Acta virol. **35** : 127 - 134, 1991

DIFFERENTIAL SUSCEPTIBILITY OF A RAT GLIOMA CELL LINE AND ITS CLONES TO HERPES SIMPLEX VIRUS TYPES 1 AND 2

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Received March 8, 1990

Summary. - A rat glioma cell line, C6-BU-1, showed differential susceptibility to herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), namely, all the HSV-1 strains tested so far persisted in this cell line but the HSV-2 strains did not. Two clones were derived from C6-BU-1 cells and designated C-17 and C-72. The C-72 as well as the parental C6-BU-1 cells supported the replication of HSV-1, but not that of HSV-2. In contrast, C-17 was highly resistant to both HSV-1 and HSV-2. Hydrocortisone treatment converted C-17 to being susceptible to HSV-1, but not to HSV-2. Therefore, C6-BU-1 cells consist of subpopulations heterogeneous in susceptibility to HSV-1 which may be possibly interchangeable.

Key words: rat glioma cell; clonal cells; susceptibility; herpes simplex virus; hydrocortisone.

Introduction

The *in vitro* replication of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) varies with the host cell. Although the majority of cell systems supports the replication of both HSV-1 and HSV-2, these viruses could not replicate in some cell lines, such as Rous sarcoma virus-transformed cells (Docherty *et al.*, 1973) and FRhL-2 cells (Minamishima and Eizuru, 1977). On the other hand, both HSV-1 and HSV-2 persist in Raji and BHK-21 cells (Robey *et al.*, 1876; Szántó *et al.*, 1976). A rat glioma cell line, C6, also has been reported to support presistent infection of HSV-1 (Schwartz and Elizan, 1973; Lancz and Zettlemoyer, 1976; Rice *et al.*, 1979; Dawson *et al.*, 1983). During studies of virus-cell interaction in C6-BU-1 cells which were derived from C6, it was noted that HSV-1 persisted in these cells with virus production, but HSV-2 was unable to produce virus progeny (Sakihama *et al.*, 1988).

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In C6-BU-1 cells persistently infected with HSV-1, only a minority of the cells showed viral cytopathic effect (CPE) (Sakihama *et al.*, 1988), suggesting that these cells consist of subpopulations heterogeneous in permissiveness to HSV-1. Meanwhile, several investigators (Morhenn *et al.*, 1973; Paran *et al.*, 1973; Costa *et al.*, 1974; Parks *et al.*, 1974; Harrell and Sydiskis, 1982) have reported that glucocorticoids influence *in vitro* infection with various viruses. In fact, Nishiyama and Rapp (1979) demonstrated that hydrocortisone was required to maintain the persistent infection of HSV-1 in Raji cells. Therefore, after establishment of clones from C6-BU-1 cells, the interaction of these clones with HSV was investigated in the absence and/or presence of hydrocortisone.

Materials and Methods

Cells and medium. A rat glioma cell line, C6-BU-1, was derived from C6, which are rat astrocytes transformed by N-nitrosomethylurea (Benda *et al.*, 1968); the cells were kindly supplied by Dr. R. Ueno (Ueno Pharmaceutical Co., Osaka). C6-BU-1 cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% foetal bovine serum (Flow Laboratories Inc., Rockville, MD), kanamycin (60 μg/ml) and 0.12 % $NaHCO_3$. The maintenance medium for the cells contained 2%, instead of 10%, foetal bovine serum. Vero cells were grown in Eagle's MEM supplemented with 5% calf serum, kanamycin (60 μg/ml) and 0.12 % $NaHCO_3$, and were used for propagation and titration of all HSV strains.

Virus. Viruses used in this study were as follows: three laboratory strains of HSV-1 (KOS, HF, and Patton), two laboratory strains of HSV-2 (Savage and YS-2), and 9 fresh isolates from the clinical materials submitted to our laboratory. The viruses were plaque-titrated as described (Sakihama *et al.*, 1988). Each fresh isolate from the clinical materials was identified as HSV-1 or HSV-2 by comparing the restriction endonuclease cleavage pattern with those of laboratory strains. Five fresh isolates (Mihara, HKU, YAM, Machiura, and MHP) were identified as HSV-1 and other four isolates (Ishii, Oishi, Muraoka, and Funatsu) as HSV-2.

