Herpes Simplex Virus Infection of in vitro Cultured Neuronal Cells (Mouse Neuroblastoma C 1300 Cells)

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SUMMARY

The nature of the restriction of herpes simplex virus replication in C 1300 neuroblastoma cells was studied. A low rate of adsorption was observed, probably due to the relatively few receptors for HSV on plasma membranes of C 1300 cells. The penetration rate of HSV to the nucleus was slow with an impaired processing of attached virus from plasma membrane to cell nucleus. Even at a high multiplicity of infection only a low percentage of the C 1300 neuroblastoma cells was permissively infected as determined by infectious centre assays. The yield of infectious HSV per virus-producing C 1300 cell was 1% of the yield from GMK control cells. The restriction in neuroblastoma cells of HSV infection could not be accounted for by sensitivity of cells to interferon or by an efficient induction of interferon. Evidence was obtained for the presence in C 1300 cells of an inhibitor of HSV replication not compatible with classical interferon. Observations on C 1300 cells maintaining many characteristics of differentiated neurons suggest that these cells may be useful as a model for studies on HSV-neuron interactions.

INTRODUCTION

Mouse neuroblastoma C 1300 cells cultured in vitro maintain many characteristics of differentiated neurons (Augusti-Tocco & Sato, 1969; Schubert et al. 1969). These include the formation of neurites, presence of neural enzymes and synthesis of neurotransmitters (Amano et al. 1972; Daniels & Hamprecht, 1974). In a previous study (Vahlne & Lycke, 1977) we reported that herpes simplex virus (HSV) replication was restricted in C 1300 cells. High multiplicity of infection caused a cytocidal type of infection but at a low multiplicity of infection cultures persistently or only occasionally releasing virus into the culture fluid were established. A similar restriction of infection was not observed with any of a number of other viruses studied. In the present report the nature of the restriction of C 1300 cells to HSV infection is investigated.

METHODS

Cells. Clone 41 A3 of mouse neuroblastoma C 1300 cells, mouse L 929 cells and green monkey kidney, GMK AH-1, cells were used. The clone 41 A3 originated from Dr G. Augusti-Tocco. The cells have been tested regularly by us for the ability to produce extended neurites in the presence of dibutyryl cyclic AMP and for the production of dopamine and 5-hydroxytryptamine. Tests for contamination of mycoplasma in cultivation have always yielded negative results. No efforts to initiate differentiation of the C 1300 cells before virus
infection were made. All cells were cultured as monolayers in 16 oz precipitation bottles and in plastic Petri dishes (Nunc, Denmark) with Eagle’s minimum essential medium (MEM) supplemented with 10% foetal calf serum, 100 International units (i.u.) of penicillin and 100 μg of streptomycin per ml. The C 1300 cells received in addition 4.5 g of dextrose per litre of medium. For maintenance, the same media supplemented with only 2% serum were used. Cell counting was performed as described previously (Vahlne & Lycke, 1977).

Preparation of cell fractions and homogenates. Cytoplasm and nuclei of cells were separated by the method of Penman (1969). Homogenization of cells was achieved by means of either a Dounce homogenizer, a Potter–Elvehjem homogenizer, or by freeze–thawing and sonification at 1.35 A for 60 s in a Raytheon ultrasonicator.

Viruses. HSV type 1, strain F, from Dr B. Roizman, was used. In studies on production of interferon and assays of interferon, vesicular stomatitis virus (VSV), Coxsackie B5, Sendai and vaccinia viruses were also used. Plaques of HSV were carried out in GMK cells grown in 5 cm plastic dishes as described previously (Lycke & Roos, 1972). HSV was labelled with 3H-thymidine and purified according to previously reported techniques (Vahlne & Blomberg, 1974). Radioactivity was assayed in a scintillation counter using Instagel as liquid scintillator.

Immunofluorescence (IF). The indirect IF was performed on cells grown on glass slides using methods and reagents previously described (Vahlne & Lycke, 1977).

