Stimulation of Herpes Simplex Type 1 Infection of C6 Cells by Trypsin–EDTA

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SUMMARY

The nature of the refractoriness of C6 glioblastoma cells to herpes simplex virus type 1 (HSV-1) infection has been studied. The cells were restricted in susceptibility to HSV-1 since only a small proportion of the cells could be infected by HSV-1 and the virus yield per cell was low. The susceptibility to infection was increased by treating the cells with trypsin–EDTA prior to infection. The cells so treated recovered resistance to the virus when incubated at 37 °C, their resistance being restored to the initial level in 2 days. This restoration was inhibited by addition of cycloheximide or puromycin. Trypsin–EDTA treatment of C6 cells increased the efficiency of adsorption of HSV-1 and the formation of stable cell–virus complexes from which the virus could not be dissociated by heparin.

INTRODUCTION

In experimentally infected animals, HSV-1 has been shown to establish a latent infection 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). The mechanism underlying the establishment of latent infection and reactivation of the virus in the nervous system has been a target for numerous studies, but little is understood concerning its nature. A reasonable suggestion may be to investigate the interactions between HSV-1 and cells of the nervous system in a simplified experimental system. As an approach along this line, the use of transformed cells of neural origin is of value because such a cell line would offer a homogeneous cell population retaining some characteristics of differentiated neural cells.

In the meantime, Schwartz & Elizan (1973) demonstrated that C6 cells derived from a rat astrocytoma (Benda et al. 1968) failed to support replication of HSV-1 in vitro, whereas implantation in animals of HSV-1-infected C6 cells initiated a chronic HSV-1 infection. This study would provide a model of latent HSV-1 infection in cells of nervous origin. Hence, the interactions between HSV-1 and C6 cells in vitro appeared worth investigating in detail.

This impetus prompted us to pursue the nature of the refractory attitude of C6 cells to HSV-1 infection, emphasis being placed on whether and how the cells could be converted to a susceptible state.

A recent study with rat glioma cells (C143) suggested that trypsin treatment of the cells, which survived HSV-1 infection, stimulated a cellular response to superinfecting HSV-1 (Doller et al. 1979). Kimura et al. (1979) also demonstrated that trypsinization was necessary for an enhanced expression of HSV-common surface antigens in cells transformed by HSV.
type 2. Therefore trypsin seemed to serve the purpose of the present study. In this study, we used trypsin–EDTA because monodispersed suspensions of C6 cells were prepared more easily with trypsin–EDTA than with trypsin alone, while the effect of trypsin was not altered in the presence of EDTA.

METHODS

Cells. The rat glioblastoma cell line C6, which had been cloned by Dr T. Wechsler, Max Planck Institute, F.R.G., was kindly supplied by Dr K. Yasui, Tokyo Metropolitan Institute for Neurosciences, Japan. Cells were cultured as monolayers in 75 cm² plastic bottles (Falcon) with Eagle's minimum essential medium (MEM) supplemented with 10% heat-inactivated foetal calf serum (FCS, Gibco), 60 μg/ml kanamycin, 2 mM-glutamate and 0.14% NaHCO₃ under a humidified 5% CO₂ atmosphere at 37 °C. For subculture, monolayers were incubated with 10 ml of trypsin–EDTA, consisting of 0.025% trypsin (Gibco), 1:250 and 0.02% EDTA in phosphate-buffered saline (PBS), for 10 min at 37 °C. Before experiments, the cells had been cloned twice sequentially and the cloned cells from the 5th to 15th passages were employed throughout the present study. In preliminary tests, the responses to HSV-1 of the original and cloned cells used were found to be similar. Also, only one lot of FCS was used since different lots were found to cause subtle changes in the cellular responses studied.

Virus. HSV-1, strain HF, was kindly donated by Dr K. Yoshino, Institute of Medical Science, the University of Tokyo, and was cloned twice in Vero cells. Virus was propagated once at a low multiplicity (0.01 p.f.u./cell) in Vero cells. The culture fluid was pooled about 30 h p.i. and clarified by centrifugation at 3000 rev/min for 10 min. Plaquing of HSV was carried out in Vero cells grown in 35 mm plastic dishes; after virus adsorption, Vero monolayers were overlaid with 1 ml of MEM containing 1% methyl cellulose, 2% FCS and 2% rabbit antiserum, and plaques were counted after 3 days by staining the cells with Wright's eosin–methylene blue solution (Merck, Darmstadt, Germany). The titre of the stock virus used ranged from 1.5 x 10⁵ to 5 x 10⁸ p.f.u./ml.

