Propagation of Semliki Forest Virus in Various Human Lymphoblastoid Cell Lines

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SUMMARY

Semliki Forest virus (SFV) propagation was studied in one marmoset and eight human lymphoblastoid cell lines. In eight of these cell lines SFV propagated well. Only in the Daudi (human) cell line virus replication was suppressed. This suppression takes place after virus adsorption but before virus inhibitory effects on cell functions.

As I intended to compare the sensitivity of various human lymphoblastoid cell lines to the antiviral and anticellular activities of human interferon (HIF) preparations, I at first attempted to propagate in these cell lines Semliki Forest virus (SFV) which is very sensitive to HIF (Hilfenhaus, Thierfelder & Barth, 1975). These preliminary studies of SFV propagation were necessary (i) because in spite of the fact that several papers have been published on the propagation of various viruses in human lymphoblastoid cell lines (e.g. Henle, Henle & zur Hausen, 1969; Wallace, 1969; Joseph, Lampert & Oldstone, 1975), there is to my knowledge no report on the propagation of SFV in these cells and (ii) because vesicular stomatitis virus, which is also very sensitive to HIF has been described to grow only poorly in at least one of these cell lines (Adams, Strander & Cantell, 1975). In this paper I briefly report on SFV propagation which is normal in eight out of nine lymphoblastoid cell lines tested while it is strongly suppressed in the human line Daudi.

Lymphoblastoid cell lines free from contamination with mycoplasma were routinely grown in RPMI 1640 medium supplemented with 10% foetal calf serum and antibiotics (standard medium). Having reached a saturation density of approx. 1.5 × 10⁶ cells/ml, cells were sedimented by low speed centrifugation and resuspended in serum-free RPMI 1640 medium containing SFV. The cell concentration of this suspension was 5 × 10⁶ cells/ml. Multiplicities of infection (m.o.i.) varied from 0.01 to 5 p.f.u./cell. Cell control cultures were incubated under the same conditions in serum-free medium lacking SFV. After a 90 min stationary incubation at 37 °C, interrupted by occasional shaking of the cell cultures, cells were washed twice with serum free medium and cultured in fresh, complete medium at a starting cell concentration of 7 to 10 × 10⁵ cells/ml. At this time and at 9 and 24 h post infection (p.i.) viable cell counts were determined and supernatant fluids taken from these cultures. The number of viable cells was measured by the trypan blue exclusion test in the Fuchs–Rosenthal haematocytometer chamber. Infectivity on SFV in the supernatant fluids was titrated in U cells (established, human, amniotic cells) by plaque assay.

The effect of SFV infection on cellular functions was measured by ¹⁴C-thymidine uptake of infected and uninfected cells. For this purpose 0.1 ml samples of cell suspensions in standard medium containing 5 × 10⁶ cells/ml were given into each well of a MicroTest plate II (Falcon Plastics, Los Angeles, CA, USA) and incubated at 37 °C in a humidified 8% CO₂ atmosphere in air overnight. We then added 50 µl/well of standard medium or of standard medium containing 1 × 10⁶ p.f.u. SFV/50 µl and incubated the plates for a further 2 h before adding 50 µl of standard medium containing 400 nCi ¹⁴C-thymidine/well (sp. act.
57 mCi/mmol) and incubating again for a further 4 h. The cells were then harvested using a Mash II apparatus (Millipore, Neu-Isenburg, Germany) and the 14C-thymidine was measured as described elsewhere (Hilfenhaus et al. 1976). It was also possible to harvest the U cell monolayer cultures with this apparatus if the capillaries, with which cells were sucked off, were gently moved over the bottom of the wells. We checked microscopically that all cells had been removed.

Kinetic studies of SFV propagation in two human cell lines, P3HR1 and SKL 1, using m.o.i. of 1.0, 0.1 and 0.01 p.f.u./cell revealed that the propagation of this virus in both cell lines was comparable to that in embryonic chicken cells (Acheson & Tamm, 1967) despite the fact that P3HR1 is an Epstein-Barr virus producer line, while SKL 1 is not. In addition, we compared SFV propagation in one marmoset and eight human lymphoblastoid cell lines which had been infected with a m.o.i. of 5 p.f.u./cell. In six lines (the marmoset line: B 95-8, and the five human lines: Diehl I, Molt-4, P3HR1, Raji and SKL 1) maximum virus yields of $\geq 1 \times 10^7$ p.f.u./ml were achieved 9 h p.i. In Kaplan and RPMI 1788 lines maximum virus yields only occurred 24 h p.i., while in Daudi cells no or only a very low virus propagation was observed. The delayed SFV propagation in Kaplan and RPMI 1788 cells might be due to a delayed infection of a high percentage of these cells, since rapid aggregation, a characteristic property of these cells, probably protects many of them from immediate infection after the virus addition.