Infectious centre assay. Monolayer cultures of C 1300 cells were infected at a multiplicity of infection of 5 p.f.u./cell. Virus was allowed to attach for 2 h at 37 °C. The cells were then washed five times in pre-warmed Hanks’ BSS and suspended by means of trypsinization in maintenance medium. Half of this suspension was serially diluted in maintenance medium containing 5% anti-HSV type 1 rabbit serum and seeded on to GMK cultures in 5 cm dishes. After sedimentation for 1 h, 5 ml Eagle’s MEM with 1% methylcellulose and 5% anti-HSV serum were carefully added. The cultures were incubated at 37 °C for 3 days and then the number of plaques counted. The other half of the C 1300 cell suspension was dispersed in five sterile plastic tubes and incubated at 37 °C. At 4 and 24 h post infection (p.i.) one and four tubes, respectively, were homogenized by freeze–thawing and sonification before the number of p.f.u. was assayed. The number of infectious centres as well as the amount of virus produced per permissively infected cell was calculated.

Assay of interferon. Production of interferon was induced in C 1300 and GMK cells. The cell cultures were infected with 2 x 10⁸ p.f.u. of HSV, or with 1 x 10⁸ p.f.u. of VSV, 4 x 10⁸ p.f.u. of Coxsackie B5, 1 x 10⁸ TCID₅₀ of Sendai, 2 x 10⁷ p.f.u. of vaccinia virus or were mock-infected. At 24 h p.i. the culture fluid was collected and the cells scraped off and transferred in a small volume of fresh medium. After mechanical homogenization in a Potter–Elvehjem homogenizer for 3 x 1 min at ice-bath temperature, the culture fluids and the cell homogenates were dialysed against 0.2 M-KCl, pH 2.2, for 20 h at 4 °C. The pH was then adjusted to 7.4 by dialysis overnight against Hanks’ BSS. The materials obtained were tested for the presence of interferon.

Tests for interferon were performed with VSV in L 929 and in GMK cells. Monolayer cultures grown in 5 cm plastic dishes were inoculated with materials to be tested for interferon and incubated for 20 h. The cells were washed with Hanks’ BSS and VSV was added at a multiplicity of infection of 0.1 to 0.5 p.f.u./cell. After adsorption for 1 h at 37 °C the cells were washed three times in Hanks’ BSS and 4 ml of maintenance medium was added. At 14 h p.i. the cultures were frozen, thawed and sonicated and the homogenates assayed for VSV content. For compatibility between results of different experiments a mouse fibroblast cell interferon of known concentration was included as a reference in each set of tests.
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Results

Replication of HSV in C 1300 cells

The multiplication of HSV in C 1300 cells is illustrated in Fig. 1. Monolayer cultures of C 1300 and GMK cells were infected at a multiplicity of infection of 5. After adsorption for 2 h the cells were washed four times with Hanks’ BSS and re-incubated with maintenance medium. At various periods, 4 cultures of each cell-type were collected. The cells were scraped off into the medium and pooled. The cell suspension was centrifuged at 1000 g for 10 min and the supernatant was assayed for released virus. The cells were homogenized in the remainder of the supernatant in a Potter-Elvehjem homogenizer for 3 x 1 min in an ice-bath and then assayed for total virus production.

The yield of virus was considerably less in C 1300 than in the GMK cells. The maximal titres of the GMK cultures exceeded the virus yields of the C 1300 cells by more than 3 log p.f.u. The amount of virus produced per permissively-infected C 1300 cell was less than that produced by the GMK cell. These findings were based on experiments performed with cell suspensions infected at a multiplicity of infection of 5. After 2 h at 37 °C to allow virus attachment, the cells were washed and assayed for infectious centres and production of
Table 1. HSV production per permissively infected cell*

