air-drying, slides were mounted with 50% glycerol in distilled water, and examined for specific fluorescence with an epifluorescence u.v. microscope (Ernst Leitz Ltd., Midland, Ontario, Canada).

Enhancement of virus infectivity by trypsin. Infected cell lysates were treated with trypsin (final concentration of trypsin at 10 μg/ml, incubation for 1 h at 37 °C) and then directly assayed by plaquing.

Survey of feline sera for antibody to rotaviruses. For measuring serum antibody titres to rotavirus, the indirect immunofluorescent test (IIFT) was developed. When MA104 cell cultures infected with feline rotavirus showed about 50% cytopathic change, monolayers were trypsinized and the cells were washed three times with PBS and resuspended in PBS (approx. 1 × 106 cells/ml). This cell suspension was dropped on to pre-cleaned slides (six drops/slide) with a digital microlitre pipette (Rainin Instrument Co., Woburn, Mass., U.S.A.), air-dried at room temperature, fixed in acetone at −20 °C for 15 min and air-dried at room temperature. Slides were stored at −20 °C until needed. Serum samples were collected from both clinically normal and clinically ill cats submitted to the Small Animal Clinic of the Cornell Veterinary Teaching Hospital. Serial twofold dilutions of test sera were dropped on to the cells. Slides were incubated for 1 h at 37 °C in a humidified chamber, and washed three times for 10 min each with PBS. After air-drying, rabbit anti-cat IgG–FITC (Nordic Immunology, Tilberg, The Netherlands) was applied for 1 h at 37 °C in a humidified chamber.
Slides were then washed, counterstained, washed and mounted as described above. The highest serum dilution that provided positive-fluorescing cells was read as the serum titre.

Animals. Initially, two adult cats approx. 1.5 years old and three SPF colony kittens approximately 3 months old were given 2 ml virus suspension orally. The titre of the virus suspension was approx. $4 \times 10^6$ p.f.u./0.1 ml. When these animals failed to show any sign of disease, a litter of kittens approx. 10 days old was infected. Again, 2 ml of the same virus suspension pool was given orally. Four of the five kittens were infected, leaving one kitten and the queen as contact exposures. In the first trial, the five animals were housed individually in Horsfall isolation units. In the second trial, the queen and her litter were all housed together in one isolation unit. After inoculation, all animals were monitored daily for fever, diarrhoea, lack of appetite, etc. Faecal samples were taken daily from the adults and handled as described earlier. Rectal swabs were substituted for faecal samples in the case of the 10-day-old kittens as the queen's cleanings eliminated samples. The rectal swabs were pre-moistened in the maintenance medium described; the rectal sample was taken, and the swab was replaced into maintenance medium in 1 dram vials. The samples were mixed in a Vortex mixer and then ultracentrifuged to form a clarified faecal suspension. Faecal samples were collected daily for approx. 7 weeks. Finally, blood samples were taken at weekly intervals. The serum antibody titre was then determined using the indirect fluorescent antibody test.

RESULTS

Detection of rotaviruses in faeces by electron microscopy

From September 1979, when a feline enteric virus screening survey was initiated in our laboratory, to August 1980, five rotavirus isolates were detected from the faecal samples of 185 hospitalized cats: one in February, two in April, one in July and one in August. Two of the five rotavirus isolates were detected by both direct electron microscopy (EM) and cell cultures. The remaining three isolates were detected only by cell cultures. All but the first isolate (Taka strain), which was used in the present study, were isolated from clinically normal adult cats.

Examination of a faecal sample taken from the hospitalized cat by direct EM revealed the presence of characteristic rotavirus particles. The feline rotavirus particles were indistinguishable from those of known rotaviruses. In negatively stained preparations both complete and incomplete particles were visualized (Fig. 1a, b). Complete particles showed a characteristic 'spoke-like' arrangement of inner capsomeres surrounded by an outer layer (Fig. 1a). Intact double-shelled particles averaged 70 nm in diam., while particles without outer capsid layers were approx. 13 nm smaller (Fig. 1b). No other virus was observed.