Virus adsorption. The cells (1.0×10^6) and virus (1.0×10^5 PFU) were mixed in a final volume of 1.0 ml in Eagle's MEM containing 5 % foetal bovine serum. Virus stability controls consisted of virus without cells in the same medium. All tubes were placed in a water bath at 37 ^{0}C and agitated at regular intervals. At designated intervals, the samples were centrifuged at 3000 rev/min for 5 min and unadsorbed virus in the supernatant fluid was immediately plaque-titrated.

Cell cloning. The clones were obtained from C6-BU-1 cells according to the modified method of Kuroki (1975). In brief, the cells (1.0×10^2 to 1.0×10^3) in 1.0 ml of growth medium containing 0.24% soft agar were seeded into each 60-mm plastic Petri dish on a feeder layer consisting of growth medium and 0.24 % soft agar. After 7 days, each colony was scraped off and transferred to a well of the 24-well tissue culture plate (Corning/Iwaki Glass, Tokyo).

Hormone inducer. Hydrocortisone 21-hemisuccinate sodium salt (Katayama Kagakukogyo Co., Ltd., Osaka, Japan) was dissolved in Eagle's MEM at a concentration of 1 mg per ml and then filtered through filter with a pore size of 0.45 μm. For working solutions, the stock solution was diluted with maintenance medium at the final concentration of 1, 10, or 100 μg/ml.

Results

Replication of HSV in C6-BU-1 cells

C6-BU-1 cells were infected with HSV-1 or HSV-2 at a multiplicity (MOI) of 0.2 and the virus yields were titrated at various intervals up to 30 days. Every strain of HSV-1 persisted in C6-BU-1 cells; as infectious virus (10^3 to 10^6 PFU/ml) it was detected even by 30 days p. i., while the infectivity of every strain of HSV-2 decreased gradually and disappeared until 20 days p. i. (Table 1). To determine whether HSV-1 and HSV-2 differed in efficiency to adsorb to C6-BU-1 cells, quantitative experiments were carried out. As shown in Fig. 1, the adsorption patterns revealed no difference between HSV-1 and HSV-2, though both adsorbed less efficiently to C6-BU-1 cells than to Vero cells.

Replication of HSV in clones derived from C6-BU-1 cells

Two clones, designated C-17 and C-72, were infected with KOS strain of HSV-1 or Savage strain of HSV-2 at a MOI of 0.2 and the virus yields were titrated daily for 10 days. The C-72 as well as the parental C6-BU-1 cells supported replication of HSV-1, but the C-17 cells did not (Fig. 2-I). On the other hand, neither C-17 nor C-72 did support the replication of HSV-2 (Fig.

Table 1. Replication of HSV-1 and HSV-2 in C6-BU-1 cells

	Days post-infection*			
	2	10	20	30
HSV-1				
KOS	2.2×10^6	5.8×10^4	4.2×10^4	4.9×10^4
Patton	4.9×10^5	5.3×10^3	1.6×10^3	9.9×10^3
HF	2.5×10^6	1.4×10^5	2.0×10^6	2.6×10^6
Mihara	2.1×10^5	7.4×10^4	4.4×10^4	1.6×10^5
HKU	1.6×10^5	7.2×10^3	4.1×10^3	1.1×10^4
YAM	5.0×10^5	5.1×10^4	1.2×10^3	5.0×10^3
Machiura	7.6×10^5	1.3×10^3	4.1×10^2	2.1×10^3
MHP	8.4×10^4	1.5×10^3	3.7×10^3	5.1×10^3
HSV-2				
Savage	1.4×10^4	1.0×10	0	0
YS-2	3.6×10^4	2.5×10	0	0
Ishii	7.8×10^4	5.0×10^0	0	0
Oishi	7.6×10^4	2.0×10	0	0
Muraoka	8.1×10^3	2.0×10	0	0
Funatsu	1.6×10^4	1.2×10^2	0	0

*The virus titre was expressed as PFU/ml

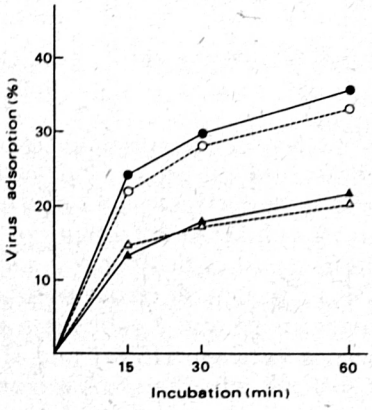

Fig. 1
Adsorption patterns of HSV-1 and HSV-2 to C6-BU-1 and Vero cells; KOS strain of HSV-1 (●) or Savage strain of HSV-2 (○) to Vero cells and HSV-1 (▲) or HSV-2 (△) to C6-BU-1 cells

2-II). Both types of HSV adsorbed similarly to both of these clones as well as to parental C6-BU-1 cells (data not shown).