To determine whether there was a strongly delayed or even no SFV propagation in Daudi cells, samples from infected Daudi cell cultures were taken at different times over a 72 h period in order to determine SFV titres. Fig. 1 shows the results of this experiment in comparison to those of analogously treated Diehl I (EBV producer) and Raji (non-EBV producer) cells. In addition to the infectivity titres we measured the viable cell counts of infected and uninfected cell cultures. In Diehl I and Raji cell cultures maximum SFV titres were obtained 9 h p.i. while 100% cell death was noted only at 24 h p.i. Contrary results were noted in Daudi cell cultures where no distinct SFV propagation and no decrease in viable cell count in the infected cell culture were to be found. The slight increase of the SFV titre 9 h p.i. and the reduced cell propagation in the infected cell culture in comparison to the uninfected control, however, were reproducible. These data would suggest that in Daudi cells very little virus propagation takes place.

Daudi cells differ from other human lymphoblastoid cells by bearing no human lymphocyte antigens on their cell surface (Poulik et al. 1974). A different cell surface of these cells in comparison to the other eight cell lines tested could also be the reason for the suppressed SFV propagation. We therefore studied the adsorption of SFV from virus fluids on to Daudi, SKL 1 and U cells. For this purpose cells ($5 \times 10^6$ cells/ml) were incubated at 37 °C in serum-free medium containing SFV ($6.5 \times 10^6$ p.f.u./ml) for 1.5 h. Then cells were removed by low speed centrifugation and the supernatant fluid was used for a second incubation with fresh cells. After this second adsorption period titres had decreased to nearly the same level in the supernatant fluids of all three cell lines tested (e.g. Daudi cells: $2.5 \times 10^4$ p.f.u./ml; SKL 1 cells: $8.0 \times 10^4$ p.f.u./ml; U cells: $1.5 \times 10^4$ p.f.u./ml), i.e. Daudi cells possess the same adsorption capacity as SKL 1 and U cells.

Since there was no lack of SFV adsorption to Daudi cells, we investigated further to see whether SFV propagation in Daudi cells is prevented before or after the virus caused inhibition of cellular functions. This can be indirectly measured by 14C-thymidine uptake of infected cells in comparison to uninfected cells (Baltimore, 1969). In Table 1 the results of 14C-thymidine uptake by infected and uninfected cells indicate that the cellular functions of SKL 1 and U cells were inhibited by SFV while those of Daudi cells were not.
Fig. 1. Comparison of virus yield and cell count after infection of (a) Daudi, (b) Diehl I and (c) Raji cells with Semliki Forest virus using a m.o.i. of 5 p.f.u./cell: •—•, virus yield; •—•, cell count of uninfected and •—•, infected cell cultures.

Table 1. $^{14}$C-thymidine uptake by various human cell lines infected with SFV

<table>
<thead>
<tr>
<th>Cell line</th>
<th>$^{14}$C-thymidine uptake</th>
<th>% inhibition of $^{14}$C-thymidine uptake in infected cell cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninfected (ct/min ± s.d.)</td>
<td>Infected (ct/min ± s.d.)</td>
</tr>
<tr>
<td>Daudi</td>
<td>7997 ± 685</td>
<td>6675 ± 1579</td>
</tr>
<tr>
<td>SKL 1</td>
<td>20106 ± 744</td>
<td>4411 ± 744</td>
</tr>
<tr>
<td>U</td>
<td>7432 ± 950</td>
<td>1743 ± 642</td>
</tr>
</tbody>
</table>

From these results one can conclude that (i) the propagation of SFV in Daudi cells is so minute, if it occurs at all, that it is insufficient to determine quantitatively the antiviral activity of HIF in these cells, (ii) the suppression of SFV propagation in Daudi cells takes place after adsorption of virus to the cells, but before the inhibition of cellular functions by the virus which precedes virus propagation in other cell lines. Similar results on virus
propagation in Daudi cells with vesicular stomatitis virus (VSV) have been published by Adams et al. (1975). Briefly, these authors infected interferon treated and non-interferon treated Daudi cells with VSV at a low multiplicity. During the first 24 h p.i. no virus propagation was detected in either culture. During the following 24 h the titres of extracellular VSV in the untreated virus control increased only 12-fold. From these results it can be concluded that the situation with VSV is the same as with SFV in Daudi cells; i.e. both viruses scarcely propagate in this cell line.

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REFERENCES


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