Isolation and propagation of rotaviruses in cell cultures

Clarified faecal suspension containing rotavirus particles was inoculated into MA104, first transfer of primary feline kidney and Vero cell cultures. MA104 cells showed 'flagging' cytopathic effect (c.p.e.), partial detachment of cells infected by virus which had not rounded up, characteristic of rotavirus replication in vitro 1 day post-infection. As incubation progressed, these cells detached and floated freely in culture fluid. First transfer of primary feline kidney cells showed c.p.e. 2 days post-infection. The c.p.e. in Vero cells was not prominent and healed by 7 days post-infection. It was found that rotaviruses could be passaged in MA104 cell cultures without the aid of trypsin. Rotaviruses have been successfully passaged more than 10 times without trypsin treatment. Virions were demonstrated in cell culture lysates by EM in all passages.
Fig. 1. Negatively stained feline rotavirus particles from feline faeces. (a) Characteristic 'spoke-like' arrangement of inner capsomeres are seen (arrows). (b) Both double- and single-shelled particles are seen.

Fig. 2. Inclusion bodies in MA104 cells infected with feline rotavirus. Both small and coalesced large bodies are seen. May–Grünewald–Giemsa stained.

**Examination for inclusion bodies**

Intracytoplasmic inclusions in different sizes and shapes were observed in infected MA104 cells. Inclusions were first detected 1 day post-infection, and when cells were not destroyed by viruses, they were still detectable 7 days post-infection. Some inclusions were small, while others were large, similar to those in cells infected with rotaviruses (Fig. 2).

**Plaque assay**

Reproducible and clear-cut plaques were formed 6 days post-infection under the overlay of CMC in the presence of trypsin (Fig. 3). Although feline rotavirus replicated and produced...
Fig. 3. Plaques produced by feline rotavirus in MA104 cells under the overlay of carboxymethylcellulose after 6 days incubation at 37 °C.

Table 2. Antigenic relationship between feline rotavirus and canine, bovine, porcine, simian and human rotaviruses by plaque reduction neutralization tests

<table>
<thead>
<tr>
<th>Rotavirus</th>
<th>Feline</th>
<th>Canine</th>
<th>Bovine</th>
<th>Porcine</th>
<th>Simian</th>
<th>Human</th>
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</thead>
<tbody>
<tr>
<td>Feline</td>
<td>7680*</td>
<td>30</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Canine</td>
<td>30</td>
<td>7680</td>
<td>ND†</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Bovine</td>
<td>30</td>
<td>ND</td>
<td>5120</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Porcine</td>
<td>30</td>
<td>ND</td>
<td>ND</td>
<td>5120</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Simian</td>
<td>30</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>7680</td>
<td>ND</td>
</tr>
<tr>
<td>Human</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>201800‡</td>
</tr>
</tbody>
</table>

* Reciprocal of antibody titre with indicated virus.
† ND, Not done.
‡ From Wyatt et al. (1980).

c.p.e. in monolayer cultures of MA104 cells in the absence of trypsin, detectable plaques were not formed even 7 days post-infection under CMC overlay without trypsin.

Antigenic relationship between feline and other mammalian rotaviruses

The antigenic relationship between feline and other mammalian rotaviruses was studied by the plaque reduction neutralization test (Table 2). Feline rotavirus was not neutralized by antiserum prepared against canine, bovine, porcine, simian and human rotaviruses. Hyperimmune feline rotavirus serum did not neutralize canine, porcine, bovine and simian rotaviruses.

Immunofluorescence

Specific immunofluorescence was demonstrated in the cytoplasm of infected MA104 cells (Fig. 4). In some infected cells the areas of immunofluorescence were small and scattered in
Fig. 4. Indirect immunofluorescent staining of MA104 cells infected with feline rotavirus. (a) Single MA104 cell with small and scattered immunofluorescent foci in the cytoplasm; protein A–FITC. (b) Small granular perinuclear fluorescence; rabbit anti-cat IgG–FITC. (c) Perinuclear large fluorescence; protein A–FITC. (d) Diffuse cytoplasmic immunofluorescence; rabbit anti-cat IgG–FITC.

the cytoplasm (Fig. 4a, b), while in other cases the areas coalesced to form large diffuse fluorescent foci (Fig. 4c, d). Rotavirus antigen was not stained by hyperimmune sera against reovirus type I, II and III; neither did hyperimmune serum to rotavirus stain reovirus antigens. Preimmunization sera had no titre against feline and canine rotaviruses by IIFT. Fluorescence was not detected in the uninfected control MA104 cells.