Preparation of ³H-thymidine-labelled HSV-1 (³H-HSV-1). Vero cells, grown in MEM plus 5% FCS (5 x 10⁶ cells), were given virus at 10 p.f.u./cell, incubated for 1 h at 37 °C for adsorption and unadsorbed virus was removed by washing. The infected cells were then fed with growth medium and incubated for 3 h. Then the cells were washed twice with MEM and 10 ml of MEM containing 2.5 μCi/ml ³H-thymidine (sp. act. 46 Ci/mmol; New England Nuclear, Boston, Mass., U.S.A.) was added. At 20 h p.i., the cells and supernatant medium were separated by a 10 min centrifugation at 10000 g. The virus was purified from the medium according to the method described by Powell & Watson (1975) with minor modifications. Briefly, virus was concentrated with polyethylene glycol (mol. wt. 5700 to 6700) at a final concentration of 8% (w/v) in the presence of 0.5 M-NaCl. The concentrated virus was added to a sucrose gradient (15 to 30%, w/w) in the presence of 0.2% FCS and spun in an SW25.2 rotor at 14000 rev/min for 60 min. To determine radioactivity, the samples were precipitated with 5% trichloroacetic acid (TCA) and centrifuged twice in TCA at 3000 g for 20 min. The precipitate was dissolved in 0.1 ml of concentrated NH₄OH by incubation at 60 °C for 30 min. The acid-insoluble radioactivity was counted in a scintillation fluid based on toluene–Triton X-100 (2:1, v/v) and expressed in d/min. In agreement with the results of a previous study (Powell & Watson, 1975), radioactivity and virus infectivity co-sedimented in the sucrose gradient. More than 92%, of the radioactivity was precipitated specifically by anti-HSV-1 rabbit serum and the specific activity of the preparation used for experiments was 960 p.f.u. per d/min. Virus particles were counted against a polystyrene latex bead standard (diam., 250 nm: Dow Chemical Co., Midland, Mich., U.S.A.) using a
uranyl acetate negative staining technique (Benyesh-Melnick et al. 1966). Electron microscopy was kindly performed by Dr T. Kanazeki, Tokyo Metropolitan Institute for Neurosciences, Japan. The ratio of particles to infectivity was about 20.

**Antiserum.** Anti-HSV-1 rabbit serum was prepared by intravenous hyperimmunization of albino rabbits with sucrose-purified HSV-1. The serum was decomplemented by heating at 56 °C for 30 min and was absorbed repeatedly with uninfected C6 cells, Vero cells and an acetone powder of rat brain. The serum, diluted 50-fold, reduced the virus titre from $1 \times 10^8$ to $1 \times 10^4$ p.f.u. on 1 h at 37 °C.

**FITC-conjugated anti-HSV-1 IgG.** Rabbit anti-HSV-1 IgG labelled with fluorescein isothiocyanate (FITC) was purchased from Toshiba-Kagaku, Japan. The FITC-IgG was absorbed as described above. The staining titre was determined by HSV-1-infected Vero cells. For experiments, the labelled IgG with 2 units of the staining titre was used.

**Virus growth studies.** C6 cells were grown as monolayers for 2 days in 35 mm plastic dishes ($3 \times 10^6$ cells). The cells were infected at an m.o.i. of 32. Adsorption was allowed to proceed at 4 °C for 1 h, after which the cells were washed three times with cold Hanks' balanced salt solution (HBSS) and incubated for 1 h at 37 °C in growth medium. The virus remaining on the surface of cells was neutralized with antiserum (dilution, 1 : 50) for 30 min at 37 °C. The cells were washed three times with HBSS and growth medium was added. The cultures were then incubated at 37 °C and at intervals a pair of cultures was removed. The cells were scraped into the medium, pooled and centrifuged at 2000 rev/min for 10 min. The sediment was disrupted in 0.5 ml of the supernatant by three cycles of freeze-thawing. The suspension was clarified by centrifugation and assayed for virus amount.