Effect of hydrocortisone on the replication of HSV in C-17

The effect of hydrocortisone on the replication of HSV in C-17 was investigated. C-17 cells were infected with strain KOS (HSV-1) or with strain Savage (HSV-2) at a MOI of 0.2. After one hour adsorption, the infected cells were fed with maintenance medium containing 1, 10, or 100 μg/ml hydrocortisone. In the presence of hydrocortisone, the virus yield of HSV-1 increased up to 10^6 - 10^7 PFU/ml at 7 or 8 days p. i., regardless of its dose but no replication of HSV-1 occurred in the absence of hydrocortisone (Fig. 3-I). In contrast, hydrocorti-

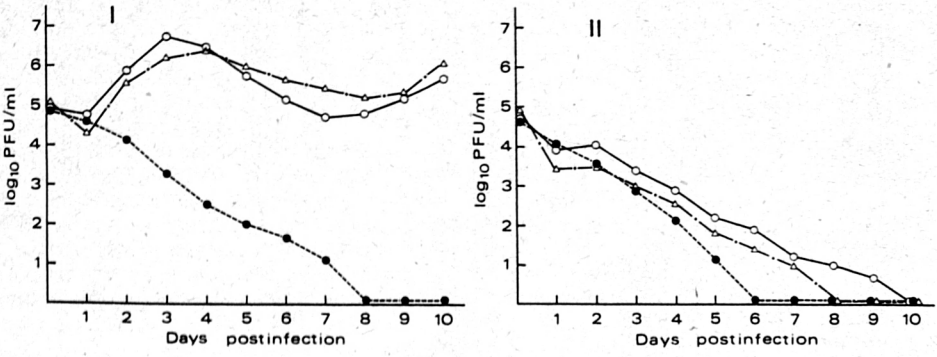

Fig. 2
Replication of KOS strain of HSV-1 (I) and Savage strain of HSV-2 (II) in parental C6-BU-1 cells (○) and its two clones, C-17 (●) and C-72 (△)

sone showed no effect on the replication of HSV-2 in C-17 cells (Fig. 3-II). Similar differential enhancement of virus replication by hydrocortisone was observed when several other laboratory strains and fresh isolates of HSV-1 and HSV-2 were tested (Fig. 4).

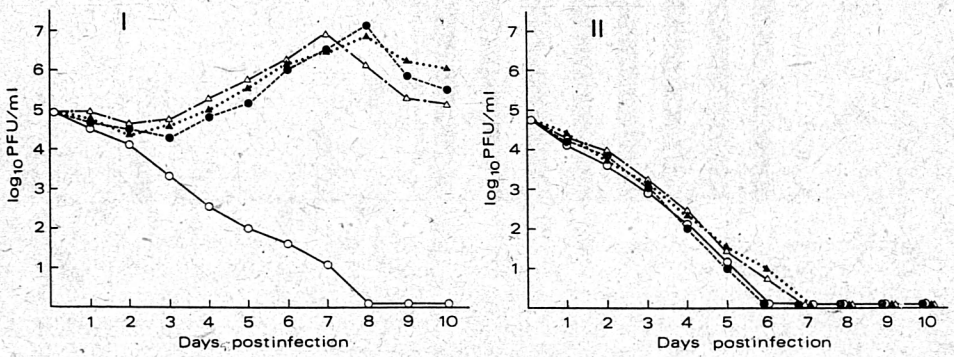

Fig. 3
Replication of KOS strain of HSV-1 (I) and Savage strain of HSV-2 (II) in C-17 in the absence (○) or presence of 1 (●), 10 (△), or 100 (▲) μg/ml of hydrocortisone