Enhancement of virus infectivity by trypsin

The p.f.u. infectivity was enhanced by 2 logs.
Table 3. Comparison of rotavirus antibody titres of feline serum samples monitored by indirect immunofluorescent test (IIFT) and plaque reduction neutralization test (PRNT)

<table>
<thead>
<tr>
<th>Serum sample no.</th>
<th>IIFT titre</th>
<th>PRNT titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 5*</td>
<td>&lt; 10†</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>320</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>640</td>
</tr>
<tr>
<td>4</td>
<td>160</td>
<td>320</td>
</tr>
<tr>
<td>5</td>
<td>160</td>
<td>1280</td>
</tr>
</tbody>
</table>

* Reciprocal of antibody titre.
† Reciprocal of 50% plaque-reduction neutralizing titre.

Survey of feline sera for antibodies to rotaviruses

From September 1979 to August 1980 a total of 61 serum samples were collected from the hospitalized cats and monitored for rotavirus antibody titre by IIFA. Twenty out of 61 (32.8%) serum samples had an antibody titre (1:5 or greater) against rotavirus. Table 3 summarizes the results of IIFA and plaque reduction neutralization tests of representative serum samples for antibodies to rotavirus.

Effects of animal inoculation

In all cats and kittens given the virus orally, virus was detected in the faeces on day 1 post-inoculation. In the two contact animals (queen and one kitten), no virus was detected until 2 days post-infection. All animals shed virus for approx. 10 to 14 days. At no time, however, when cats were shedding virus did any cat manifest signs of disease. Temperatures remained normal, and bowels remained firm. After this period, all of the adults and the 10-day-old kittens ceased to shed virus. Serum antibody titre response by these animals was also very good. The three 3-month-old kittens differed in that they evidenced periodic shedding of virus and serum antibody response was poor. These three cats are being monitored, and additional research is being performed to determine the significance of the unusual response seen.

It would seem, therefore, that feline rotavirus does infect cats, but the significance of the virus as a cause of actual disease is still being investigated through further work.

DISCUSSION

McNulty et al. (1978 b) reported that five out of six cats examined from the Belfast area in Northern Ireland had antibody titres to rotavirus by IIFT. Since IIFT detects only group-specific antibodies, they could not decide whether the antibody in cats was a result of infection with human or other animal rotaviruses, or a result of infection with feline rotavirus. Recently, rotavirus was visualized by EM in a faecal sample of a cat with diarrhoea in England (Chrystie et al., 1979). It was shown that this rotavirus reacted with the rotavirus group-specific but not human type-specific antibodies by enzyme-linked immunosorbent assay. However, its relationship to other non-human rotaviruses was not determined.

The results of the present study confirm the existence of a rotavirus which is distinct from canine, bovine, porcine, simian and human rotaviruses. The homologous and heterologous titres in this study differ much more than the differences in titres found by Thouless et al. (1977). The possible explanations are: (i) difference in sensitivity between the plaque reduction neutralization test and the immunofluorescent focus reduction neutralization test; (ii) difference in virus (strain)/cell culture systems; and (iii) difference in reagents employed (e.g. animal species used to produce hyperimmune serums, passage history of rotaviruses).

The investigation of rotaviruses isolated from various species of animals and birds has been
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hampered by their fastidious growth properties in cell cultures. An introduction of pancreatic proteolytic enzymes to the study of rotaviruses was a major breakthrough for their propagation in cell cultures (Babiuk et al., 1977; Theil et al., 1977; Almeida et al., 1978; Barnett et al., 1979; Clark et al., 1979; Schoub et al., 1979). Recently, several rotaviruses have been successfully propagated to high titre in cell cultures, including simian (Ramia & Sattar, 1979, 1980), bovine (Clark et al., 1979), porcine (Theil et al., 1977) and human (Esparza et al., 1980; Kapikian et al., 1980; Wyatt et al., 1980) rotaviruses.