<table>
<thead>
<tr>
<th>Cells</th>
<th>C 1300</th>
<th>GMK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells</td>
<td>$4 \times 10^6$</td>
<td>$1 \times 10^7$</td>
</tr>
<tr>
<td>Number of infectious centres</td>
<td>$5.8 \times 10^6$</td>
<td>$91 \times 10^6$</td>
</tr>
<tr>
<td>% productively infected cells</td>
<td>3.4</td>
<td>91</td>
</tr>
<tr>
<td>P.f.u. per infectious centre 4 h p.i.</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>P.f.u. per infectious centre 24 h p.i.</td>
<td>$0.4 \pm 0.06$</td>
<td>$64 \pm 5$</td>
</tr>
</tbody>
</table>

* Monolayer cultures were infected at a m.o.i. of 5. After 2 h adsorption the cells were washed and suspended in maintenance medium. Half the suspension was diluted in medium containing 2% rabbit anti-HSV-serum and seeded on to GMK cells. Plaques were read on day 3 (infectious centres). The other half was incubated at 37 °C for determination of virus production.

† Mean ± s.e. mean (n = 4).

virus. The experiments indicated that, per permissively infected cell, the GMK cells produced 135 times more virus than the C 1300 cells (Table 1).

To see if the restricted replication of HSV in C 1300 cells was explicable to any extent by failure of virus attachment, the adsorption of HSV to C 1300 and to GMK cells was compared by the method of Docherty et al. (1972). C 1300 and GMK cells were suspended at a concentration of $10^8$ cells in 0.9 ml of pre-warmed Eagle’s MEM in sterile plastic tubes and infected by adding 0.1 ml of a virus suspension with $10^6$ p.f.u. of HSV. The first sample was drawn immediately after the addition of virus and the following after intervals of 15 to 240 min. During this period the suspensions of infected cells were agitated in a waterbath at 37 °C. Samples of 100 μl were each added to 9.9 ml volumes of cold medium and centrifuged for 10 min at 1000 g. The amounts of infective virus in the supernatants were titrated. As controls, the residual infectivity of virus added to tubes with culture medium only was observed. The cell-free specimens were treated in the same way as the infected cell suspensions.

Fig. 2 demonstrates that only very small amounts of virus attached to the C 1300 cells. In fact the concentration of virus which remained unassociated with the C 1300 cells even exceeded the virus titres detectable in the cell-free controls. The experiments were also repeated with 3H-labelled HSV. To $10^8$ suspended cells was added $10^6$ ct/min of labelled virus suspension, corresponding to $2 \times 10^6$ p.f.u. of virus. At various times, samples (100 μl) were taken and the cells were washed four times in Hanks' BSS by means of centrifuging for 10 min at 1000 g. After the final washing, the cells were dissolved in 0.5 ml of a 10% solution of Triton X-100 and assayed for radioactivity. The low adsorption rate of HSV to the C 1300 cells was confirmed (Fig. 3). Thus, there seemed to be only minute amounts of HSV which attached to C 1300 cells.

In addition, evidence was obtained for an impaired processing of attached virus from the cell surface to the nuclei of C 1300 cells. Twelve monolayer bottle cultures of C 1300 and of GMK cells were infected, each with $10^6$ ct/min of purified 3H-thymidine-labelled HSV. After adsorption of virus for 2 h at 37 °C the cells were washed five times in Hanks' BSS and three bottles were harvested at the time. The remaining 9 bottles were incubated in sets of three for 3, 4 and 9 h p.i. The cells were scraped off with a rubber policeman and pooled. Nuclei and cytoplasmic fractions were then separated and radioactivity was assayed. About 1% of inoculated virus attached to C 1300 cells, while 40% of the virus added to GMK remained cell-associated after washing. The relative concentrations of labelled virus DNA associated with fractions of cell cytoplasm versus nuclei are presented in Fig. 4. In HSV-infected GMK
Fig. 2. C 1300 and GMK cells (1 \times 10^8 each) were suspended in 0.9 ml of pre-warmed Eagle's MEM, and infected with 10^6 p.f.u. of HSV in a volume of 0.1 ml. The infected cell suspensions were then agitated in a waterbath at 37 °C and at various times 100 μl samples were taken, immediately diluted in 9.9 ml Eagle's MEM and centrifuged at 1000 g for 100 min. The supernatants were assayed for virus by plaquing on GMK cultures. Residual infectivity of virus added to tubes with: □—□, C 1300 cells or ▲—▲, GMK cells.

cells more of the radioactivity was found in the cell nuclei than in the cytoplasmic fractions at 4 h p.i. In C 1300 cells, on the other hand, most of the labelled virus DNA remained associated with the cytoplasmic fractions throughout the experiment.