**Fluorescein-labelled antibody (FA) staining.** The cells infected with HSV-1 were monodispersed by mild trypsinization, washed twice by centrifugation in medium, then spread on glass slides, air dried, fixed 10 min in acetone at room temperature and stored at −80 °C until staining. Virus antigens in the cells remained unreduced for at least 6 months when the preparations were kept at −80 °C. For staining, the cells on slides were exposed to FITC-anti-HSV-1 IgG at 37 °C for 1 h. For calculation of the percentage of fluorescent cells, 500 to 1000 cells were counted when 10 to 90% cells were fluorescent or 2000 to 3000 cells were counted when the proportion of fluorescent cells was outside this range.

**Measurement of cell-associated virus.** (i) In the first method C6 cells in 60 mm plastic Petri dishes ($1.6 \times 10^7$ cells) were washed once with 5 ml of MEM-HEPES, i.e. MEM plus 1% FCS and 20 mm-N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES), pH 7.2, before addition of 0.5 ml of a virus suspension (m.o.i. of 0.03). To test virus stability, 0.5 ml of the virus suspension was placed in dishes in the absence of cells. The dishes were placed at 37 °C and shaken at 100 rev/min (Gyrotory shaker G2) continuously. At an appropriate time a 5 µl sample was removed from each dish and placed in 1 ml of MEM-HEPES. The samples were centrifuged at 2000 rev/min for 10 min and unadsorbed virus in the supernatant was titrated in Vero cells. (ii) In the second method C6 cells and Vero cells in 35 mm plastic dishes ($3 \times 10^6$ and 1.2 × $10^6$ cells, respectively) were washed once with 2 ml of MEM-HEPES. A 0.1 ml portion of the same medium containing about $4 \times 10^4$ d/min of $^3$H-HSV-1 was then added to each dish (m.o.i. of 12). After incubation at 37 °C for the specified times, four cultures were removed and washed three times with 2 ml of HBSS. The cells of two cultures were lysed with 1% sodium lauryl sulphate containing 10 mm-EDTA and 10 mm tris-HCl (pH 7.9) by incubation for 1 h at 37 °C. Acid-insoluble radioactivity was counted as described. Another part of the cultures was subjected to an additional incubation at 37 °C for 1 h in MEM-HEPES containing 10 unit/ml of heparin (Novo, Bagsvaerd, Denmark). Then cultures were processed to count radioactivity as described. The cells were not detached from the surface of dishes during experiments. Non-specific binding of the labelled virus to the surface of the dishes was negligible.
Measurement of detachment of virions from cell surface. Cultures of C6 cells and Vero cells, as well as a ³H-HSV-1 suspension, were prepared as described above. Adsorption was allowed to proceed at 4 °C for 30 min, after which the cells were washed three times with cold HBSS. Then, the cultures were incubated at 37 °C in MEM-HEPES. Prolonged culture of C6 cells at a low temperature occasionally resulted in detachment of cells. At intervals a pair of cultures was removed, manipulated as described above and cell-associated radioactivity was determined.

RESULTS

Replication of HSV-1 in C6 cells

Initially we examined whether HSV-1, strain HF, could replicate in C6 cells under our experimental conditions, in which special care was taken to synchronize virus replication and to reduce residual virus remaining on the surface of cells after adsorption. It was evident that HSV-1 replicated in C6 cells, showing a pattern of growth comparable to that of a single cycle of HSV-1 replication which was well delineated in most permissive cells (Darlington & Granoff, 1973), that is, eclipse lasted for 4 to 6 h and the exponential growth phase ended at 16 to 20 h p.i. The yield of virus in C6 cells was low, not exceeding the input virus titre, in contrast to the fact that 5 × 10⁶ Vero cells usually produced more than 1 × 10⁹ p.f.u. of HSV-1 (Fig. 1a).

Fig. 1(b) demonstrates the time course of appearance of fluorescent cells which paralleled the virus growth curve. The number of cells synthesizing virus antigens was only 36 to 40% of the total cells although the multiplicity of infection was 160 p.f.u./cell in this experiment. In contrast, when Vero cells were infected at a multiplicity of 5 p.f.u./cell, almost all cells showed virus antigens consistently.
Stimulation of HSV-infection in C6 cells

