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PERSISTENT HERPETIC INFECTION OF HeLa CELLS STUDIED BY IMMUNOFLUORESCENCE AND CYTOCHEMICAL METHODS

J. LEŠŠO, J. SZÁNTÓ

Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia

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Summary. — The production of infective virus and viral antigen during the 6th—15th passage of HeLa cells persistently infected with herpes simplex (HS) virus was studied. The persistent infection was induced and maintained by cultivating the acutely infected cells in a medium with 40% of human serum containing specific antibodies. The persistently infected cultures contained only relatively small amounts of both intracellular virus (ICV) and viral antigen as detected by the fluorescent antibody (FA) method. Extracellular virus (ECV) in the culture fluid was not detected. In a comparison with the acute infection, the viral antigen could be detected in persistently infected cells even at very low titres of ICV. As shown by cytochemical assay, the activities of alkaline phosphatase (ALP), acid phosphatase (ACP), nonspecific esterase (NE), membrane adenosine triphosphatase (ATPase) and succinic dehydrogenase (SD) were very low in persistently infected cells. With the exception of ALP they were increased only in individual foci of cells where also a marked accumulation of viral antigen and morphological changes were observed.

Introduction

The effect of specific antibodies in the induction of persistent infection in cells fully susceptible to the given virus was proved in poliovirus-infected HeLa cells (Ackerman and Kurtz, 1955). By this method, however, it was impossible to obtain completely resistant cells. A persistent HS virus infection of HeLa and Detroit 6 cells was induced by use of human serum (Wheeler, 1960; Fernandez, 1960; and Szántó, 1963). Gingsberg (1958) reported that the persistent infection of HeLa cells with an adenovirus could be induced by means of a culture medium containing 40% of human serum. A change of this medium for another one containing 40% of immune rabbit serum resulted in the appearance of cytopathic changes in the cells.

The aim of the present study was to follow in HeLa cells persistently infected with HS virus the production of the infective virus along with FA assay of the viral antigen and the cell metabolism by cytochemical assay of the activities of some important enzymes.

Materials and Methods

Virus. The HSZP strain of HS virus described previously (Leššo and Szántó, 1969) was employed.

Cell cultures and their infection. We used a line of HeLa cells (Gey *et al.*, 1952) maintained in our Institute since 1955. The induction and some properties of the persistent infection of HeLa cells with the HSZP strain of HS virus were described (Szántó, 1963). The persistent infection of these cells could be induced after virus infection in the presence of 40% of human serum. This medium was added to the culture after virus adsorption and the culture was passaged every 7—10 days with two changes of the medium during this period. The production of the infective virus and viral antigen was followed during the 6th—15th passage, daily for 5 days and then 10, 19 and 21 days after seeding.

Virus titration was carried out in tube cultures on the basis of the cytopathic effect (Szántó, 1963).

The FA method was described (Leššo and Szántó, 1969). For the assay of viral antigen only the conjugate prepared from hyperimmune rabbit serum was used.

Cytochemical methods for the assay of ACP, ALP, NE, membrane ATPase, and SD were the same as those used previously (Leššo and Mayer, 1968).

Results

Detection of HS virus by titration and FA methods

The results of several repeated experiments on the kinetics of ICV in HeLa cells persistently infected with HS virus are illustrated in Fig. 1. It can be seen that the titres of infective virus in the cells were generally low, with maxima on the 2nd and 21st day after seeding of the infected cells. The titre of ICV started to decrease from the 4th day and reached the lowest level on the 10th day. From this point it increased again up to the 21st day. We failed to demonstrate ECV in the culture medium.

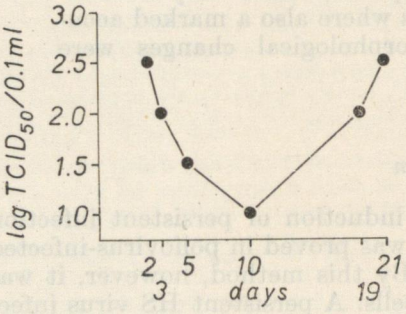

Fig. 1.