Table 2 demonstrates that in agreement with the results reported above the relative number of cells which developed specific HSV-IF after inoculation was significantly lower in C 1300 than in GMK cultures.

**Influences of interferon**

The possibility that restrictiveness of C 1300 cells to HSV multiplication was mediated by sensitivity to interferon or by an increased production of interferon was studied in a series of experiments. Fig. 5 illustrates that VSV infections in C 1300 and L 929 cells were equally sensitive to interferon and that infection of C 1300 cells with HSV was only slightly influenced with very large doses of interferon. In addition, Table 3 demonstrates that C 1300 cells were poor producers of interferon. With the viruses tested more interferon was always induced in the HSV supporting GMK cells than in C 1300 cells. Thus, the observed restriction of HSV replication in C 1300 cells was probably not accounted for by sensitivity to, or induction of, interferon.

**Effect of irradiation on HSV infection of C 1300 cells**

A suspension of 10^8 C 1300 cells in 15 ml of cell culture medium was exposed to irradiation with 4000 rads from a 60Co source. After irradiation the cells were seeded into small glass bottles and incubated at 37 °C for 48 h. Cultures of C 1300 cells not irradiated, but otherwise treated as the irradiated cells, served as controls. The cultures were infected with HSV at multiplicities of infection corresponding to 10, 1 and 0.1 p.f.u./cell. After 1 h of adsorp-
Fig. 3. C 1300 and GMK cells (1 × 10^6 each) were suspended in 0.9 ml of pre-warmed Eagle's MEM, and infected with ³H-thymidine labelled and purified HSV (10^6 ct/min, 2 × 10^8 p.f.u.). After varying periods of time 100 μl samples were taken and the cells were washed four times in Hanks' BSS by means of centrifugation at 1000 g for 10 min. After the final washing, the cells were dissolved in 0.5 ml of a 10% solution of Triton X-100 and assayed for radioactivity in Instagel. Radioactivity bound to C 1300 cells •—• and GMK cells △—△. Open circles represent controls with culture medium only.

Fig. 4. Monolayer cultures of C 1300 and GMK cells were infected with ³H-thymidine labelled and purified HSV (1 × 10^6 ct/min; 1 × 10^7 p.f.u.). After adsorption for 2 h at 37 °C the cells were washed five times in pre-warmed Hanks' BSS. At various times 3 cultures of each cell type were harvested. The cells were scraped off with a rubber policeman and pooled. Nuclei and cytoplasmic fractions were separated and radioactivity was assayed. The relative distribution between cytoplasm and nuclei of cell-associated labelled virus DNA is shown. ○—○, Radioactivity found in C 1300 cytoplasm; ⋄—⋄, C 1300 nuclei; □—□, GMK cytoplasm and ■—■, GMK nuclei.
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Table 2. HSV-specific immunofluorescence (IF)*

<table>
<thead>
<tr>
<th>Time p.i. (h)</th>
<th>C 1300</th>
<th>GMK</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>23±2†</td>
<td>100</td>
</tr>
<tr>
<td>24</td>
<td>23±1</td>
<td>100</td>
</tr>
</tbody>
</table>

* GMK and C 1300 cells grown on coverslips were infected with HSV at a m.o.i. of 5. After 4 h adsorption, the cells were washed four times and further incubated in maintenance medium.
† % IF positive cells. Mean ± s.e. mean (n = 6).

