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The antiviral activity exerted by vaccinia virus on the growth of herpes simplex virus in BS-C-1 cells

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Abstract

The growth of herpes simplex virus type 2 (HSV-2) in BS-C-1 cells, was inhibited following super-infection with vaccinia virus. This inhibition was efficiently induced by both the intracellular mature virus (IMV) form of vaccinia virus and the extracellular enveloped virus (EEV), containing an additional external viral membrane. Treatment of vaccinia IMV with the detergents NP-40, Brij-58 or *n*-octyl-α-D-glucopyranoside, abolished its ability to inhibit the growth of HSV-2. Ultraviolet irradiation of vaccinia virus, that completely inactivated the infectivity of the virus, resulted in partial loss of the capability to inhibit the growth of HSV-2: 16-fold more irradiated virus was needed for the inhibition. Electron microscopy showed that the irradiated vaccinia virus adsorbed and penetrated into the HSV-infected cells but remained morphologically intact within the cells for at least 22 h. When the steps in the growth of HSV affected by the irradiated vaccinia virus were followed, it was found that while the synthesis of HSV DNA was partially decreased, the synthesis of HSV proteins was very strongly inhibited and virus particles were not formed. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Herpes simplex virus; Vaccinia virus; Double-infection

1. Introduction

Understanding of the interaction between various viruses during co-infection, is of scientific and may be also of medical importance. Different viruses growing in the same cells can complement, recombine, or interfere one with another. Vaccinia virus, a member of the pox virus family, replicates its DNA and matures in the cytoplasm of the infected cells. The lack of nuclear involvement in vaccinia virus DNA synthesis was demonstrated using enucleated cells, following treatment of the cells with the drug cytochalasin B (Pennington and Follett, 1974). Therefore, vaccinia virus has to encode many of the proteins and enzymes required for its replication, several of which are integrated into the progeny virus particles, in order to function during the initial steps of infection (as reviewed by Moss, 1996).

Several forms of vaccinia virions, distiguished by the membranes they contain, are produced by the infected cells (as reviewed by Moss, 1996). The

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intracellular mature virion (IMV) contains the virus DNA within the core, two lateral-bodies and is surrounded by two tightly apposed membranes. The IMV is then wrapped by cellular cisternae, derived from the *trans*-Golgi network, thereby acquiring two additional membranes. These four-membrane-enveloped structures are then transported to the periphery of the cells, where the outer membrane fuses with the cell plasma membrane thus producing two forms of externalized virions: cell-associated-enveloped virus (CEV) that mediates cell-to-cell infection, and released extracellular enveloped virion (EEV) responsible for long-range transmission. Biochemical analysis of the EEV- and IMV-associated proteins revealed that the first contains at least six additional polypeptides, missing from IMV.

The transcription, replication of viral DNA and assembly of capsids of herpes simplex virus (HSV), another DNA-containing virus, take place in the nucleus (as reviewed by Roizman and Sears, 1996). Viral DNA is transcribed throughout the growth cycle of the virus by host RNA polymerase II, aided by viral factors. The synthesis of the viral gene products is regulated in a cascade fashion. Several of the proteins made are enzymes and DNA-binding proteins, involved in viral DNA replication and many others are structural proteins included within the virion. The DNA is generally formed by a rolling-circle mechanism, yielding concatamers which are then cleaved into monomers and packaged into pre-assembled capsids. These nucleocapsides acquire infectivity upon budding through the inner lamella of the nuclear membrane.

Both vaccinia virus and herpes virus cause a rapid shut-off of host macromolecular metabolism early in infection. They affect mainly host DNA and protein synthesis, while the synthesis of host ribosomal RNA is only somewhat reduced. The first phase of the host-cell protein synthesis shut-off does not require de novo protein synthesis (Moss, 1968; Esteban and Metz, 1973; Fenwick, 1984). The shut-off of the host-cell protein synthesis is faster and more efficient in HSV-2-infected cells than in cells infected with HSV-1 (Fenwick et al., 1979).

In the present study we followed infection of HSV in BS-C-1 cells which were later infected with vaccinia virus, in order to find out whether the growth of HSV is affected and if this is the case, to shed some light on the factors and mechanisms which may be involved.

2. Materials and methods

2.1. Cells and viruses

BS-C-1 (ATCC CCL 26), a cell-line originating from green monkey kidney, was grown in DMEM (Dulbecco's minimum essential medium) containing 7.5% fetal bovine serum (FBS). Vaccinia virus (WR strain) and herpes simplex virus type 2 (HSV-2, Curtis strain), were used.

2.2. Chemicals and radioactive isotopes

Isatin-β-thiosemicarbazone (IBT) and acyclovir were kindly donated by Wellcome Laboratories, UK. Brij-58 (polyoxyethylene-20-cetyl ether) and *n*-octyl α-glucoside (*n*-octyl α-D-glucopyranoside) were obtained from Sigma, St. Louis, MO. Thymidine-methyl ^{3}H (49.2 Ci/mmol) was purchased from Rotem Industries, Dimona, Israel. L-^{35}S-methionine (1000) Ci/mM) was obtained from Amersham Life Science, Buckinghamshire, UK.