The production of HS virus in persistently infected HeLa cells (intracellular virus)

In following the viral antigen in cells by the direct FA method, we observed small amounts of this antigen located on the nuclear membrane and in the cytoplasm as a diffuse and granular fluorescence (Figs 2 and 3). Viral antigen was present in approximately 10% of cells 2 days after seeding. The antigen was seen in groups of 10—20 cells scattered throughout the cell monolayer. Three to four days after seeding, an increased amount of the antigen was observed in approximately 15% of cells. A marked decrease of the amount of fluorescent antigen in cells was found on the 10th day, when also the number of cells in the preparations was lower than at the earlier time intervals. On the 19th—21st day the viral antigen was present again in approx. 15% of cells.

Enzymic activities in HeLa cells persistently infected with HS virus

The activities of the above-mentioned enzymes were followed during the first 5 days after seeding the cultures. The results illustrating the enzymic

Table 1. Cytochemical assay of enzymic activities and the detection of viral antigen by the FA method in HeLa cells persistently infected with HS virus

Days after seeding	Viral antigen present in % of cells	ALP		ACP		NE		ATPase		SD	
		I	C	I	C	I	C	I	C	I	C
1	5	0	±	+	+	+	+	+	+	+	+
2	10	0	+	++	++	++	++	+	+	+	+
3	15	0	+	+	++	+	++	+	+	+	+
4	15	0	+	+	++	+	++	+	+	+	+
5	5	0	+	+	++	+	++	+	+	++	+

I = infected cells; C = control cells.
± Very poor reaction present in a few cells.
+ Weak reaction present in 5—10% of cells.
++ Strong reaction present approximately in 20—40% of cells.
0 Negative result.

activities together with the detection of viral antigen are given in Table 1. No activity of ALP was found in cells persistently infected with HS virus. This is in contrast to uninfected cells or cells acutely infected with HS virus, which display certain activity of ALP (Figs 4 and 5). ALP could not be found even in those groups of cells which contained fluorescent antigen and displayed some morphological changes. These observations were performed so that cell preparations were first stained with FA and then treated by the appropriate azocopulation method for the enzyme assay.

Reduced activities of ACP and NE were observed in persistently infected cells as compared to uninfected or acutely infected cells (Figs 6 and 7). Increased activities of NE, ACP, ATPase and SD were observed only in some groups of cells which also contained a rather large amount of fluorescent antigen and displayed morphological changes.

Discussion

Mayer and Blaškovič (1962), in a review on the persistent infection, postulate that the latter phenomenon is established in cells fully susceptible to a given virus provided the following factors become effective in the given system: (1) specific antibodies, (2) non-specific serum inhibitors of virus and serum factors in the nutritional medium which maintain the cells in an optimal metabolism, and (3) deficiency states of cells. The importance of specific antibodies for the induction of a persistent infection in HeLa cells with HS virus was already mentioned.

The persistent infection in the present experiments was induced and maintained in the presence of 40% of human serum containing antibodies against HS virus. In this persistent infection we found only relatively small amounts of fluorescent antigen and of infective virus in cells; at the same time, ECV in the culture medium could not be demonstrated. The latter

finding, reported also by Wheeler (1960) and Szántó (1963) in HeLa and Detroit-6 cells infected with HS virus, and by Black and Melnick (1955) in the case of B virus, supports the view that the dissemination of the infection in the culture proceeds mainly from cell to cell. We observed in the course of 21 days of our persistent infection also a kind of cyclic periodicity, characterized by virus synthesis and antigen formation, cell destruction and renewed cell growth. This is agreement with our finding that the amount of ICV and viral antigen dropped to a minimum on the 10th day after seeding the culture when, however, also the number of cells in the preparations was lower than on the preceding days. On the 19th and 21st day, the titre of ICV and the amount of the fluorescent antigen increased again.