Table 3. Interferon induction in C 1300 cells*

<table>
<thead>
<tr>
<th>Test materials</th>
<th>C 1300</th>
<th>GMK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluid phase</td>
<td>Cell phase</td>
</tr>
<tr>
<td>HSV</td>
<td>95±9†</td>
<td>82±5†</td>
</tr>
<tr>
<td>VSV</td>
<td>82±6</td>
<td>69±12</td>
</tr>
<tr>
<td>Vaccinia</td>
<td>64±8</td>
<td>75±7</td>
</tr>
<tr>
<td>Coxsackie B5</td>
<td>84±6</td>
<td>87±9</td>
</tr>
<tr>
<td>Sendai</td>
<td>72±6</td>
<td>71±8</td>
</tr>
</tbody>
</table>

* Assays for interferon induced in C 1300 and GMK cells were performed with VSV in L 929 and GMK cells, respectively. Materials to be assayed for interferon were diluted 1:5 in Hanks’ BSS and added to L 929 and GMK cultures 20 h before VSV inoculation. VSV yields are given as % of untreated control cells. Standard error of the mean of 3 different test materials are also given.
† VSV yields in L 929 cells.
‡ VSV yields in GMK cells.

Fig. 5. Monolayer cultures of C 1300 and L 929 cells were treated with various amounts of mouse fibroblast interferon for 20 h. The cells were then washed and infected with VSV or HSV (multiplicity of infection = 1). After adsorption for 1 h the cells were washed four times with warm Hanks’ BSS. Maintenance medium was added and the cells were incubated at 37 °C for 14 h (L 929 cells) or 20 h (C 1300 cells). The cells were then scraped into the medium, freeze-thawed and sonicated. Total virus content was assayed as plaques in GMK cultures. (a) C 1300 cells infected with: •—•, HSV or ○—○, VSV; (b) L 929 cells infected with VSV. Bars indicate s.e. mean (n = 8).
Fig. 6. C1300 cells suspended in culture medium were exposed to an irradiation of 4000 rads from a 60Co source and seeded on to small glass bottles. After incubation at 37 °C for 48 h the cells were infected with HSV. After 1 h of adsorption the cells were washed four times with Hanks' BSS. Maintenance medium was added and the cells were incubated at 37 °C. After various periods 4 cultures were collected and frozen. Before titration the cultures were thawed and the cells were scraped off and sonicated in the medium. Bars indicate s.e. mean (n = 4). Open symbols indicate HSV production of irradiated cells. Filled symbols indicate HSV production of non-irradiated C1300 cells. Multiplicity of infection = 0.1 (■, □), 1 (▲, △) and 10 (●, ○).

Non-attached virus was removed by washing the cultures four times with Hanks' BSS. Maintenance medium was added and the cultures were incubated. Four cultures of irradiated as well as of non-irradiated cells were collected at various times and frozen. Before titrations of infective virus the cultures were thawed, and the cells scraped off and sonicated in the medium.

The virus yields were higher in the irradiated cells up to 12 h p.i. (Fig. 6). There was no effect on titres of samples collected at 16 h p.i. or later, suggesting that the virion producing capacity of the cells was not affected.
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Table 4. Production of HSV and Coxsackie B5 virus in GMK cells pre-treated with materials from homogenized C 1300 or GMK cells*

<table>
<thead>
<tr>
<th>Virus</th>
<th>C 1300 cell homogenate</th>
<th>GMK cell homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1†</td>
<td>2</td>
</tr>
<tr>
<td>HSV</td>
<td>5.5±0.4</td>
<td>2.7±0.6</td>
</tr>
<tr>
<td>HSV</td>
<td>33.3±3.4</td>
<td>80.0±10.3</td>
</tr>
<tr>
<td>Coxsackie B5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Coxsackie B5</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Four different homogenates of C 1300 and GMK cells (2 × 10⁶ cells per type of cell and material) were prepared. The mechanically disintegrated cells, diluted in Hanks’ BSS, were centrifuged at 100,000 g for 60 min. The supernatants were mixed 1:2 or 1:4 with Eagle’s MEM containing 0.003 % DEAE dextran, and added to each of 8 GMK cultures. After 24 h incubation at 37 °C the cell cultures were washed four times with Hanks’ BSS and infected with HSV or Coxsackie B5 virus at a multiplicity of infection of 1 p.f.u./cell; 24 h later the cultures were frozen. Before titration the cells were thawed and scraped off into the culture medium and sonicated. The virus titres are given as % of untreated control cultures, means and s.e. mean.
† Experiment number.