Table 1. Response of C6 cells aged 30 min and 2 days to HSV-I

<table>
<thead>
<tr>
<th>M.o.i.*</th>
<th>Cell age</th>
<th>Fluorescent cells (%)</th>
<th>Virus produced (x 10^6 p.f.u.)</th>
<th>P.f.u./FA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:6</td>
<td>30 min</td>
<td>70:8 ± 0:6</td>
<td>117 ± 5</td>
<td>0:55</td>
</tr>
<tr>
<td></td>
<td>2 days</td>
<td>13:1 ± 0:4</td>
<td>16 ± 5</td>
<td>0:40</td>
</tr>
<tr>
<td>3:0</td>
<td>30 min</td>
<td>10:9 ± 0:5</td>
<td>17 ± 8</td>
<td>0:52</td>
</tr>
<tr>
<td></td>
<td>2 days</td>
<td>1:8 ± 0:2</td>
<td>1:1 ± 0:4</td>
<td>0:21</td>
</tr>
</tbody>
</table>

* After adsorption for 1 h at 37 °C, the virus remaining on the surface of the cells was neutralized with antiserum (dilution, 1:50) for 30 min at 37 °C.
† Number of fluorescent cells (FA) = total cell number (3 x 10⁶) x % fluorescent cells/100.

In addition, it was demonstrated that the infectious centres were about 10% or less of the fluorescent cells and only a few infectious particles were produced in each infectious centre as determined by the method of Vahlne & Lycke (1978) modified by the use of Vero cells for plaquing.

No difference was observed in susceptibility of C6 cells to the stock HSV-I virus compared to the virus produced in C6 cells. To prepare the latter virus, C6 cells were given the stock virus and residual virus remaining on the surface of cells after an adsorption period of 1 h at 37 °C was neutralized with antiserum and further treated with 0.25% trypsin so as to abolish contamination of the progeny virus by the input virus. Virus obtained from the medium at 16 h p.i. was again propagated in Vero cells to obtain a high concentration of virus. It was also observed that three strains of HSV-I freshly isolated and propagated in chick embryonic cells or human embryonic lung cells also showed growth characteristics in C6 cells similar to the virus stocks described above.

Response to HSV-I of C6 cells aged 30 min and 2 days

A comparison was made between two differently aged cells to see if the percentage of fluorescent C6 cells and the yield of virus per fluorescent cell were influenced by cell age (the period of culture after trypsin-EDTA treatment). C6 cell monolayers in 35 mm dishes (3 x 10⁶ cells) cultured for 2 days and 30 min, respectively, were prepared in such a way that the differently aged cells were comparable in number. Virus yields and the number of fluorescent cells were estimated 20 h and 12 to 15 h p.i., respectively. As shown in Table 1, the younger cells were more susceptible to the virus than the older culture, but the yield per infected cell was almost equal.

The dose response relationship between the percentage of fluorescent cells and m.o.i. indicated that the response of 30 min cells was about 10 times higher than that of 2 day cells. The two dose response curves were linear and parallel to each other in a log-log plot, indicating one-hit dose responsiveness of C6 cells to HSV-I (data not shown). The age-dependent relationship of the response was not influenced by the multiplicity of infection up to 20 p.f.u./cell. The effect of trypsin-EDTA treatment was positive only when it was applied to the cells prior to infection.

Recovery of resistance to HSV-I

The time required to recover the resistance to HSV-I in C6 cells was determined. C6 cells grown as monolayers for 2 days were treated with trypsin-EDTA for 10 min at 37 °C. Then the cells were resuspended in growth medium, washed by centrifugation and seeded into 17 mm wells (Falcon, 5 x 10⁶ cells). The cultures were then incubated at 37 °C and, at intervals, a pair of cultures was removed. The number of cells was counted and the cultures were infected with HSV-I in such a way that the m.o.i. of 10 was comparable for cells of
different ages. After an adsorption period of 1 h at 37 °C, the cells were washed three times with HBSS and virus remaining on the surface of cells was neutralized with antiserum (dilution, 1:50) for 30 min at 37 °C. The cells were stained with FITC-anti-HSV-1 IgG 12 to 15 h later. The recovery process started about 2 h after incubation at 37 °C and resistance to HSV-1 progressively increased with age reaching the maximum level in about 2 days (Fig. 2).