2.3. Virus infection

Monolayers of BS-C-1 cells in 5-cm diameter plastic Petri dishes were infected with virus, in a vol. of 0.3 ml. Following 30 min at 37°C, the cells were washed twice and 5 ml DMEM supplemented with 2% FBS, were added. The cultures were harvested 22 h after infection with a rubber policeman and kept frozen at −70°C.

2.4. Plaque assay

Confluent monolayers of cells, in 3 cm-diameter plastic Petri dishes, were infected with 0.2 ml of virus dilutions. After adsorption for 1 h at 37°C, the cultures were overlaid with Eagle's minimum

essential medium (MEM), supplemented with 0.7% Noble agar (Difco Laboratories, Detroit, Mich.) and 5% FBS. On the fifth day following infection, the cultures were fixed with 20% formalin in phosphate-buffered saline and stained with 0.1% crystal-violet in 0.1 M citric acid.

2.5. Preparation of extracellular enveloped virus (EEV) and intracellular mature virions (IMV)

EEV and IMV were isolated from cells 2 days after infection. EEV were collected by centrifugation of clarified culture medium in a TST28 rotor at 24 000 rpm for 45 min at 4°C. IMV were derived from the cytoplasm of Dounce-homogenized cells and purified through a cushion of 36% sucrose, under similar centrifugation conditions (Joklik, 1962). The pellet was then layered on sucrose gradient (25–40%) and centrifuged in an TST28 rotor at 13 500 rpm for 35 min at 4°C. The virus band was collected, diluted in buffer and centrifuged as before.

2.6. Treatment of vaccinia virus with detergents and preparation of virus cores

Purified virus was treated for 30 min with NP40 (0.5%) in 50 mM of 2-mercaptoethanol, 25 mM Tris pH 7.5 (Tas and Martini, 1983) and the cores were sedimented in an SW41 rotor at 28 000 rpm for 45 min and washed under similar centrifugation conditions.

Purified virus preparations were treated with Brij-58 (1%) or with *n*-octyl-α-glucoside (2%) for 20 min at 37°C and then centrifuged in a TST55.5 rotor at 28 000 rpm for 45 min. The pellets were suspended in 10 mM Tris pH 7.5 and the centrifugation was repeated.

2.7. Ultraviolet (UV) irradiation

Virus purified suspension (0.8 ml), containing 4×10^8 plaque forming units (PFU), in a 2 cm-diameter dish, located 9 cm underneath a UV lamp (Philips 6 W type 103314), were irradiated for 10 min, when no infectious virus could be detected any more.

2.8. Formation of HSV particles

BS-C-1 cultures were labeled with ^{3}H-thymidine (3 μCi/ml) starting at 2 h after infection with HSV and harvested 20 h later. The cells were suspended in 0.6 ml of RSB (10 mM KCl, 1.5 mM $MgCl_2$ 10mM Tris pH 7.7) and sonicated for 1 min in a Bransonic model 12 ultrasonic cleaner. The broken cells were layered on a 12–52% (wt./wt.) sucrose gradient in Tris buffered-saline (0.85% NaCl, 0.2 M Tris, pH 7.3) and centrifuged in a TST rotor at 26 000 rpm for 1 h at 4°C. Fractions (10 drops) were collected from the bottom of the tube. Trichloroacetic acid precipitable radioactivity of half of each fraction, and of the rest following digestion with deoxyribonuclease (50 μg/ml) in the presence of 10 mM $MgCl_2$ for 30 min at 37°C, were determined.

2.9. CsCl gradient analysis of HSV DNA

Infected cells were labeled with ^{3}H-thymidine (3 μCi/ml) starting at 2 h after infection. The cells were harvested 22 h postinfection, centrifuged at low speed ($600 \times g$), washed and suspended in SSC (150 mM NaCl and 15 mM sodium citrate, pH 7.2). Sodium dodecyl sulfate (SDS) and proteinase-K were added at final concentrations of 1% and 0.3 mg/ml, respectively. After incubation for 4 h at 37°C, the DNA was centrifuged in a CsCl density gradient in buffer containing 1 mM EDTA and 10 mM Tris pH 8 in a Ti 50 rotor at 35 000 rpm for 72 h at 20°C. Fractions (10 drops) were collected from the bottom of the tube. Radioactivity of the fractions was determined after trichloroacetic acid precipitation.

2.10. Gel electrophoresis of viral polypeptides

BS-C-1 cells maintained in DMEM containing 1/20 of the regular methionine concentration supplemented with 7.5% dialyzed FBS were labeled with ^{35}S-methionine (6 μCi/ml), starting at 2 h after infection. The cultures were harvested 20 h later and the polypeptides from the cell extracts were dissociated with 2% sodium dodecyl sulfate (SDS) and 5% 2-mercaptoethanol at 100°C and then resolved by electrophoresis in 7.5% polyacry-

lamide gel (Laemmli, 1970). The gel was dried and exposed to X-ray film (X-Omat AR, Eastman Kodak, Rochester, NY).

2.11. Transmission electron microscopy

Infected cells were mixed with 2% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4 for 2 h at room temperature. The samples were sedimented, postfixed in 1% OsO_4 and rinsed in water. They were then stained with 2% aqueous uranyl–acetate for 1 h at 4°C. Dehydration with ethanol was followed and the samples were embedded in Araldite embedding resin. Ultrathin sections were cut with an LKB ultramicrotome, layered on copper grids and stained with 2% uranyl–acetate (prepared in 50% methanol) and then with 0.2% lead-citrate. Sections were viewed with a Philips CM 12 electron microscope at an accelerated voltage of 100 kV.

