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Evidence for an Inhibitor of Herpes Simplex Virus in Transformed Hamster Cells (39629)

ELIZABETH W. DOLLER

Department of Microbiology, The Milton S. Hershey Medical Center, The Pennsylvania State University, College of Medicine, Hershey, Pennsylvania 17033

Introduction. Cells transformed by a virus are often resistant to superinfection by the homologous virus (1). It has been postulated that a specific repressor is responsible for the resistance to superinfection of simian virus 40 (SV40) and adenovirus-transformed cells (2-4). Previous work from this laboratory demonstrated that Herpes simplex virus type 2 (HSV-2) can transform hamster cells (5) and that these cells are resistant to superinfection by HSV-2 (6). In this report, evidence is presented for a soluble inhibitor, obtained from the HSV-2-transformed cells, which inhibits HSV replication in permissive cells.

Materials and methods. Extracts were prepared from normal hamster embryo fibroblast (HEF) cells and HSV-2-transformed HEF cells (333-8-9 and 333-8-9,T-1). The characteristics of the HSV-2-transformed cells have been described (5, 6). These cells maintain virus-specific antigens (5) and contain virus DNA (7). A suspension of 10^8 cells/ml grown in medium 199, supplemented with 10% fetal calf serum (FCS), 10% tryptose phosphate broth, 0.075% $NaHCO_3$, 100 units/ml of penicillin, and 100 μg/ml of streptomycin, was sonicated for 1 min and centrifuged at 100,000g for 1 hr. The supernatant was removed and stored at $-76°$ prior to testing for inhibitory activity in plaque reduction assays.

In one type of assay, confluent monolayers of HEF cells were treated simultaneously with 10^2 plaque forming units (PFU) of HSV-1 (strain Patton) or HSV-2 (strain 333), 10 μg of poly-L-ornithine (poly-O; mol wt 120,000), and 0.05 ml of extract. Controls received virus, poly-O, and medium 199, supplemented as described above, instead of extract. After a 1-hr adsorption period, Eagle's medium with 0.5% methyl cellulose, 5% FCS, and 0.225% $NaHCO_3$ was added. Plaques were counted 48 hr postinfection.

In a second assay, confluent monolayers of HEF cells were pretreated with 10 μg of poly-O and 0.1 ml of extract for 24 hr. The monolayers were then washed and infected with 10^2 PFU of HSV. After virus adsorption, Eagle's medium containing methyl cellulose was added. Plaques were counted 48 hr postinfection. To determine the effect of temperature on the extracts, they were heated at 37 and 56° for 30 min before being used in the plaque reduction test.

The replication of HSV-2 in the presence of extracts was also examined. Confluent monolayers of HEF cells were treated with 0.2 ml of extract and 20 μg of poly-O. After 24 hr, the extract was removed and 10^3 PFU of HSV were added. After 1 hr, the unadsorbed virus was removed by washing. Fresh extract and poly-O were readded and then replenished every 24 hr. Samples were harvested 2, 24, 48, and 72 hr postinfection and assayed for infectious virus in primary rabbit kidney cells. The replication of vesicular stomatitis virus (VSV) was also studied according to this procedure to determine whether the extracts would inhibit an interferon-sensitive virus.

Results and discussion. HEF extract caused no reduction in plaques compared to untreated controls when added at the time of virus inoculation (Table I). However, extract from the transformed cells (333-8-9,T-1) reduced the plaque number of HSV-1 and HSV-2 to approximately 40% of the control (Table I). The extracts were not toxic to the HEF cell monolayers which readily incorporated neutral red. The inhibitor in the transformed cell extract is probably a soluble component of the cell, since the activity was found in the supernatant of the sonicated cell suspension that had been centrifuged at 100,000g for 1 hr.

