Mink Lung Cells: A Non-primate Cell Line Highly Susceptible for Varicella-Zoster Virus

(Accepted 15 May 1978)

SUMMARY

Mink lung cells (MvILu) are highly susceptible to varicella-zoster virus (VZV). The titres of cell-free VZV suspensions reached $1 \times 10^5$ p.f.u./ml at 3 days post-infection, with subsequent cell degeneration, if MvILu cells were infected with a multiplicity of infectious virus of $0.01$ p.f.u./cell. In contrast, during the same period and under the same conditions the titres of cell-free VZV were $10^2$ to $10^3$ times lower when grown on human foreskin fibroblasts. A fast and reliable plaque assay and a neutralization test for VZV on MvILu cells, were developed.

Most investigators have used human embryonic fibroblast or simian cell cultures to propagate varicella-zoster virus (VZV; Taylor-Robinson & Caunt, 1972). It has been recognized that VZV frequently remains cell-associated (Weller et al. 1958) and the highest yield of infectious virus was obtained from human embryonic diploid fibroblast cultures after ultrasonic treatment (Caunt, 1963; Schmidt & Lennette, 1976). This report describes the fast and extensive propagation of VZV on mink lung cells (MvILu).

Mink (Mustela vision) lung cells were obtained from Dr C. Bergholz, Chicago and propagated in tissue culture using conditioned RPMI 1640 medium supplemented with 10% (v/v) inactivated foetal calf serum (RPMI-FCS-10). Human foreskin fibroblast (HFF) cell cultures were obtained from Dr F. Deinhardt, München, and grown in RPMI-FCS-15 as described (Darai & Munk, 1976). VZV strain Ellen was obtained from the American Type Culture Collection and strain Braun from Dr Enders, Stuttgart. Both strains had been propagated on HFF cells. When several cell lines from a variety of different species and organs were investigated for the propagation of VZV, it was found that MvILu cells were highly susceptible to this virus. In unfixed MvILu cells, infection is readily detectable as areas of increased refractility. This c.p.e. develops fast and is visible by phase contrast microscopy.

This finding allowed the development of a short-term plaque assay which was carried out as follows: mink cells, freshly trypsinized, were seeded into Linbro plastic plates (6 x 4 wells, each well containing $5 \times 10^4$ cells, 16 mm diam. well) and incubated in a 5% (v/v) CO$_2$/air atmosphere at 37 °C. After 24 h, the MvILu cells reached confluency. One ml portions of virus suspension were serially diluted in 10-fold dilutions of each sample which were made in RPMI-1640 without FCS, and 0.1 ml of each dilution of virus material was inoculated into four wells of a Linbro plastic plate containing confluent monolayers of MvILu cells. After 1 h of adsorption at 37 °C all the cultures were overlaid with 0.1 ml of 1.5% methyl cellulose/well in RPMI-FCS-10. Afterwards the cultures were maintained for 3 days at 37 °C in a 5% (v/v) CO$_2$/air atmosphere. On day 4 p.i. the cultures were washed with PBS buffer once, fixed with 5% (v/v) formaldehyde and stained with 1% (w/v) crystal violet, and the number of virus plaques were counted.

Kinetics of virus growth on MvILu cells in comparison to HFF cells were determined as follows: monolayers of HFF and MvILu cells were infected with VZV strain Ellen at
Table 1. *Titres of varicella-zoster virus in cell-free supernatants of mink lung cells (MvILu) and of human foreskin fibroblast cells (HFF)*

<table>
<thead>
<tr>
<th>Days p.i.</th>
<th>HFF (p.f.u./ml)</th>
<th>MvILu (p.f.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$1.0 \times 10^4$</td>
<td>$1.0 \times 10^4$</td>
</tr>
<tr>
<td>2</td>
<td>$1.6 \times 10^4$</td>
<td>$6.8 \times 10^4$</td>
</tr>
<tr>
<td>3</td>
<td>$3.1 \times 10^4$</td>
<td>$2.9 \times 10^4$</td>
</tr>
<tr>
<td>4</td>
<td>$6.4 \times 10^4$</td>
<td>$9.0 \times 10^4$</td>
</tr>
<tr>
<td>5</td>
<td>$4.9 \times 10^5$</td>
<td>$2.0 \times 10^5$</td>
</tr>
<tr>
<td>6</td>
<td>$9.2 \times 10^5$</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>$4.4 \times 10^6$</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>$1.0 \times 10^7$</td>
<td></td>
</tr>
</tbody>
</table>

* Day when infected cell cultures degenerated completely.

![Photograph of plaques of varicella-zoster virus strain Ellen on mink lung cells (MvILu) which were washed, fixed and stained 4 days p.i. A single well (diam. = 16 mm) of a Linbro plate is shown.](image-url)

a m.o.i. of 0.01 p.f.u./cell. Virus was absorbed for 1 h, at 37 °C and the cells were washed three times with Hanks’ solution and re-fed with conditioned RPMI-FCS-10 medium. Thereafter the cells were transferred to the incubation temperature of 37 °C for several days. The infected cell cultures were harvested daily by freezing and thawing three times and by centrifuging at 800 g for 15 min at 4 °C. Cell-free supernatants were used for virus titrations by plaque assay as described above and the results are given in Table 1. It is evident that at the relatively low m.o.i. used in this experiment, the maximal titre of $2.93 \times 10^7$ p.f.u./ml on MvILu cells was reached by 3 days p.i. In contrast, the highest titre of $9.15 \times$
10^5 p.f.u./ml on HFF cells was measured at 6 days p.i., i.e. 3 days later than on MvILu cells. Essentially the same results were obtained with VZV, strain Braun. Plaques measured 0.5 to 1 mm in diam. with a clear and distinct morphology (Fig. 1).

The high susceptibility of MvILu for VZV reported here and the corresponding plaque formation in plaque assays which were reduced or inhibited after treatment with a variety of human anti-VZV convalescence sera was applied successfully to a rapid neutralization test for VZV. The neutralization test was performed using a procedure described for herpes simplex virus and was slightly modified as follows: human sera were diluted (1:5, 1:10, 1:20, 1:40, etc.) in a Falcon microtitre plate with RPMI 1640 with 100 × TCID_{50} of VZV per 50 μl. The serum-virus mixture was incubated in a 5% (v/v) CO_2/air atmosphere at 37 °C for 2 h. Subsequently 5 × 10^5 MvILu cells in 100 μl RPMI-FCS-20 were added to each sample of the serum-virus mixture per well. The infected cultures were incubated at 37 °C in a 5% (v/v) CO_2/air atmosphere. After 5 days of incubation, the cells were stained with 1% (w/v) crystal violet and the titres of the neutralizing antibodies were determined. Similar results were obtained when VZV, strain Braun, was used.

These findings directed our attention to the possible susceptibility of MvILu cells for propagation of human cytomegaloviruses (CMV) and to the development of a rapid and efficient plaque assay for human CMV, analogous to the method described here for VZV (G. Darai & R. M. Flugel, unpublished data) In addition, MvILu cells were also used for propagation of Epstein-Barr virus (EBV). Preliminary results indicate that EBV can be grown on MvILu cells to high titres (G. Darai & M. Flugel, unpublished data).

We wish to give special thanks to Professor Klaus Munk for his generous support. We also thank Ms Ulrika Gramlich for excellent technical assistance and Mrs Jutta Stippinger for skilful preparation of the manuscript.

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(Received 28 March 1978)