Based on their characteristics in cell culture, rotaviruses can be divided into two groups: (1) those that can only grow very poorly without proteolytic enzyme treatment like some strains of bovine rotavirus, or cannot go into multiple rounds of replication in cell cultures without the aid of proteolytic enzymes and (2) those that can go into multiple rounds of replication in cell cultures without the aid of proteolytic enzymes. Human, porcine, chicken, turkey and some strains of bovine rotaviruses belong to group 1. Feline, canine (Y. Hoshino et al., unpublished results) and simian rotaviruses belong to group 2. Since viruses in group 2 cannot undergo multiple cycle replication in the semi-permissive cells without proteolytic enzymes (Graham & Estes, 1980; Y. Hoshino & F. W. Scott, unpublished results), this may not be a strict classification. This dependency of rotavirus upon proteolytic enzymes in vitro may reflect the in vivo situation of rotavirus replication and induction of clinical disease. If so, this grouping will contribute to the understanding of pathogenesis and natural history of rotaviruses.

It is significant that all the viruses in group 1 are known to cause mild to severe diarrhoea in young animals experimentally, while induction of diarrhoea in experimental animals with viruses in group 2 has been less successful (England & Poston, 1980; Soike et al., 1980; present report). It is also noteworthy that attempts to enhance intestinal susceptibility by oral administration of pancreatic enzymes in two rhesus macaques were unsuccessful (Soike et al., 1980). The report that simian (group 2) rotavirus was more acid-labile than bovine (group 1) rotavirus (Rodger et al., 1977) may further support the significance of this classification. Physicochemical and pathological studies to clarify these points are in progress in our laboratory.

Although rotaviruses in group 2 can propagate in cell cultures in the absence of proteolytic enzymes, virus infectivity and yields can be increased in the presence of proteolytic enzymes (Graham & Estes, 1980; present report). Furthermore, group 2 rotaviruses that can propagate in cell cultures cannot produce detectable plaques in the absence of trypsin (Ramia & Sattar, 1979, 1980; Smith et al., 1979; present report). The exact mechanism of this phenomenon is unknown. The results of the present study support the hypothesis that the proteolytic enzyme acts on the virus extracellularly. But the exact mechanism of the proteolytic enzymes action on the virus particles is unknown. It is speculated that the proteolytic enzyme treatment changes virions to allow either enhanced virus adsorption and penetration into cultured cells or more efficient propagation once the virus has penetrated the cells.

Barnett et al. (1979) reported that trypsin treatment of Madin–Darby bovine kidney cells before infection with bovine rotavirus followed by thorough washing did not result in an increased rotavirus infectivity as compared to non-treated controls. Graham & Estes (1980) reported that trypsin pretreatment of MA104 cells did not result in enhancement of simian rotavirus SA-11 infectivity. However, it should be mentioned that enhancement of rotavirus infectivity is different from induction of multiple rounds of viral replication in cell cultures. Further studies are to be pursued on the relationship between rotavirus and proteolytic enzymes using the system of rotavirus in group 2 and semi-permissive cells.

It has been reported that intracytoplasmic inclusions were produced by simian rotavirus SA-11 and O agent in primary vervet monkey kidney cells (Malherbe & Strickland-Cholmley, 1967) and by bovine rotavirus in bovine embryonic kidney cells (Welch & Twiehaus, 1973). These inclusions were small and discrete with clearly defined round or oval outline, and they
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did not coalesce to form large bodies like those seen in cells infected with reoviruses. The inclusions induced by feline and canine (Y. Hoshino et al., unpublished results) rotaviruses had different sizes and shapes. Some were small and scattered in the cytoplasm like those reported for simian and bovine rotaviruses, while others coalesced to form large inclusions. The size and shape of inclusion bodies may vary depending upon each virus–cell culture system. We observed coalesced large inclusions in simian rotavirus–MA104 and bovine rotavirus–MA104 systems.

Serological evidence has been reported for rotavirus infection in cats (McNulty et al., 1978b). The results of our seroepidemiological survey show that approximately one-third of the cats in the Ithaca, New York area have antibody titres against feline rotavirus. This implies that asymptomatic infection of cats with feline rotavirus is fairly common. Since cats as pets have close contact with humans, and since rotaviruses from one species can infect members of some other species (Bridger et al., 1975; Middleton et al., 1975; Mebus et al., 1976; Torres-Medina et al., 1976; Snodgrass et al., 1977), it will be interesting to examine further any possible relationship between human and feline rotaviruses, and to perform seroepidemiological surveys of human serum samples for antibody titres against feline rotaviruses.

Any possible relationship between the feline rotavirus and the chronic arthritis suffered by the cat from which the isolate was obtained is unknown. Pathogenesis studies of feline rotavirus are in progress in our laboratory.

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REFERENCES


Feline rotavirus


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