2.12. Immunofluorescence staining

Infected cells were washed with buffered-saline and fixed with acetone. Detection of HSV antigens was performed with pathoDx herpes typing kit (Diagnostic Products Corp. Los-Angeles, CA).

Table 1
Growth of HSV-2 and vaccinia virus in BS-C-1 cells in single and double infection, as determined by plaque assay in the presence of acyclovir or IBT

Virus[a]	Virus titer (PFU/ml)[b]			
	0 h[c]	22 h		
		−	+ Acyclovir	+ IBT
HSV-2	5.0×10^4	2.3×10^6	6.0×10^4	1.9×10^6
Vaccinia	1.3×10^5	3.5×10^8	3.3×10^8	3.0×10^4
HSV + vaccinia	−	6.0×10^5	4.5×10^5	1.6×10^5
Vaccinia + HSV	−	3.8×105	1.0×105	4.0×105

[a] Crude (unpurified) preparations of the viruses were used. When two viruses infected the cells, a 30-min interval was allowed between the first and the second.

[b] The plaque assay was performed in the presence and absence of acyclovir (100 μM) or IBT (28 μM).

[c] Virus titer at the end of the adsorption period.

3. Results

3.1. Growth of HSV-2 in BS-C-1 cells super-infected with vaccinia virus

In the present study, the growth of HSV-2 in BS-C-1 cells, super-infected with vaccinia virus, was followed. In order to distinguish between the progeny of these two viruses made in the cells, specific inhibitors of either one of the two viruses, were added to the agar-overlay of the cell monolayers, when performing the plaque assay. The two specific inhibitors of the growth of HSV and of vaccinia virus used, were: acyclovir (Elion et al., 1977; Schaeffer et al., 1978) and isatin-β-thiosemicarbazone (IBT) (Bauer, 1972; Levinson, 1973; Pfau, 1982), respectively. The specific inhibition of HSV by acyclovir (100 μM) and of vaccinia virus by IBT (28 μM), are clearly demonstrated in the plaque assay of the viruses produced, when one type of virus infected the cells (Table 1); (under these conditions the antiviral agents did not cause toxicity to the cells). When BS-C-1 cells were infected with HSV and 30 min later with vaccinia virus, HSV was inhibited by 92.9% and vaccinia virus by 99.9% (Table 1). (The progeny virus made in the mixed infection was determined by plaquing in the presence of acyclovir, giving rise to mostly vaccinia virus plaques, and in the presence of IBT enabling the appearance of mainly HSV plaques). Inhibition in the growth of the two viruses was also observed when infection with vaccinia virus preceded that with HSV (Table 1).

We wished to find out whether an 'early' or a 'late' function of vaccinia virus is responsible for the inhibition of HSV. For this aim we used IBT, which inhibits the 'late' stages during vaccinia virus growth (Woodson and Joklik, 1965; Katz, 1987). The results (Table 2) show that the pres-

Table 2
Growth of HSV-2 and vaccinia virus in BS-C-1 cells in single and double infection, in the absence and presence of IBT

Time of vaccinia virus infection[a] (h)	IBT (21 μM) [b] present during growth	Virus titer (PFU/ml)[c]			
		Single infection[d]	−	+Acyclovir	+IBT
−	−	1.1×10^6	−	−	−
+1	−	−	3.3×10^5	5.5×10^4	3.2×10^5
+2	−	−	5.0×10^5	2.4×10^5	1.5×10^5
+1	+	−	1.8×10^5	1.3×10^4	1.8×10^5
+2	+	−	3.8×10^5	7.5×10^4	1.5×10^5

[a] Crude (unpurified) preparations of the viruses were used. Vaccinia virus was added at the time indicated.
[b] This concentration of IBT was sufficient to inhibit the growth of vaccinia virus under these culture conditions (without agar).
[c] The plaque assay was performed in the absence and presence of acyclovir (100 μM) or IBT (28 μM).
[d] Represents HSV titer.

ence of the drug did not affect the level of inhibition of HSV growth caused by vaccinia virus, suggesting that either the virus particle by itself is responsible, or an 'early' step, taking place during the growth of the virus, is responsible for the interference of vaccinia virus with HSV replication.

We then proceeded with characterization of the structural components of vaccinia virus required for the inhibition of HSV growth.

3.2. Inhibition of HSV-2 growth by vaccinia virus particles pretreated or not with detergents

The capability of the IMV and EEV forms of vaccinia virus to inhibit the growth of HSV, was then followed. The intracellular IMV were purified from the cytoplasm of the vaccinia-infected cells, while the EEV, released from the cells and which contain several additional membrane polypeptides missing from IMV, were obtained from the media of the infected cultures. The extent of inhibition of HSV growth induced by the EEV and by IMV were quite similar (87.7 and 91.9%, respectively), suggesting that the additional envelope surrounding the EEV does not contribute to the inhibition of HSV growth. An increase in the m.o.i. of vaccinia virus resulted in greater inhibition of HSV growth, and vaccinia virions were capable of inhibiting the growth of HSV-2 even when added to the cultures 3 h following the infection with HSV-2 (results not shown).

