Replication of Porcine Cytomegalovirus in the 19-PFT Cell Line
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ABSTRACT. Replication of porcine cytomegalovirus was examined in fibroblast- and epithelial-like cell lines of the 19-PFT cell line derived from pig fallopian tube. The virus grew well in the fibroblast-like cell line than the epithelial-like cell line. Cytomegaly cytopathic effects of the virus were clearly observed under the microscope after dispersion of the infected cell culture by trypsin-verse and it was demonstrated that cytomegaly cytopathic effects could be used for infectivity titration. Intracellular inclusions were formed in the infected cells and herpetic virus particles were observed in the nucleus and cytoplasm when infected cells were observed under the transmission electron microscope. Infected cells formed characteristic red plaque.—KEY WORDS: 19-PFT cell line, porcine cytomegalovirus, replication.


It is well known that cell-free virus of porcine cytomegalovirus (PCMV) replicates only in the culture of porcine lung macrophages but not in primary porcine cell cultures and established porcine cell lines [5]. Bouillant and Greig [2], Animal Diseases Research Institute of Japan, reported that 2 kinds (epithelial-like and fibroblast-like) of cell line (PFT) were established from pig fallopian tube. They showed that many animal viruses including PCMV replicated in the cell line [1, 2]. However, further details of replication of PCMV in the cell line have not been reported. This paper describes the replication and some properties of PCMV in the PFT cell line. Fibroblast- and epithelial-like cell lines, designated as 19-PFT cell line, were kindly supplied by Dr. A. M. P. Bouillant. Fibroblast-like (19-PFT-F) and epithelial-like (19-PFT-E) cell lines were used at the 24th to 30th and the 70th to 72nd passage levels, respectively.

Monolayer cultures in tissue culture plastic flask (225 cm², Corning U.S.A.), were dispersed with 0.2% trypsin-verse. Dispersed cells were washed once with growth medium and suspended at 10⁷/ml of cell density in growth medium. Fifty, 3, and 0.1 ml of the cell suspension were added to the tissue culture plastic flask (225 cm²), plastic petri dish (50 mm in diameter) with or without coverslips (9 × 18 mm), each well of 6 wells plastic plate (Costar U.S.A.) and each well of 96 multwells plastic plate, respectively. The tissue culture flasks were incubated at 37°C. The dish and plate cultures were incubated in a humidified incubator at 37°C containing 5% CO₂.

The growth and maintenance media were Eagle’s minimum essential medium (Nissui Co., Ltd., Japan) containing 0.3% dehydrated tryptose phosphate broth (Difco U.S.A.), 5% of inactivated bovine serum, 2% of 7.5% sodium bicarbonate, 100 units/ml of penicillin, 100 µg/ml of streptomycin, and 1 µg/ml of amphotericin B.

The 01 strain of PCMV isolated from lung macrophages of a fattening pig was used in this study. Infective titer of the virus was determined as follows. Serial tenfold dilutions of the viral material were prepared in the growth medium. One-tenth ml of each dilution was inoculated into each of 2 well cultures in 96 multwells plate per dilution. The plate was incubated in the CO₂ incubator. After 7 days of culture, medium was renewed and reincubated. At 14 days after incubation, medium in each well was discarded, and to the well was added 0.05 ml of 0.2% trypsin-verse. The plate was placed at room temperature for 10 min, and cells in each well were observed under an inverted microscope. Infected cells were observed as cytomegalic cells. The viral dilution showing 50% of cytomegalic cells was calculated by Kärber’s method [3]. Reciprocal dilution was taken as the infective titer and expressed as the mean median tissue culture infective dose (log-TCID₅₀/ml).

Plaque formation was done as follows. Serial tenfold dilutions of the virus material were prepared in the growth medium. One-fifth ml of each dilution was inoculated onto the monolayer of the 19-PFT-F cells in the 6 wells plate. After virus adsorption at 37°C for 1 hr, each monolayer was overlaid with 2.5 ml of the overlay medium which was the growth medium containing 0.8% Bactoagar (Difco U.S.A.). The plate culture was incubated in the CO₂ incubator. Fresh overlay medium, 2.5 ml was added to the monolayer every 5 days of incubation. After 10 and 15 days of culture, 2.5 ml of second overlay medium containing 0.005% neutral red was added to the monolayer. Plaques were observed after 11 and 18 days of culture.

Antiserum was made in 5-day-old germ-free piglets by nasal inoculation of the J1 strain of PCMV [4] and inactivated at 56°C for 30 min. For a direct fluorescent antibody test (FAT), the antiserum was conjugated with fluorescein isothiocyanate by the routine method. FAT was performed by the method described previously [4].

