Established Cell Line Sensitive to Influenza C Virus

(Accepted 5 December 1978)

SUMMARY

Various strains of influenza C virus grew productively in an established line of monkey kidney cells (LLCMK2) without prior adaptation. When trypsin was added to the medium, higher virus yields were obtained than in other cell cultures. All influenza C virus strains tested formed well defined plaques under the agar overlay medium containing trypsin. Infectivity determined by plaque assay in LLCMK2 cells was higher than that determined by amniotic inoculation of fertile hens' eggs.

We have reported that a strain of influenza C virus formed plaques on MDCK cells under the agar overlay containing trypsin (Nerome & Ishida, 1978). Its usefulness is, however, limited to C/JJ/50 strain, because other strains did not form plaques reproducibly and the haemagglutinin (HA) titre was somewhat low. Because of the high sensitivity of an established line of Rhesus monkey kidney cells (LLCMK2) to Sendai virus (Sugita et al. 1974), we tested these cells and found that they were superior to MDCK cells in their susceptibility to various influenza C viruses and in their higher sensitivity.

The influenza C viruses tested were Taylor/1233/47, JJ/50, Yamagata/64, Kanagawa/1/76 and Miyagi/1/77. All strains had been propagated in the amniotic cavity of 8- and 10-day-old fertile hens' eggs. Both MDCK and LLCMK2 cells were grown in Eagle's MEM supplemented with 10% newborn calf serum.

Plaque assay was by the method described previously (Tobita et al. 1975). Briefly, confluent monolayers in 60-mm Falcon dishes were washed with phosphate buffered saline (PBS, pH 7.2) and infected with serial dilutions of virus. After adsorption for 40 min at room temperature, dishes were covered with 5 ml of agar overlay medium consisting of Earle-based Eagle's MEM containing 2.2 mg/ml of NaHCO3, 0.2% bovine serum albumin, twofold concentration of glucose, 20 µg/ml of crystalline trypsin (Sigma), 300 µg/ml of DEAE dextran and 0.8% of purified agar (Difco) and incubated under 5% CO2 at 34°C. After 5 to 6 days incubation, cultures received 2.5 ml of overlay medium containing 0.007% neutral red and 1% purified agar and plaques were observed on the following day.

For growth experiments under fluid medium, washed monolayers were inoculated with virus at a multiplicity of 0.01 to 0.05 p.f.u./cell. After adsorption, the monolayers were washed three times with PBS and overlaid with Eagle's MEM containing 2.2 mg/ml of NaHCO3, 0.2% bovine serum albumin and 10 µg/ml of trypsin. EID50 was determined by inoculating serial tenfold dilutions of a sample into the amniotic cavities of 9-day-old fertile hens' eggs and by testing the amniotic fluid for the presence of HA after 2 day incubation at 34°C.

Growth of various influenza C virus strains was studied by titration of haemagglutinin in the culture fluid. When trypsin was included in the fluid overlay medium, all strains tested grew and released HA into culture fluid. In the absence of trypsin, however, no HA activity was detected, except for the Kanagawa strain which yielded a low titre of HA. The optimal concentration of trypsin was 10 to 20 µg/ml. With all strains tested at a multi-
Fig. 1. Plaques of influenza C viruses produced in LLCMK₂ cells (7 days after infection). (a) Taylor/1233/47; (b) JJ/50; (c) Yamagata/64; (d) Kanagawa/1/76; (e) Miyagi/1/77.

Table I. Comparison of infectivity titres of influenza C virus strains in fertile hens' eggs, MDCK, and LLCMK₂ cells

<table>
<thead>
<tr>
<th>Virus strains*</th>
<th>EID₅₀</th>
<th>p.f.u. in MDCK</th>
<th>p.f.u. in LLCMK₂</th>
<th>p.f.u. in LLCMK₂:EID₅₀ ratio (log₁₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/Taylor/1233/47</td>
<td>5.80</td>
<td>—</td>
<td>6.28</td>
<td>+0.48</td>
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<tr>
<td>C/JJ/50</td>
<td>6.16</td>
<td>6.70</td>
<td>7.19</td>
<td>+1.03</td>
</tr>
<tr>
<td>C/Yamagata/64</td>
<td>6.16</td>
<td>—</td>
<td>7.23</td>
<td>+1.07</td>
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<tr>
<td>C/Kanagawa/1/76</td>
<td>6.39</td>
<td>—</td>
<td>7.30</td>
<td>+0.91</td>
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<tr>
<td>C/Miyagi/1/77</td>
<td>6.27</td>
<td>—</td>
<td>7.15</td>
<td>+0.88</td>
</tr>
</tbody>
</table>

* Virus strains were propagated in the amniotic cavity of fertile hens' eggs.
† No reproducible plaque formation.

In Table I, the infectivity of five strains of influenza C virus determined in three host

plicity between 0.01 and 0.05 p.f.u./cell, HA of titre 512 to 1024 was released in 5 to 7 days.

For plaque assay, the optimal concentration of trypsin was again 10 to 20 μg/ml. In addition, 300 μg/ml of DEAE dextran was required for increased plaque size. Plaques of five strains of influenza C virus are shown in Fig. 1. Taylor/1233/47, JJ/50, Kanagawa/1/76 and Miyagi/1/77 strains formed clear plaques of 3 to 4 mm in diam. after 7 days incubation at 34 °C. Plaques of Yamagata/64 strain were heterogeneous in size and slightly turbid. The number of plaques was proportional to the virus concentration, indicating that a plaque was initiated by a single infectious virus particle (data not shown).

In Table 1, the infectivity of five strains of influenza C virus determined in three host
systems are compared. Infectivity determined by plaque assay in LLCMK₂ cells was five- to tenfold higher than that determined in fertile hens' eggs. The JJ/50 strain also formed plaques in MDCK cells but its plaquing efficiency was twice as high in LLCMK₂ cells as in MDCK cells.

Although various cell culture systems are now available for the propagation of influenza C virus (Chakraverty, 1974; O'Callaghan et al. 1977; Nerome & Ishida, 1978), LLCMK₂ cells appear to be more useful because of their susceptibility to various strains, greater virus yield and higher sensitivity.

We are grateful to Dr A. Sugiura, Institute of Public Health, Tokyo, for his comments on the manuscript.

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REFERENCES


(Received 4 October 1978)