Table 5. Effect of C 1300 and GMK homogenates on HSV and VSV plaque formation in GMK cultures*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Virus</th>
<th>C 1300 cell homogenate</th>
<th>GMK cell homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virus</td>
<td>1†</td>
<td>2</td>
</tr>
<tr>
<td>Virus and cell material inoculated</td>
<td>HSV</td>
<td>69.8±8.1</td>
<td>57.0±6.3</td>
</tr>
<tr>
<td>simultaneously</td>
<td>VSV</td>
<td>—</td>
<td>103.0±4.4</td>
</tr>
<tr>
<td>Cultures pre-treated for 24 h</td>
<td>HSV</td>
<td>34.9±4.6</td>
<td>—</td>
</tr>
<tr>
<td>with cell material</td>
<td>VSV</td>
<td>111.5±8.1</td>
<td>—</td>
</tr>
</tbody>
</table>

* Homogenates of C 1300 and GMK cells were prepared and centrifuged as described in the footnote to Table 4. Supernatants of the homogenates diluted 1:2 or 1:4 in Eagle’s MEM containing 0.003 % DEAE dextran, were either added simultaneously with 100 p.f.u. of HSV or VSV to 8 GMK cultures, grown in 5 cm plastic Petri dishes, or the GMK cultures were pre-treated with the diluted supernatants for 24 h and then washed twice in Hanks’ BSS prior to infection. After 1 h of adsorption at 37 °C, 5 ml of Eagle’s MEM containing 1 % methylcellulose and 2 % calf serum was carefully added and the cultures were re-incubated. Plaques were read 3 days later. The titres are given as % of untreated control cultures, means and s.e. mean.
† Experiment number.

Evidence for HSV inhibiting factors of C 1300 cells

C 1300 and GMK cells (2 × 10⁶) were suspended in 10 ml Hanks’ BSS and homogenized in an ice-bath with a Potter–Elvehjem homogenizer for 3 × 1 min. The homogenates were centrifuged at 100,000 g for 60 min and the supernatants collected and tested for possible HSV inhibiting effect.

Table 4 shows that GMK cells treated with material from homogenized C 1300 cells produced only 5.5 to 27.8 % of the yields of untreated controls. The corresponding figures with GMK cell homogenates ranged from 33.3 to 86.5 %. No such influence on Coxsackie B5
virus infection was discernible. Similarly, the influence of C 1300 cell preparations on HSV and VSV plaque formation (Table 5) suggested the presence in C 1300 cells of a virus-inhibiting factor operating against HSV but not against VSV infection.

**DISCUSSION**

HSV multiplies in essentially all types of cells of the susceptible animal, including the different specific cells of the nervous system (Mannweiler & Palacios, 1969). Several observations have emphasized, however, the relatively slow progression of HSV infection in neuronal cells, studied both in cultures of organized mammalian nerve tissue (Leestma et al. 1969; Dubois-Dalcq & Buyse, 1972) and in cultured dissociated neuronal cells (Stevens, et al. 1972). In experimentally infected animals, HSV has been shown to establish latent reactivatable infections in sensory ganglia (Stevens & Cook, 1971), the central nervous system (Cook & Stevens, 1976) and sympathetic ganglia of the autonomic nervous system (Price et al. 1975).