To determine whether the recovery process would require cellular protein synthesis, monolayers of C6 cells aged 30 min (2 x 10⁶ cells in 35 mm dish) were cultured for 3 h in the presence of 10 μg/ml of cycloheximide (Boehringer, Mannheim-Waldhof, F.R.G.) or 40 μg/ml of puromycin (Boehringer). As illustrated above, the recovery process progressed during this period in the absence of the inhibitors. In the presence of the inhibitors, however, this progress was inhibited and the susceptibility of C6 cells to infection of HSV-1 remained at the original level. Following release of the block by the inhibitors, recovery resumed, indicating reversibility of the inhibitors' action (Table 2).

**Adsorption of HSV-1 to C6 cells**

The adsorption efficiency was determined by quantifying the number of infectious virus particles remaining unadsorbed to the cells (Fig. 3) and by counting the cell-associated radioactivity of ³H-HSV-1 (Fig. 4a).

Comparison of Fig. 3 with Fig. 4(a) indicates that the difference in kinetic curves of HSV-1 adsorption between two differently aged C6 cells was statistically significant. Therefore, the rate of adsorption and the amount of virus adsorbed to the cells aged 30 min were higher than those to the cells aged 2 days. The curves indicating adsorption of virus to the older cells did not reach the level of adsorption to the younger cells within the time of the experiment. In these experiments, the virus receptor sites of the cells of both ages were not saturated because the amount of ³H-HSV-1 adsorbed to the cells increased in proportion to the input multiplicity of infection in a range exceeding 10 p.f.u./cell.
Stimulation of HSV-infection in C6 cells

Table 2. Effect of cycloheximide (10 μg/ml) and puromycin (40 μg/ml) on the recovery of resistance to HSV-1 in C6 cells*

<table>
<thead>
<tr>
<th>Cell age (h)</th>
<th>% Fluorescent cells (± standard deviation, n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>0.5</td>
<td>93.7 ± 1.0</td>
</tr>
<tr>
<td>3.5</td>
<td>44.6 ± 1.5</td>
</tr>
<tr>
<td>6.5</td>
<td>30.2 ± 2.8</td>
</tr>
<tr>
<td>9.5</td>
<td>17.7 ± 1.4</td>
</tr>
<tr>
<td>12.5</td>
<td>19.2 ± 1.2</td>
</tr>
</tbody>
</table>

* M.o.i. = 20.

Fig. 4. Kinetics of interaction between ³H-HSV-1 and cells. (a) Cell-associated radioactivity of ³H-HSV-1 counted immediately after the adsorption period; (b) heparin-stable cell-associated radioactivity. Of the virus which had attached during 60 min, about 96% (19500 d/min), 85% (12800 d/min) and 80% (8000 d/min) remained to be associated with Vero cells, C6 cells aged 30 min and C6 cells aged 2 days, respectively, after incubation in MEM-HEPES without heparin. ○—○, C6 cells aged 30 min; ●—●, C6 cells aged 2 days; ▲—▲, Vero cells.

Formation of heparin-stable cell–virus interaction

An attempt was made to determine the efficiency of progression of HSV-1 from the initial attachment step to subsequent steps of infection. Heparin served this purpose, because it interfered with a function of cell membrane and released HSV-1 virions attached to the surface of BSC1 cells (Hochberg & Becker, 1968).

Comparison of Fig. 4(a) with 4(b) clearly indicates that a larger proportion of input virus was released from C6 cells, especially after 2 day culture, than from Vero cells, and that the rate of progression of HSV-1 to a heparin-stable state, as well as the final amount of such stable virus–cell complexes, was also significantly higher in the 30 min than in the 2 day culture of C6 cells.

In this experiment, in which adsorption was allowed to proceed at 37 °C, only a small
proportion of virus was released from C6 cells and Vero cells by incubation in MEM-HEPES without heparin.

**Detachment of HSV-1 from cell surfaces**

Fig. 5 shows the pattern of detachment of $^3$H-HSV-1 at 37 °C. The greater part of the adsorbed HSV-1 was released from the surface of C6 cells after incubation at 37 °C. The rapid release was followed by a slow release. The reduction of the rate may be viewed as a result of progression of virus to a more stable complex with cells. Comparison of Fig. 5 with Fig. 4(a) suggests that the rate of dissociation, which proceeded rapidly during the first 15 min, may correspond to the dissociation constant of the cell–virus complex formed in the initial attachment step, because only a small proportion of virus progressed to a heparin-stable complex during this period. Trypsin–EDTA treatment of C6 cells did not cause an obvious difference in the dissociation kinetics of HSV-1. In contrast, the virus attached to Vero cells strongly resisted dissociation by the incubation in medium.