Discussion

Raji cells and BHK-21 cells have been reported to support the persistent infection of HSV-1 and HSV-2 without any artificial manipulation (Robey *et al.*, 1976; Szántó *et al.*, 1976). The limitation was, however, that such persistent infection could be induced only with certain HSV strains. In our study, C6-BU-1 cells supported the persistent infection of laboratory strains and also of fresh HSV-1 isolates, but not of HSV-2 strains which were unable to produce virus progeny. Thus, C6-BU-1 cells showed a differential susceptibility to HSV-1 and HSV-2. Linnavuori and Hovi (1983) reported on the importance of interferon (IFN) in the restriction of HSV replication in human monocyte cultures. However, no IFN was detected in C6 cells infected persistently with HSV-1 (Lancz and Zettlemoyer, 1976; Dawson *et al.*, 1983) and exogenous IFN had little effect on the persistent infection in C6 cells (Lancz and Zettlemoyer, 1976). In addition, the basic pattern of the differential susceptibility of C6-BU-1 cells to HSV did not change with MOI (data not shown). Therefore, it is tempting to assume that IFN does not play an important role in the differential susceptibility of C6-BU-1 cells. Adsorption experiments could not explain the differential susceptibility of C6-BU-1 cells to HSV-1 and HSV-2. Rice *et al.*

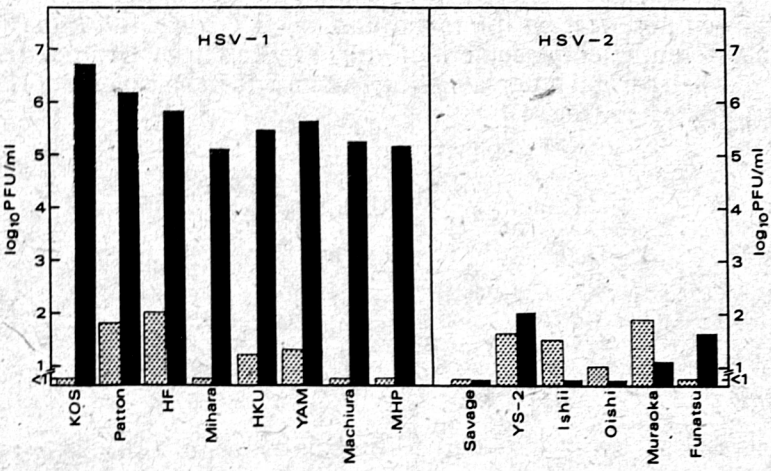

Fig. 4
Replication of various strains of HSV-1 and HSV-2 in C-17 in the absence (dotted columns) or presence (striated columns) of 10 μg/ml of hydrocortisone
Virus yields were determined at day 7 p. i.

(1979) showed that minimal viral DNA synthesis occurred in C6 cells infected persistently with HSV-1. Therefore, the presence or absence of viral DNA synthesis might explain the differential susceptibility of C6-BU-1 cells to HSV-1 and HSV-2.

In this study, it was shown that C6-BU-1 cells consisted of subpopulations heterogeneous in susceptibility to HSV-1. One clone, C-72, supported the persistent infection of HSV-1 as did the parental C6-BU-1 cells. Similar result was also reported with HSV-1 versus human B cell line JOK-1 (Thiele *et al.*, 1989). This phenomenon may be explained by two ways. First, the susceptibility of C-72 to HSV-1 infection may depend on distinct phases of cell cycle. Second, some C-72 cells might lose their susceptibility and become resistant to HSV-1 immediately after cloning. Another clone, the C-17, showed resistance to both HSV types. Hydrocortisone treatment, however, converted this clone to support the replication of HSV-1 but not that of HSV-2. Nishiyama and Rapp (1979) also reported the differential effect of hydrocortisone on the replication of HSV-1 and HSV-2 in Raji cells, though the mechanisms were not investigated. Tanaka *et al.* (1984 *a, b*) demonstrated that dexamethasone enhanced the synthesis of immediate-early antigens and of viral DNA of human cytomegalovirus. As mentioned before, HSV-1 adsorbed at a similar rate to both C-17 and C6-BU-1 cells. In addition, even pretreatment with hydrocortisone converted C-17 to being susceptible to HSV-1 (data not shown). Therefore, it is tempting to say that some cellular factor(s) acts at early stage after adsorption on viral proteosynthesis or DNA synthesis enhancing the replication of HSV-1, though the precise mechanism remains unclear.

In conclusion, we suggest that subpopulations of C6-BU-1 cells were interchangeable becoming from susceptible to resistant to HSV-1 infection or vice versa, which mechanism maintains the persistent infection of HSV-1 in C6-BU-1 cells.

References

Benda, P., Lightsbody, J., Sato, G., Levine, L., and Sweet, W. (1968): Differentiated rat glial cell strain in tissue culture . *Science* **161**, 370 - 371.