It is possible to remove the outer membranes of IMV, thus obtaining the virus core. This can be done by treatment of purified preparations of the virus with the non-ionic detergent NP-40 in the presence of a reducing agent, such as 2-mercaptoethanol (Easterbrook, 1966). When HSV-infected cells were treated with vaccinia virus cores, no significant inhibition of the growth of HSV was observed, as compared to the effect exhibited by intact EEV and IMV. A similar loss of the capability of vaccinia virus to inhibit the growth of HSV was noted when the virus was treated with two other reagents: Brij-58 (Payne, 1978) and *n*-octyl-α-glucoside (Gould et al., 1981), affecting the integrity of the virus membranes (data not shown).

3.3. Inhibition of HSV-2 growth by UV-irradiated vaccinia virus

We then wished to find out whether the infectivity of vaccinia virus is essential for the anti-HSV effect. For this study, purified preparations of vaccinia virus were used. The rate of inhibition of the growth of HSV caused by purified virus preparations (Table 3) was significantly higher than that induced by crude virus preparations, which we previously used (Tables 1 and 2). Purified preparations of vaccinia virus irradiated by UV under the conditions described in Section 2 lost their infectivity (8.6 $\log_{10}$). This UV-irradiated vaccinia virus inhibited the growth of HSV,

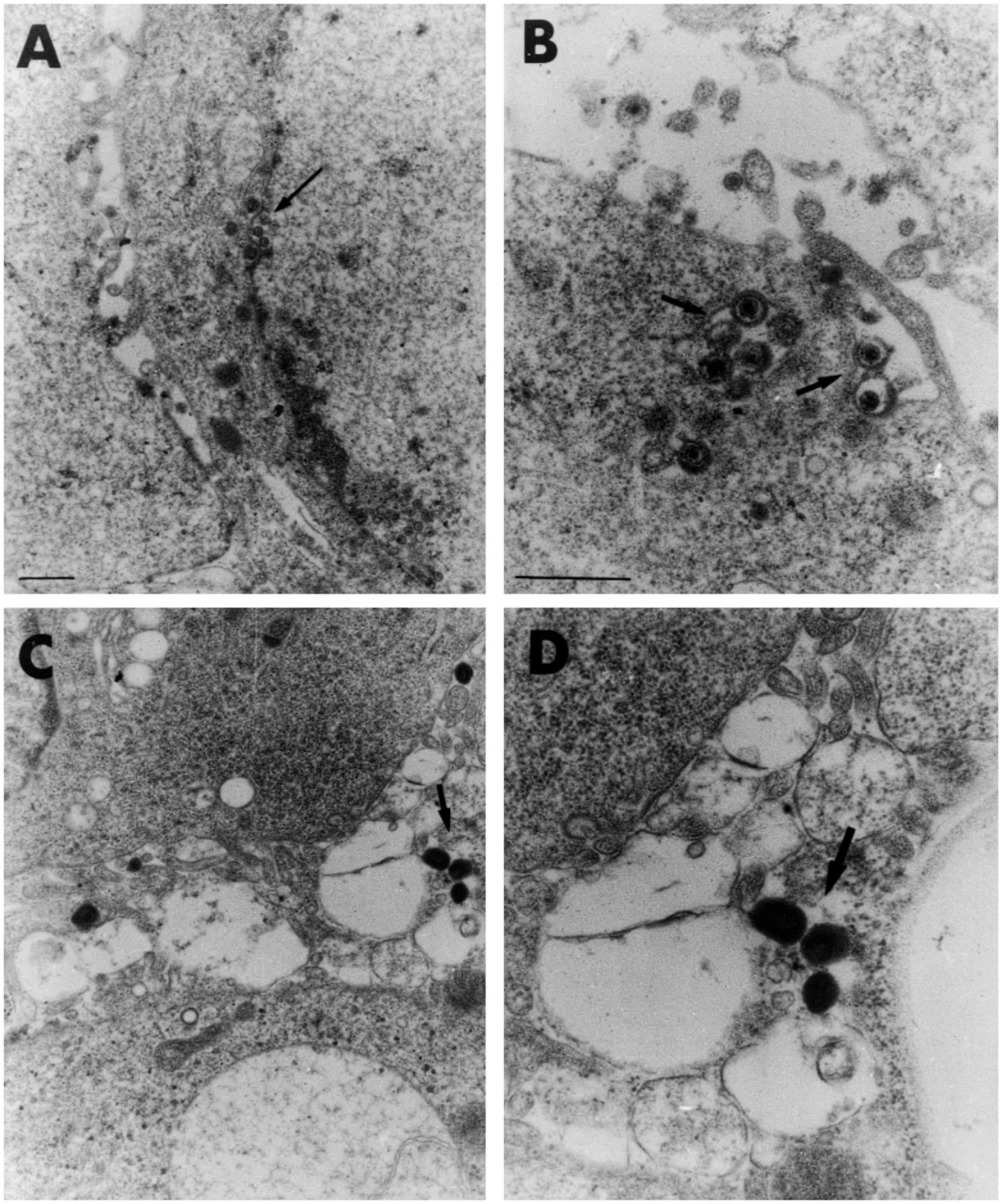

Fig. 1. Electron micrographs of ultrathin sections of BS-C-1 cells infected with HSV-2 (A,B), (arrows point at HSV-2 mature particles); infected with HSV-2 and then treated with UV-irradiated vaccinia virus (C,D), (arrows point at vaccinia virus particles). Bars, 0.5 μm.