All the 19-PFT-F cells had fluorescent nuclear antigen, but about 20% of the 19-PFT-E cells were positive at 5 days after inoculation when coverslip cultures were inoculated with 10⁴.5 TCID₅₀ of the cell-free virus of the 01 strain and observed by the FAT. On the basis of these results, the 19-PFT-F cell line was used throughout the subsequent experiments.

Comparison of infective titers by cultivation period was done by the method described above using the 19-PFT-F cells. Infective titers at 7, 10, 12, 14, 16, and 18 days after inoculation were 10³.5, 10³.5, 10⁴.0, 10⁴.5, 10⁴.5, and 10⁴.5, respectively. The cultivation period for virus titration was
therefore determined for 14 days.

Growth curve of the OF1 strain was determined in the 19-PFT-F cells. Petri dish cultures were inoculated with 0.4 ml containing $10^{4.1}$ TCID$_{50}$ of the virus, incubated at 37°C for 1 hr, washed 3 times with 3 ml of the growth medium, received 5 ml of the growth medium, and reincubated at 37°C. The growth medium was renewed at intervals 5 days. Cells in the dish culture from 1 to 11 days after inoculation were stripped off by rubber policeman. Cells and fluid were harvested and centrifuged at 2,000 rpm for 5 min. Supernatant fluid was discarded, and the cells were suspended in 2 ml of the growth medium, and stored at −70°C until used for virus titration. Before virus titration, stored viral materials were thawed, mixed well, and centrifuged at 2,000 rpm for 5 min. The supernatant fluid was used for titration. Simultaneously, coverslip cultures in the petri dishes were infected with the same dose of the virus. Two coverslip cultures were harvested from 1 to 11 days after inoculation, washed with phosphate buffered saline, fixed with acetone or Bouin’s solution for FAT and staining with hematoxylin and eosin. The results of the growth curve of the virus are shown in Fig. 1. Infective titer of the virus began to increase 4 days after inoculation and reached a maximum at 10 days. Fluorescent antigen in the nucleus appeared 2 to 6 days after inoculation and disappeared at 7 days. On the other hand, antigen in the cytoplasm appeared 3 days after inoculation and lasted up to 11 days. In hematoxylin and eosin staining preparation, intranuclear inclusion was found 2 days after inoculation and all the cells had intranuclear inclusion at 5 days. Cells with inclusion became larger than normal cells and syncytia were formed at 11 days.

Many nucleocapsids and enveloped virus particles with characteristics of herpesvirus were noticed in the nucleus and cytoplasm, respectively, when infected cells were observed under the transmission electron microscope.

Minute red plaques were observed under the microscope at 11 days after inoculation (Fig. 2). Plaques with red periphery, 0.5 to 2.5 mm in diameter, were formed at 18 days after inoculation (Fig. 3).

When the viral materials were heated at 50°C for 0, 5, 10, 20, and 30 min, infective titers were $10^{4.5}$, $10^{3.5}$, $10^{2.5}$, $10^{1.5}$, and $<10^{-0.5}$, respectively.

No decrease in infective titer was found after sonication or 3 times of freezing and thawing at −70°C and 37°C.

Stability of the virus was determined at 4°C and 37°C. As shown in Table 1, the infective titer decreased gradually from 48 to 96 hr at 4°C. At 37°C, the infective titer did not change up to 4 hr, but disappeared at 24 hr after incubation.

The virus was specifically neutralized by the antiserum but not by the normal serum.

Porcine lung macrophage cultures are suitable substrate for the growth of PCMV. However, the disadvantages of lung macrophage cultures are that they do not replicate and therefore demand primary cultures that must be screened for contaminant viruses, including PCMV and mycoplasma.

<table>
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<tr>
<th>Temperature</th>
<th>Time in hours</th>
<th>Infectivity (log-TCID$_{50}$/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>3.5</td>
</tr>
<tr>
<td>4°C</td>
<td>24</td>
<td>3.5</td>
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<tr>
<td></td>
<td>48</td>
<td>2.5</td>
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In the present study, it was confirmed that the 19-PFT-F cell line was very susceptible for the growth of PCMV but the 19-PFT-E cell line was less susceptible than the 19-PFT-F cell line. It was presumed that the 19-PFT-F cell line could be used for growth of PCMV instead of porcine lung macrophage cultures. The cell line was also available for cloning of PCMV by the plaque method. Cytomegalic cytopathic effects of PCMV were clearly observed under the microscope after dispersion by trypsin-versene and it was demonstrated that cytomegalic cytopathic effects could be used for infectivity titration of PCMV without Giemsa or fluorescent antibody staining.

REFERENCES