Costa, J., Yee, C., Troost, T., and Rabson, A. S. (1974): Effect of dexamethasone on herpes simplex virus type 2 infection *in vitro*. *Nature (London)* **252**, 745 - 746.

Dawson, G. J., Elizan, T. S., Mowshowitz, S. L., and Cohen, R. (1983): Virus-host cell interaction in rat C6 glial cell cultures persistently infected with herpes simplex virus. *Intervirology* **19**, 95 - 104.

Docherty, J. J., Mitchell, W. R., and Thompson, C. J. (1973): Abortive herpes simplex virus replication in Rous sarcoma virus transformed cells. *Proc. Soc. Exp. Biol. Med.* **144**, 697 - 704.

Harrell, A. J., and Sydiskis, R. H. (1982): The effect of dexamethasone on the replication of herpes simplex virus in human gingival fibroblast cultures. *J. Baltimore Coll. Dent. Surg.* **35**, 9 - 13.

Kuroki, T. (1975): Agar plate culture and Lederberg-style replica plating of mammalian cells. *Methods Cell Biol.* **9**, 157 - 178.

Lancz, G. J., and Zettlemoyer, T. L. (1976): Restricted replication of herpes simplex virus in neural cells. *Proc. Soc. Exp. Biol. Med.* **152**, 302 - 306.

Linnavuori, K., and Hovi, T. (1983): Restricted replication of herpes simplex virus in human monocyte cultures: role of interferon. *Virology* **130**, 1 - 9.

Minamishima, Y., and Eizuru, Y. (1977): Susceptibility of primate diploid cells to human herpesviruses. *Microbiol. Immunol.* **21**, 667 - 671.

Morhenn, V., Rabinowitz, Z., and Tomkins, G. M. (1973): Effects of adrenal glucocorticoids on polyoma virus replication. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1088 - 1089.

Nishiyama, Y., and Rapp, F. (1979): Regulation of persistent infection with herpes simplex virus *in vitro* by hydrocortisone. *J. Virol.* **31**, 841 - 844.

Paran, M., Gallo, R. C., Richardson, L. S., and Wu, A. M. (1973): Adrenal corticosteroids enhance production of type-C virus induced by 5-iodo-2-deoxyuridine from cultured mouse fibroblasts. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2391 - 2395.

Parks, W. P., Scolnick, E. M., and Kozikowski, E. H. (1974): Dexamethasone stimulation of murine mammary tumor virus expression: a tissue culture source of virus. *Science* **184**, 158 - 160.

Rice, M., Holland, L., and Wagner, E. K. (1979): Viral nucleic acid synthesis in HSV infected neural cells. *Arch. Virol.* **59**, 345 - 355.

Robey, W. G., Graham, B. J., Harris, C. L., Madden, M. J., Pearson, G. R., and Vande Woude, G. F. (1976): Persistent herpes simplex virus infections established in two Burkitt lymphoma derived cell lines. *J. gen. Virol.* **32**, 51 - 62.

Sakihama, K., Eizuru, Y., and Minamishima, Y. (1988): Interaction of herpes simplex virus type 2 with a rat glioma cell line. *Microbiol. Immunol.* **32**, 933 - 947.

Schwartz, J., and Elizan, T. S. (1973): Chronic herpes simplex virus infection. Initiation in hamsters upon implanatation of infected nonpermissive glial cells. *Arch. Neurol.* **28**, 224 - 230.

Szántó, J., Leššo, J., and Lackovič, V. (1976): Persistent infection of BHK cells with herpes simplex virus types 1 and 2 in the absence of specific anti-herpetic antibody. *Acta virol.* **20**, 40 - 47.

Tanaka, J., Ogura, T., Kamiya, S., Yoshie, T., Yabuki, Y., and Hatano, M. (1984*a*): Dexamethasone enhances human cytomegalovirus replication in human epithelial cell cultures. *Virology* **136**, 448 - 452.

Tanaka, J., Ogura, T., Kamiya, S., Sato, H., Yoshie, T., Ogura, H., and Hatano, M. (1984*b*): Enhanced replication of human cytomegalovirus in human fibroblasts treated with dexamethasone. *J. gen. Virol.* **65**, 1759 - 1767.

Thiele, K., Mittnacht, S., and Kirchner, H. (1989): Persistent replication of herpes simplex virus type 1 in JOK-1 cells. *J. gen. Virol.* **70**, 1907 - 1911.