Table 3
Super-infection of HSV-infected cells with live or UV-irradiated vaccinia virus

Vaccinia virus[a]	m.o.i.	HSV titer (PFU/ml)[b]
–	–	1.2×10^7
Live	2	5.0×10^4
UV-irradiated	2	2.5×10^7
UV-irradiated	8	4.0×10^6
UV-irradiated	16	3.8×10^5

[a] Purified virus preparations were used.
[b] When live vaccinia virus was used, the titer of HSV was determined in the presence of IBT (28 μM). The m.o.i. of HSV was 2 PFU/cell.

although a greater amount of virus was required in order to achieve a comparable level of inhibition to that induced by live virus (Table 3). Electron microscopy showed the presence of morphologically intact UV-irradiated vaccinia virus particles, either attached to the surface of the cells or located within the plasma membrane, even as late as 22 h following infection (Fig. 1C, D).

In addition to the significant inhibition in the growth of HSV-2 induced by the irradiated-vaccinia virus, the cytopathic effects, usually seen in cultures of HSV-infected cells, i.e. rounding of the cells and then their detachment from the surface of the culture dish (Fig. 2A), did not take place in the co-infected cells (Fig. 2B), the morphology of which resembled that of uninfected cell monolayer.

3.4. The stage in HSV-2 growth affected by UV-irradiated vaccinia virus

The stages during the growth of HSV in BS-C-1 cells, affected by the treatment with the UV-irradiated vaccinia virus, were then followed. These experiments were performed with BS-C-1 cells infected with HSV at a m.o.i. of 2 PFU/cell and treated 30 min later with U.V.-irradiated vaccinia virus of an initial infectivity titer (prior to irradiation) of 32 PFU/cell. First, the formation of HSV particles under these conditions was followed. HSV-infected cells treated with UV-irradiated vaccinia virus, were labeled with ^{3}H-thymidine and harvested after 22 h. The extracts of the cells

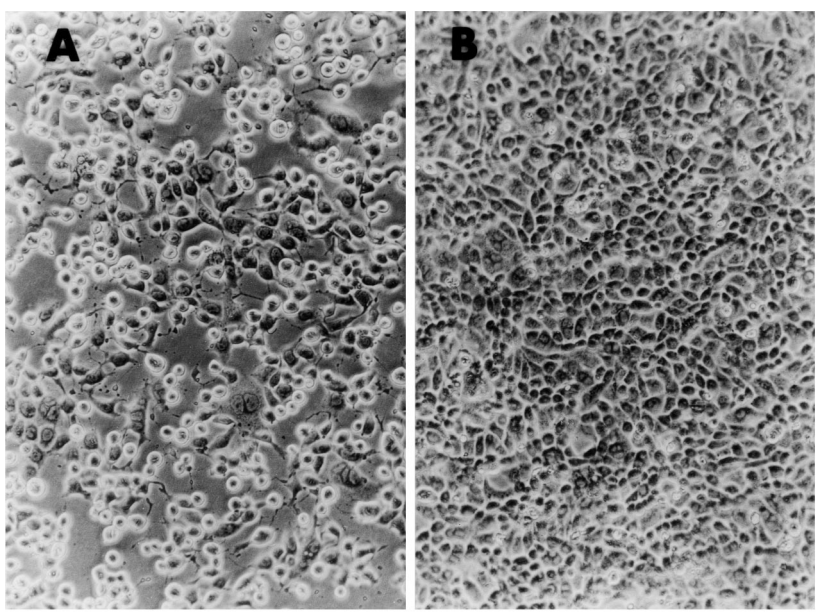

Fig. 2. BS-C-1 cell monolayers infected with HSV-2 (A); infected with HSV-2 followed by UV-irradiated vaccinia virus (B). The panels show representative light-microscopy fields at approximately 400 × magnification.

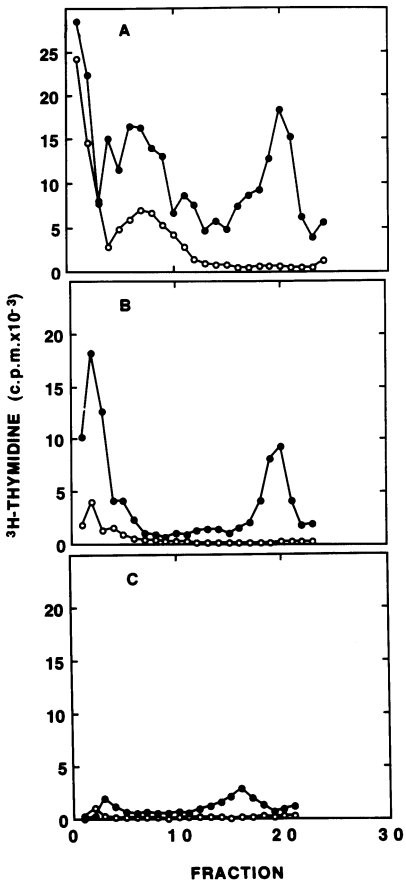

Fig. 3. Formation of HSV-2 particles. Infected cells labeled with ^{3}H-thymidine were analyzed by sucrose gradient centrifugation. Fractions were collected from the bottom of the gradients. (A) HSV-infected cells. (B) HSV-infected cells treated with UV-irradiated vaccinia virus. (C) HSV-infected cells in the presence of acyclovir (100 μM). The trichloroacetic acid precipitable radioactivity of half of each fraction (●), and of the other half following deoxyribonuclease digestion (○), are shown.

