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REPLICATION AND PLAQUE FORMATION OF PARAINFLUENZA VIRUSES IN AN ESTABLISHED LINE OF MONKEY KIDNEY CELLS

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Summary. — All four types of parainfluenza virus produced distinct plaques in an established line of monkey kidney cells ($LLCMK_2$) under agar overlay containing trypsin and DEAE dextran. Parallel titration of these viruses in $LLCMK_2$ and primary cynomologous monkey kidney (MK) cells showed that $LLCMK_2$ cells were about tenfold more sensitive than MK cells. When trypsin was added to the fluid medium, the virus yield in $LLCMK_2$ cells was significantly higher than in MK cells.

Key words: *parainfluenza virus*; *plaque formation*; *monkey kidney cells*

Introduction

Although several reports have described the propagation of human parainfluenza viruses in various cultured cells (Johnson *et al.*, 1960; Lennette and Schmidt, 1969; Itho *et al.*, 1970; Morimoto *et al.*, 1970), these cells were not uniformly susceptible to all types of parainfluenza viruses. Studies of parainfluenza viruses, therefore, are still hampered by the lack of sensitive and reliable plaque assay methods. Sugita *et al.* (1974) reported the plaque formation of Sendai virus in an established line of rhesus monkey kidney cells ($LLCMK_2$). We later found that $LLCMK_2$ was also susceptible to various strains of influenza C virus (Nerome *et al.* 1979). In this communication we report that $LLCMK_2$ is a useful host system for the plaque assay and growth of parainfluenza virus representing all four serotypes.

Materials and Methods

Following strains of parainfluenza viruses were employed in these studies: strain 67-10 of type 1, Greer strain of type 2, HA-1 strain of type 3, strain 66—348 of type 4A, and strain 68—334 of type 4B. All these strains had been propagated in primary monkey kidney cells. $LLCMK_2$ cells were grown in Eagle's MEM containing 10% newborn calf serum. Primary cynomolgous monkey kidney cells (MK) were grown in LE medium (Earle's solution containing 0.5% lactalbumin hydrolysate) with 10% calf serum.

Plaque assay on monolayers of $LLCMK_2$ cells was done as previously described (Tobita *et al.*, 1975; Nerome *et al.*, 1979). Monolayer cultures for virus propagation and plaque assay were prepared in 60-mm Falcon dishes. Washed monolayers were inoculated with 0.2 ml of appropriately

Table 1. Comparison of infectivity titre of parainfluenza viruses in $LLCMK_2$ cells and primary cynomologous monkey kidney cells

Parainfluenza Virus		Titres (log_{10}/ml)		PFU/TCD_{50} ratio
Type	Strain	PFU*	TCD_{50}**	
1	67–10	6.20	5.20	+ 1.00
2	Greer	7.15	5.20	+ 1.95
3	HA-1	7.53	6.33	+ 1.20
4A	66–348	5.11	4.30	+ 0.81
4B	68–334	4.23	3.00	+ 1.23

* PFU in $LLCMK_2$ under the agar overlay medium containing 10 μg/ml trypsin.

** TCD_{50} titrations based on haemadsorption were performed in primary cynomolgus monkey kidney cells maintained under a fluid medium containing 10 μg/ml trypsin.

diluted virus. After an adsorption period of 40 min, 5 ml of overlay medium was added. The overlay medium was Eagle's MEM with double strength glucose containing 2.2 mg/ml of $NaHCO_3$, 0.2% bovine serum albumin, 10 μg/ml of crystalline trypsin (Sigma), 300 μg/ml of DEAE dextran and 0.8% of purified agar (Difco). After 6-day incubation under 5% CO_2 at 34° C, 2.5 ml of agar overlay medium containing 0.007% of neutral red and 1% purified agar (Difco) was added and plaques were counted on the following day.

For multicycle-growth experiments under the fluid medium the monolayers were washed and infected with virus at multiplicity 0.01 PFU/cell. After 40 min absorption at room temperature, dishes were washed several times and cells fed with 5 ml of Eagle's MEM containing 2.2 mg/ml of $NaHCO_3$, 0.2% bovine serum albumin and 10 μg/ml of crystalline trypsin (Sigma).

Titration on primary MK cells was done by the 50% endpoint method. Tube cultures of MK cells were washed and 0.1 ml of virus in tenfold dilution series was inoculated. Four tubes were jsed for each dilution. After adsorption for 40 min at room temperature, 1 ml of Eagle's MEM containing 10 μg/ml of crystalline trypsin was added. After the incubation at 34 °C for 7 days, the cultures were tested for haemadsorption with guinea pig erythrocytes.

Results and Discussion

As shown in Fig. 1, (PLATE XIX), under the agar overlay containing trypsin all strains tested produced distinct plaques. The addition of DEAE dextran further increased the plaque size. Under the optimal condition with 10 μg/ml of trypsin and 300 μg/ml of DEAE dextran, plaques of all virus strains measured 2–3 mm in diameter on the seventh day. To determine whether the number of plaques was proportional to the virus concentration, serial 2-fold dilutions were made in maintenance medium and assayed in $LLCMK_2$ cells. A linear relationship was observed between the number of plaques and the virus concentration, indicating that one plaque was initiated by a single infectious particle (data not shown). The ratio of plaque counts in $LLCMK_2$ cells to TCD_{50} in primary MK cells was further investigated using viruses, which had been passaged in MK cells. The PFU yield in the former was about tenfold higher than the TCD_{50} yield in the latter (Table 1).

Multiple growth was studied in $LLCMK_2$ cells under the fluid medium containing trypsin. Infection with type 1, 2, and 3 virus strains resulted in an extensive cytopathic effect (CPE), characterized by the appearance of giant cells, and the release of haemagglutinating activity. Haemagglutinin

titre in the culture fluid reached 256 to 512 with type 2 and 3 virus strains, and 1,024 with type 1 virus. When trypsin was omitted, the development of CPE was slow and haemagglutinin titer in the fluid was less than 1 : 16. Type 4A and B virus strains similarly caused CPE, positive haemadsorption, and release of infectious virus up to $10^{7.7}$ PFU/ml. However no haemagglutinating activity was detected in the culture fluid. The reason is not known.

Several investigators reported the enhancing effect of proteolytic enzymes on propagation of human parainfluenza viruses (Sabina and Munro, 1969; Itoh *et al.*, 1970; Morimoto *et al.*, 1970). By what mechanism trypsin enhances the replication of parainfluenza viruses and whether it involves the activation of F protein by proteolytic cleavage as it is the case with Sendai virus (Homma and Tamagawa, 1973; Scheid and Choppin, 1974) has to be studied. Despite of the above mechanism, we conclude that $LLCMK_2$ represent so far the most covenient host cell system for the assay and propagation of human parainfluenza viruses.

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