Mouse neuroblastoma, C 1300, cells have maintained characteristics of differentiated neurons although subcultivated numerous times in vitro. These cells therefore afford interesting possibilities for studies of HSV infection in neurons. Recently, we reported (Vahlne & Lycke, 1977) that HSV caused a cytocidal type of infection in C 1300 cells only at a high multiplicity of infection. At a low multiplicity of infection persistently infected cultures were observed which could be subcultivated. In addition, we were able to establish C 1300 cell sublines which did not demonstrate HSV replication and specific cytopathic changes until the 41st and 48th passages. The restriction of virus infection in C 1300 cells seemed specific for HSV. No similar phenomenon was observed for the single representatives of adeno-, entero-, myxo- and poxvirus groups tested.

From results reported in the present study it is obvious that relatively few receptors for HSV exist on the plasma membranes of the 41 A3 clone of C 1300 cells. This might explain why infection of a number of cells sufficient to reveal cytopathic changes required a high multiplicity of infection. Possibly the insignificant adsorption of HSV to C 1300 cells also contributes to the ease with which HSV could be cleared from persistently infected cultures by HSV antibodies (Vahlne & Lycke, 1977).

Furthermore, when the distribution of infecting parental virus DNA between fractions of cell nuclei and cytoplasm was studied, a slow and probably inefficient transfer of virus DNA to the nucleus of HSV-infected C 1300 cells was demonstrable. Thus the penetration of HSV, in addition to attachment, seemed inadequate. In GMK cells approximately 60% of the virus DNA was found associated with the nuclear fraction at 4 h after HSV inoculation. In contrast, only about 30% of parental HSV DNA was detectable in nuclear fractions of C 1300 cells up to 9 h p.i. The relatively low yields of infective HSV in C 1300 cultures could partly be explained by the fact that only few cells (1-4% at a multiplicity of infection of 5 p.f.u./cell) became permissively infected. However, per virus-producing cell, less than 1% of the HSV yield of the GMK cells was synthesized in C 1300 cells, suggesting an inefficient HSV synthesizing capacity of the neuroblastoma cells.

This restriction of virus replication was shown not to be mediated by interferon. It is of interest that the restricted replication of HSV in C 6 glioblastoma cells could not be attributed to a virus-inhibiting action of interferon (Lancz & Zettlemoyer, 1976). St. Jeor & Rapp (1973) showed that pre-treatment of human embryonic lung cells with the thymidine analogue 5-iodo-2'-deoxyuridine (IdUrd) enhanced CMV production in these cells and that this effect was probably due to interference of a non-interferon cellular product inhibitory
for CMV replication, i.e. cellular phenotypic changes might have occurred, resulting in the elimination or faulty production of a cellular product. One drawback encountered when cells pre-treated with IdUrd are used for DNA virus replication studies is that any remaining drug would interfere with virus DNA synthesis and might thus obscure the eventual elimination of virus inhibitory products. To avoid the hazard of residual IdUrd, irradiation of the cells prior to infection was chosen. The HSV yield of irradiated cells as compared to non-irradiated cells was higher up to 12 h p.i., indicating a shortening of the eclipse phase in a limited number of cells, although the virus production capacity of the irradiated cultures was not substantially affected. Similar effects of HSV replication in u.v.-irradiated monkey kidney CV-1 cells have been reported (Coppey & Nocentini, 1976). In these cells the HSV-specified thymidine kinase activity was shown to increase faster during the exponential phase of virus DNA synthesis than in non-irradiated cells. Consistent with our results on the irradiated cells, we could extract from C 1300 cells an inhibitor of HSV not compatible with classical interferon but which influenced HSV replication. Previously non-interferon-like inhibitors of HSV replication have been extracted from HSV type 2-transformed cells (Doller, 1977). Inhibitors of two other herpes viruses, Epstein-Barr virus and cytomegalovirus, have been demonstrated in extracts of human D 98 cells (Glaser et al. 1975).

Schwartz & Elizan (1973) have described permissiveness of IMR-32 human neuroblastoma cells to HSV infection. Whether the restrictiveness of the 41 Aa clone of C1300 mouse neuroblastoma cells is a characteristic for this cell clone or displays more general properties of neuronal cells cannot be stated at present.

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