The Multiplication of an Influenza C Virus in an Established Line of Canine Kidney (MDCK) Cells

(Accepted 9 November 1977)

SUMMARY

JJ/5o and four other strains of influenza C virus grew in an established line of canine kidney (MDCK) cells. Multicycle virus growth was markedly enhanced by the addition of trypsin to the culture medium and these viruses could be passaged serially in this system. The addition of appropriate concentrations of trypsin to the agar overlay medium enabled plaquing of influenza C/JJ/5o virus. Titration by plaque assay on MDCK cells was more sensitive than that by intra-amniotic inoculation of fertile hens' eggs.

Influenza C virus is usually grown in the amniotic cavity of fertile hens' eggs. It does not grow in most cell cultures which are susceptible to other influenza viruses. Rhesus monkey kidney cells have been reported to be susceptible to influenza C viruses (Chakravertty, 1974). However, a basic disadvantage of primary cell culture systems is their variability and the heterogeneity of their cellular constituents. Therefore, suitable systems for influenza C virus in cell lines are desirable for biological studies and would be of great value in genetic and biochemical studies.

A wide variety of influenza A and B viruses grow in MDCK cells maintained in medium containing trypsin (Tobita, 1975; Tobita et al. 1975). In this communication we report that various strains of influenza C virus also replicate, and one strain produces plaques, in MDCK cells in the presence of trypsin.

The following influenza C viruses that had been propagated in the amniotic cavity of fertile hens' eggs at 34 °C were employed; Taylor/I233, JJ/5o, Yamagata/64, Kanagawa/1/76 and Miyagi/I/77. Infectivity was determined by intra-amniotic inoculation of 9-day-old fertile hens' eggs. MDCK cells were grown and maintained by the method described (Tobita, 1975; Tobita et al. 1975). Confluent monolayers were washed twice with phosphate-buffered saline (PBS, 0·01 M, pH 7·2) and infected with virus at a multiplicity of about 0·1 fifty % egg infectious dose (EID₅₀) per cell. After 30 min of adsorption at room temperature, cells were washed twice with PBS and overlaid with the fluid maintenance medium containing various concentrations of crystalline trypsin (Sigma). During the incubation at 34 °C samples of culture fluid were removed at daily intervals for the assay of haemagglutinin (HA) activity.

The results are shown in Fig. 1. Without the addition of trypsin, virus growth was extremely slow as shown by the appearance of HA in the culture fluid. The addition of trypsin markedly enhanced virus growth. The more trypsin was added, up to the concentration of 20 µg/ml, the faster the virus grew. With a concentration of 10 µg/ml or higher, the HA titre of the culture fluid reached 1:256 and the infectivity 10⁶¹ EID₅₀/0·2 ml. Fig. 2 shows the one-step growth curve. Replicate monolayer cultures of MDCK cells were infected at a multiplicity of 1 EID₅₀/cell, which corresponded to 6 p.f.u./cell (see below). After adsorption for 30 min at room temperature, monolayers were washed and maintenance medium containing 10 µg/ml trypsin was added. At different times of
Fig. 1. Growth curves of JJ/50 strain of influenza C virus in the presence of graded concentrations of trypsin: \(\triangle \rightarrow \triangle\), 20 \(\mu\)g; \(\bullet \rightarrow \bullet\), 10 \(\mu\)g; \(\Delta \rightarrow \Delta\), 2 \(\mu\)g; \(\mathbf{■} \rightarrow \mathbf{■}\), 0.08 \(\mu\)g/ml; \(\square \rightarrow \square\), 0.4 \(\mu\)g; \(\bigcirc \rightarrow \bigcirc\), absence of trypsin. For details, see the text.

Fig. 2. One-step growth curves of influenza virus C/JJ/50 in the presence of trypsin (10 \(\mu\)g/ml). \(\bigcirc \rightarrow \bigcirc\), Infectivity in medium; \(\square \rightarrow \square\), HA in medium; \(\bullet \rightarrow \bullet\), infectivity in cells; \(\mathbf{■} \rightarrow \mathbf{■}\), HA in cells.
incubation at 34 °C, cultures were removed and the culture fluid was harvested. Cells were washed and resuspended in 1 ml of PBS and cell-associated virus was liberated by sonication. Infectivity and HA titres in the fluid and cells were determined separately. As shown in Fig. 2, infectious virus appeared in the culture fluid at 6 h after infection. A feature that distinguishes influenza C virus from other influenza viruses is that very little infectivity and HA activity remained associated with the cells. This suggests that either influenza C virus is released from the cell as soon as it is formed or the virus associated with the cell is inactive. When infected monolayers were maintained under the agar overlay, influenza C virus produced plaques. For optimal plaque production, the concentration of trypsin in the agar overlay was 20 μg/ml and that of DEAE-dextran 300 μg/ml. Otherwise, the method of plaque assay was the same as that in the previous report (Tobita, 1975; Tobita et al. 1975). After 5 to 6 days incubation at 34 °C, the secondary overlay medium containing neutral red was added and the plaques were observed the next day. Plaques were turbid but clearly visible and about 3 mm in diam. The number of plaques were proportional to the virus concentration (data not shown). The efficiency of plaque formation assay was compared with that of egg infectivity titration. A virus stock of C/JJ/50 strain contained $10^{7.32}$ p.f.u./ml and $10^{6.60}$ EID$_{50}$/ml. Thus, plaque assay on MDCK cells was about sixfold more sensitive than titration in the amniotic cavity.

In addition to the C/JJ/50 strain, freshly prepared Taylor/1233, Yamagata/64 and the recently isolated strains, Kanagawa/1/76 and Miyagi/1/77, also grew in MDCK cells. The HA titres of the culture fluids were 64 or higher when trypsin was included at a concentration of 20 μg/ml in the maintenance medium. The present system provides a useful tool for the detailed study of influenza C virus. The mechanism of the growth-enhancing effect of trypsin on influenza C virus is currently being studied.

The authors wish to thank Dr A. Sugiura, Institute of Public Health, Tokyo for his helpful discussion and comments on the manuscript.

Department of Virology and Rickettsiology  
National Institute of Health of Japan  
10–35, 2-Chome, Kamiosaki, Shinagawa-ku, Tokyo 141  
Japan  

REFERENCES


(Received 28 July 1977)