Comparison of Fig. 3 and 4(a) with Fig. 5 suggests that cell-associated and extracellular virus established an equilibrium and, as a consequence, a fraction of infectious virus could not be associated with C6 cells aged 2 days despite the presence of unsaturated receptor sites.

**DISCUSSION**

The susceptibility of C6 cells to infection with HSV-1 was low; only a small proportion of cells was infected with HSV-1 and the yield per cell in C6 cells was 50 to 400 times lower than in Vero cells. In the cells in which infection was established, HSV-1 replicated showing a pattern of growth comparable to that of a single cycle of HSV-1 replication in most
permissive cells. The growth pattern of HSV-1 in C6 cells in our experiments differs from those reported in previous studies, in which HSV-1 did not replicate in C6 cells cultured in vitro (Schwartz & Elizan, 1973), or replicated after a significantly prolonged eclipse (Lance & Zettlemoyer, 1976). These differences may be due to different experimental conditions such as incomplete removal of superficially adsorbed virus.

Trypsin-EDTA treatment of cells increased the number of cells synthesizing virus antigens and virus-yielding cells, but did not change the yield of virus per infected cell. The increase in the susceptibility of C6 cells to HSV-1 infection may be ascribed to proteolytic action of trypsin because our preliminary results indicated that trypsin treatment alone increased the response of C6 cells to the levels comparable to those by trypsin-EDTA treatment, while EDTA treatment alone was not effective in the experimental conditions described. These results are consistent with the observations that C6 cells require protein synthesis to recover resistance to HSV-1.

It has been reported that large amounts of glycoproteins and glycosaminoglycans were stripped from the cell surface by trypsin, while EDTA did not remove cell-surface components (Vogel, 1978). Possibly, trypsin may affect the surface structure of C6 cells. It is also possible that trypsin is taken up by cells and its activity is expressed intracellularly (Hodges et al. 1973; Maizel et al. 1975). The present results showed that the effect of trypsin-EDTA was evident only when it was applied to the cells prior to infection. Therefore, the possible alteration of intracellular metabolism or destruction of inhibitors for virus replication induced by intracellular trypsin may not explain the stimulation of the cellular response observed here.

When HSV-1 was attached to C6 cells, it first formed a reversible complex which could be dissociated by incubation at 37 °C in medium. The binding strength of the initial virus-receptor complex in the case of C6 cells was significantly lower than that of Vero cells. As the incubation time was prolonged at 37 °C, a part of the cell-associated virus progressed to an irreversible complex from which it could not be dissociated by heparin. The rate of formation of the heparin-stable complex and the amount of virus forming the stable complex with C6 cells was significantly lower than with Vero cells. Therefore, the low susceptibility of C6 cells to HSV-1 infection may be ascribed to the low efficiency of the virus to progress from a reversible complex to an irreversible complex with the cells. Proteolytic digestion of surface components may modify the cellular membrane of C6 cells in such a way that HSV-1 forms reversible and irreversible complexes at an increased rate.

The stimulative effect of trypsin treatment of cells on adsorption has been reported for vesicular stomatitis virus (Schloemer & Wagner, 1975) and adenovirus type 2 (Philipson et al. 1968). The effect of trypsin has been explained by better exposure of virus receptors or increased negative charge of the cell surface (Schloemer & Wagner, 1975). The latter possibility seems less likely for C6 cells responding to HSV-1 because the dissociation kinetics of HSV-1 was comparable before and after trypsin-EDTA treatment of C6 cells.

The nature of the heparin-stable complex is unclear. Trypsinization may cause rearrangement of the receptor subunits on the cell surface in order to provide a proper orientation in favour of the HSV-1 virion as described by Lonberg-Holm & Philipson (1974). An alternative possibility is that trypsin influences penetration of HSV-1 into cells, which has been explained to occur by fusion (Morgan et al. 1968) or pinocytosis (Dales & Silverberg, 1969). Further studies are required to understand the effect of trypsin-EDTA on formation of the heparin-stable complex of HSV-1 with C6 cells.

Our results suggested that the spreading of HSV-1 infection in astrocytoma is accelerated by protease production caused by inflammation. This possibility may well explain the finding of Schwartz & Elizan (1973) that the implantation of the HSV-1-infected C6 cells caused a chronic HSV-1 infection in animals.
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REFERENCES


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