In this type of persistent infection we were able fairly easily to detect the fluorescent antigen in spite of rather low titres of infective virus. In the case of the acute infection we detected the fluorescent antigen if the titre of infective virus was at least 10^3 $TCID_{50}/0.1$ ml, whereas in the persistent infection the critical level of ICV was lower. This fact can be explained by the presence in persistently infected cells of non-infective or incomplete virus particles, detectable by the FA method. It is also possible that the production of viral antigen is not conditioned by the formation of infective virus particles in this infection. Aurelian and Roizman (1964) reported that, in abortive infection, HS virus was able to adsorb onto dog kidney cells but unable to cause the production of an infective progeny virus. Spring *et al.* (1968) in a study of an abortive HS virus infection showed the presence of non-enveloped nucleocapsids and the absence of reduplication of virions near the nuclear membrane, the latter being characteristic of the productive infection.

In folowing the metabolism of peristently infected HeLa cells, we found low activities of ALP, ACP and NE as compared to uninfected cells. The activities of all the enzymes tested were generally lower in the persistent infection than in the acute infection. This fact signalizes a reduced metabolism in cells persistently infected with HS virus. It is difficult at present to estimate the importance of these facts for the course and maintenance of a persistent infection in this system. Nevertheless, our observation on increased activities of ALP and SD in human amnion cells persistently infected with an attenuated tick-borne encephalitis virus (Leššo and Mayer, 1968) is of interest. The metabolic response of cells to viral infection is apparently very different and depends on the cell substrate, the infecting virus and the course of the infection in the cells.

References

Ackerman, W. W., and Kurtz, H. (1955): Observations. *J. exp. Med.* **102**, 555.

Aurelian, L., and Roizman, B. (1964): The host range of herpes simplex virus. Interferon, viral DNA and antigen synthesis in abortive infection of dog kidney cells. *Virology* **22**, 452.

Black, F. L., and Melnick, J. L. (1955): Micro-epidemiology of poliomyelitis and herpes B infections. *J. Immunol.* **74**, 236.

Fernandez, C. G. (1960): Persistence of herpes simplex virus in HeLa cells. *Nature (Lond.)* **185**, 268.

Gey, G. O., Coffman, W. O., and Kubicek, M. T. (1952): Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. *Cancer Res.*, **12**, 264.
Gingsberg, H. S. (1958): The significance of the viral carrier state in tissue culture systems. *Prog. med. Virol.* **1**, 36.
Leššo, J., and Mayer, V. (1968): Immunofluorescent and cytochemical study on tick-borne encephalitis virus-infected cells. *Acta virol.* **12**, 128.
Leššo, J., and Szántó, J. (1969): Reproduction of herpes simplex virus in HeLa cells studied by immunofluorescence and cytochemical methods. *Acta virol.* **13**, 278.
Mayer, V., and Blaškovič, D. (1962): Latent and persistent viral infections at the cellular level (in Slovak). *Biológia (Bratislava)* **17**, 60.
Spring, S. B., Roizman, B., and Schwartz, J. (1968): Herpes simplex virus products in productive and abortive infection. II. Electron microscopic and immunological evidence for failure of virus envelopment as a cause of abortive infection. *J. Virology* **2**, 384.
Szántó, J. (1963): Course of persistent infection of HeLa and Detroit 6 cells with herpes simplex virus. *Acta virol.* **7**, 385.
Wheeler, C. E. (1960): Further studies on the effect of neutralising antibody upon the course of herpes simplex infection in tissue culture. *J. Immunol.* **84**, 394.

Explanation of Photomicrographs:

Figs 2, 3, 4, 6 and 7: HeLa cells, persistently infected with HS virus, 3—4 days after seeding.
2 — Groups of cells with a diffuse fluorescence of viral antigen in the cytoplasm. Direct FA method. ×250.
3 — A group of cells with diffuse and granular fluorescence of viral antigen on the nuclear membrane and in the cytoplasm. Direct FA method. ×450.
4 — Negative reaction for ALP; the azocopulation method. ×300.
6 — Reaction for ACP; Gomory's method. ×300.
7 — Reaction for membrane ATPase. ×300.
Fig. 5. Reaction for ALP in uninfected HeLa cells. A low, but significant enzymic activity present in groups of cells. The azocopulation method. ×300.