were analyzed for the presence of HSV particles, by sucrose gradient centrifugation, followed by deoxyribonuclease digestion, as described in Section 2. One of the prominent effects induced by the vaccinia virus was the failure of HSV to form particles (Fig. 3B), in contrast to the deoxyribonuclease-resistant virus particles formed during single infection with HSV (Fig. 3A). The peak, formed by fractions 5–9 in Fig. 3A, contains also particles, the DNA of which was partially sensitive to deoxyribonuclease and consequently its level was partially decreased following digestion of the DNA by the enzyme. Deoxyribonuclease-resistant virus particles, as expected, were not observed in an HSV-infected culture treated with 100 μM of acyclovir (Fig. 3C). Electron microscopy failed to reveal either mature or immature HSV particles, in the vaccinia virus-treated cells (Fig. 1C,D), in contrast to their presence during a single infection with HSV (Fig. 1A,B).

The synthesis of HSV DNA in the infected cells can be followed by labeling the cells with ^{3}H-thymidine. The distinguished densities of the viral and cellular DNA (1.712 and 1.695 g/ml, respectively) due to their different GC contents, enabled us to follow the effect of vaccinia virus on HSV DNA even without application of blotting technology. Only a partial inhibition of HSV DNA synthesis was induced by the UV-irradiated vaccinia virus (Fig. 4B), as compared with a control of a single infection with HSV (Fig. 4A). Acyclovir (100 μM) as expected, completely inhibited the DNA synthesis of HSV, while it only slightly affected the synthesis of the host-cell DNA (Fig. 4C).

When the polypeptides synthesized in the cells were analyzed by gel electrophoresis, following labeling with ^{35}S-methionine, a clear shift from polypeptides synthesized in BS-C-1 cells (Fig. 5, lane 2) to those made following HSV infection (Fig. 5, lane 3), was observed. A very significant inhibition of a wide-range of HSV polypeptides occurred in the irradiated-vaccinia virus treated cells (Fig. 5, lane 5), in contrast to the more selective inhibition caused by acyclovir (Fig. 5, lane 4), blocking the synthesis of HSV late polypeptides. The extensive inhibition of the protein synthesis of uninfected BS-C-1 cells induced by the irradiated vaccinia virus is also shown (Fig. 5, lane 1). The failure to synthesize HSV polypeptides following infection, when the cells were treated with UV-irradiated vaccinia virus, was also demonstrated by treatment of acetone-fixed infected cells with fluorescent antibodies directed against two (41 and 79 Kda) HSV late proteins (data not shown).

4. Discussion

The present study shows that vaccinia virus, when added to BS-C-1 cells pre-infected with HSV, is capable of inhibiting the growth of HSV. The anti-herpes activity induced by vaccinia virus depends on an early function taking place during the growth of vaccinia virus, since IBT, a specific inhibitor of vaccinia virus late functions and maturation (Woodson and Joklik, 1965; Katz, 1987), did not affect this activity. The vaccinia virus had to adsorb to the cells, since its anti-herpes activity was abolished upon neutralization by specific antiserum against vaccinia virus (our unpublished results). However, following attachment and penetration into the HSV-infected cells, the UV-irradiated vaccinia virus failed to uncoat its membranes, a process normally accomplished within the first 2 h following infection with live virus (Joklik, 1964; Dales, 1965) but remained intact for at least 22 h, as viewed by electron microscopy (Fig. 1C,D).

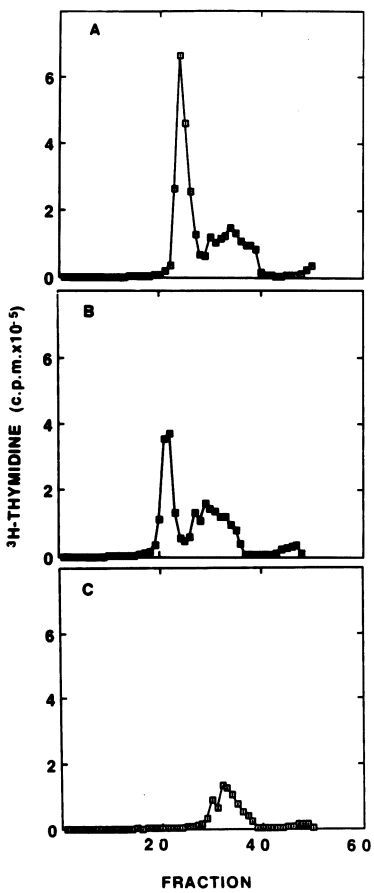

Fig. 4. HSV DNA in CsCl density gradient. BS-C-1 cells were labeled with ^{3}H-thymidine and the DNA was analyzed by CsCl density centrifugation. (A) HSV-infected cells. (B) HSV-infected cells treated with UV-irradiated vaccinia virus. (C) HSV-infected cells in the presence of acyclovir (100 μM). Fractions were collected from the bottom of the gradients. The left high peaks in A and B are of HSV DNA (1.712 g/ml) and the right lower peaks in A and B and that of C, are of cellular DNA (1.695 g/ml).

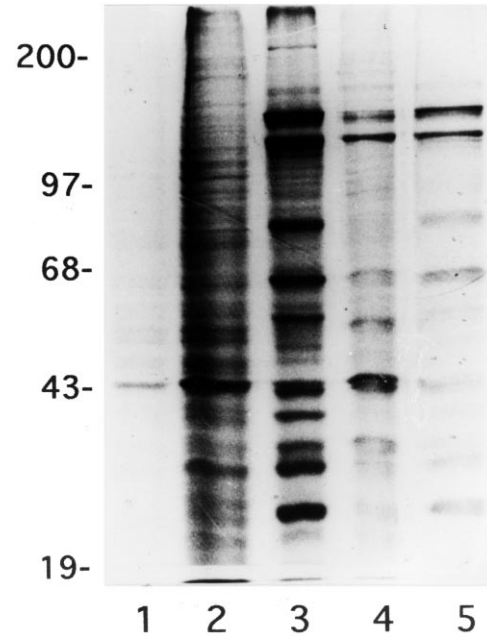

Fig. 5. Gel electrophoresis of ^{35}S-methionine-labeled polypeptides. Lanes: 1, BS-C-1 cells treated with vaccinia virus. 2, BS-C-1 cells. 3, HSV-infected cells. 4, HSV-infected cells in the presence of acyclovir (100 μM). 5, HSV-infected cells treated with UV-irradiated vaccinia virus. Molecular weight markers ran in parallel are expressed in Kda.

While the additional viral membrane present in the EEV form of vaccinia virus, did not enhance the anti-herpes activity induced by IMV, the integrity of the membrane of the latter was crucial, since stripping it off by several detergents, abolished its anti-herpes activity. It is possible that the detergent treatment of the virions caused either a loss of structural proteins required to inhibit the growth of HSV or prevented the penetration of

the virus cores into the cells or gene expression by the virus. The vaccinia virus inducing the anti-HSV effect had not to be infectious, since UV-irradiated vaccinia virus could still cause it, although at a lesser efficiency; more than 16-fold of such virus was needed in order to obtain a comparable level of inhibition to that exhibited by live vaccinia virus (Table 3). It is possible that the larger amount of the irradiated virus required had to compensate for decreased gene expression induced by the UV-treated virus (Gershowitz and Moss, 1979; Bablanian et al., 1981).

We do not know at present whether the anti-herpes activity exerted by vaccinia virions is due to the shut-off of the host-cell protein synthesis induced by vaccinia virus, a phenomenon previously described by Moss (1968) and Esteban and Metz (1973). Moss (1968) suggested that a component of the vaccinia virion may be required for the inhibition of the host-cell protein synthesis, since an UV-irradiated vaccinia virus was also able to cause this inhibition which was lost upon heating or following detergent treatment. Experiments we carried out showed that the anti-HSV activity of the virion was also lost upon treatment of the virus by detergents, partially retained after incubation at 56°C for 30 min and completely eliminated by heating at 100°C. Two of the proteins associated with the outer surface of IMV, the peripheral p14 (product of gene A27L) (Rodrigues and Esteban, 1987; Rodrigues and Smith, 1990; Sodeik et al., 1995) and the integral p32 (product of gene D8L) (Niles and Seto, 1988; Lai et al., 1991), may have a role in the anti-herpes activity exerted by the vaccinia virions.

When the stage in HSV growth, affected by irradiated vaccinia virus, was followed, it was found that early steps in the growth of HSV were not strongly affected. The finding that the vaccinia virus could be added to the HSV-infected cells even at 3 h following HSV and still able to exert its inhibitory effect, supports this conclusion. Furthermore, the synthesis of HSV DNA synthesis was only partially inhibited, under these conditions. In addition to the significant decrease observed in the infectivity of the progeny HSV made in the vaccinia virus-treated cells, virus particles were not formed under these conditions, as determined by sedimentation of particles through sucrose gradients and by electron microscopy. The failure to form HSV particles may be due to the efficient inhibition exerted by the irradiated vaccinia virus on the synthesis of HSV polypeptides.

The present study shows that the ability of vaccinia virus to suppress the protein synthesis is not restricted to that of the host-cell, as was previously shown by Moss (1968) and Esteban and Metz (1973), but can be extended to that of other viruses, such as HSV. The mechanism by which vaccinia virus inhibits the protein synthesis of the host-cell and that of other viruses, while allowing the synthesis of its own proteins, when live vaccinia virus is infecting the cells, needs further elucidation. Although the target for the inhibition of HSV infection by vaccinia virus has not been defined yet, the findings obtained so far suggest that it has to take place following HSV DNA synthesis but prior to the formation of HSV particles.

References

Bablanian, R., Coppola, G., Scribani, S., Esteban, M., 1981. Inhibition of protein synthesis by vaccinia virus. III. The effect of ultraviolet-irradiated virus on the inhibition of protein synthesis. Virology 112, 1–12.

Bauer, D.J., 1972. Thiosemicarbazones. In: Bauer, D.J. (Ed.), Chemotherapy of Virus Diseases (Int. Encyclopedia Pharmcol. Ther. 1, Sect. 61). Pergamon, London, pp. 35–113.

Dales, S., 1965. Relation between penetration of vaccinia, release of viral DNA and initiation of genetic functions. Perspect. Virol. 4, 47–71.

Easterbrook, K.B., 1966. Controlled degradation of vaccinia virions in vitro: an electron microscopic study. J. Ultrastruct. Res. 14, 484–496.

Elion, G.B., Furman, P.A., Fyfe, J.A., de Miranda, P., Beauchamp, L., Schaeffer, H.J., 1977. Selectivity of action of an antiherpetic agent 9-(2-hydroxyethoxymethyl)-guanine. Proc. Natl. Acad. Sci. USA 74, 5716–5720.

Esteban, M., Metz, D.H., 1973. Early virus protein synthesis in vaccinia virus-infected cells. J. Gen. Virol. 19, 201–216.

Fenwick, M., 1984. The effects of herpesviruses on cellular macromolecular synthesis. In: FraenkelConrat, N., Wagner, R.R. (Eds.), Comprehensive Virology. Plenum, New Yrok, pp. 359–390.

Fenwick, M., Morse, L.S., Roizman, B., 1979. Anatomy of herpes simplex virus DNA. XI. Apparent clustering of functions effecting rapid inhibition of host DNA and protein synthesis. J. Virol. 29, 825–827.

Gershowitz, A., Moss, B., 1979. Abortive transcription products of vaccinia virus are guanylylated, methylated and polyadenylated. J. Virol. 31, 849–853.

Gould, R.J., Ginsberg, B.H., Spector, A.A., 1981. Effects of octyl-β-glucoside on insulin binding to solubilized membrane receptors. Biochemistry 20, 6776–6781.

Joklik, W.K., 1962. The purification of four strains of poxvirus. Virology 18, 9–18.

Joklik, W.K., 1964. The intracellular uncoating of poxvirus DNA. I. The fate of radioactively-labeled rabbitpox virus. J. Mol. Biol. 8, 263–276.

Katz, E., 1987. Thiosemicarbazones: inhibition of the growth of poxviruses and requirement for the growth of an isatin-β-thiosemicarbazone dependent mutant. Rev. Clin. Basic Pharmacol. 6, 119–130.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.) 227, 680–685.

Lai, C., Gong, S., Esteban, M., 1991. The 32-kilodalton envelope protein of vaccinia virus synthesized in Escherichia coli binds with specificity to cell surfaces. J. Virol. 65, 499–504.

Levinson, W., 1973. Inhibition of viruses, tumors and pathogenic microorganisms by isatin-beta-thiosemicarbazone and other thiosemicarbazones. In: Carter, W.A. (Ed.), Selective Inhibitors of Viral Function. CRC Press, Cleveland, pp. 212–226.

Moss, B., 1968. Inhibition of HeLa cell protein synthesis by the vaccinia virion. J. Virol. 2, 1028–1037.

Moss, B., 1996. Poxviridae: the viruses and their replication. In: Fields, B.N., Knipe, D.M., Howley, P.M., et al. (Eds.), Fundamental Virology. Lippincott-Raven, New York, pp. 1163–1197.

Niles, E.G., Seto, J., 1988. Vaccinia virus gene d8 encodes a virion transmembrane protein. J. Virol. 62, 3772–3778.

Payne, L., 1978. Polypeptide composition of extracellular enveloped vaccinia virus. J. Virol. 27, 28–37.

Pennington, T.H., Follett, E.A.C., 1974. Vaccinia virus replication in enucleate BSC-1 cells: particle production and synthesis of viral DNA and proteins. J. Virol. 13, 488–493.

Pfau, C.J., 1982. The thiosemicarbazones. In: Came, P.E., Caliguiri, L.A. (Eds.), Handbook of Experimental Pharmacology Berlin. Springer-Verlag, Berlin, pp. 147–203.

Rodrigues, J.F., Esteban, M., 1987. Mapping and nucleotide sequence of a vaccinia virus gene that encodes a 14-kilodalton fusion protein. J. Virol. 61, 3550–3554.

Rodrigues, J.F., Smith, G.L., 1990. IPTG-dependent vaccinia virus: identification of a virus protein enabling virion envelopment by Golgi membrane and egress. Nucleic Acid Res. 18, 5347–5351.

Roizman, B., Sears, A. E., 1996. Herpes simplex viruses and their replication. In: Fields, B.N., Knipe, D.M., Howley, P.M., et al. (Eds.), Fundamental Virology. Lippincott-Raven, New York, pp. 1043–1107.

Schaeffer, H.J., Beauchamp, L., de Miranda, P., Elion, G.B., Bauer, D.J., Collins, P., 1978. 9-(2-hydroxyethoxymethyl)guanine activity against viruses of the herpes group. Nature (Lond.) 272, 583–585.

Sodeik, B., Cudmore, S., Ericsson, M., Esteban, M., Niles, E., Griffiths, G., 1995. Assembly of vaccinia virus virus: incorporation of p14 and p32 into the membrane of the intracellular mature virus. J. Virol. 69, 3560–3574.

Tas, P.W.L., Martini, O.H.W., 1983. Inhibition of the elongation step of protein synthesis by vaccinia virus. FEBS Lett. 153, 427–430.

Woodson, B., Joklik, W.K., 1965. The inhibition of vaccinia virus multiplication by isatin-β-thiosemicarbazone. Proc. Natl. Acad. Sci. USA 54, 946–953.