Plaque reduction also occurred in HEF cells pretreated with 333-8-9 or 333-8-9, T-1 extract for 24 hr prior to infection (Table

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TABLE I. THE EFFECT OF CELL EXTRACTS ON PLAQUE FORMATION BY HSV IN HAMSTER CELLS NOT PRETREATED WITH EXTRACT.[a]

Extract	Virus	Average number of plaques[b]	Percentage of control[c]
None[d]	HSV-1	42	100
	HSV-2	49	100
HEF	HSV-1	TNTC[e]	100
	HSV-2	46	94
333-8-9, T-1	HSV-1	17	41
	HSV-2	21	44

[a] Virus, extract, and poly-L-ornithine were added to HEF cultures simultaneously.

[b] The average number of plaques was based on six replicate cultures.

[c] Percentage of control = average number of plaques with extract/average number of plaques without extract × 100.

[d] No extract was added to these cultures.

[e] Too numerous to count.

II). Although HEF extracts occasionally caused some reduction in plaque number, the transformed cell extracts consistently caused much greater reductions. It is possible that the pretreatment allows the cell to more adequately incorporate the inhibitor from the medium. Since the extracts were removed prior to infection in this assay, the extract apparently does not reduce the number of plaques by inactivating extracellular virus. The extracts could be diluted only twofold before more than 50% of the activity was lost. Therefore, all further experiments were carried out using undiluted extract.

The effect of the cell extracts on replication of HSV-2 was also examined. The data in Fig. 1 show that the titer of HSV-2 in HEF cells treated with HEF extract reached almost 10^4 PFU/ml. The titer of HSV in cells treated with 333-8-9 or 333-8-9,T-1 extract fell below the input value and never increased. Thus, the transformed cell extracts also inhibited replication of HSV.

Plaque reduction assays were performed with extracts that had been heated at 37 or 56° for 30 min before being added to HEF monolayers. As indicated in Fig. 2, the inhibitory effect of the transformed cell extracts was lost when extracts were heated to 56°; that is, the number of plaques in cultures receiving transformed cell extract heated at 56° was similar to the number of plaques in cultures with HEF extract. Heating the extracts at 37° for 30 min did not significantly alter the inhibitory activity. The data presented here are for inhibition of HSV-2, but similar results were obtained with HSV-1.

The growth of VSV in HEF cells treated with transformed cell extracts or HEF extract was compared to growth in cells receiving no extract. VSV grew equally well under all conditions employed (data not shown). These experiments indicate that the inhibition of HSV in the transformed cells is not due to classicial interferon, since interferon inhibits VSV and is heat-stable at 56°. The

TABLE II. THE EFFECT OF CELL EXTRACTS ON PLAQUE FORMATION BY HSV IN HAMSTER CELLS PRETREATED WITH EXTRACT.[a]

Extract	Virus	Average number of plaques[b]	Percentage of control[c]
None[d]	HSV-1	72	100
	HSV-2	45	100
HEF	HSV-1	51	71
	HSV-2	31	69
333-8-9	HSV-1	0	0
	HSV-2	1	3
333-8-9, T-1	HSV-1	2	3
	HSV-2	10	21

[a] Extract and poly-L-ornithine were added to HEF cultures 24 hr prior to infection.

[b] The average number of plaques was based on four replicate cultures.

[c] Percentage of control = average number of plaques with extract/average number of plaques without extract × 100.

[d] No extract was added to these cultures.

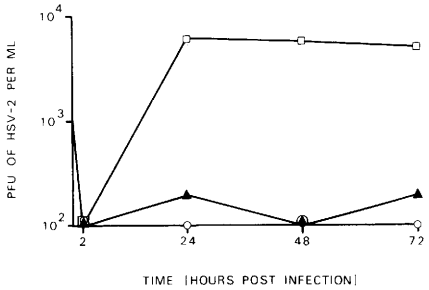

FIG. 1. Replication of HSV-2 (strain 333) in HEF cells continuously treated with cell extracts. Normal HEF cells were treated with cell extracts 24 hr prior to infection with 10^3 PFU of HSV-2. After virus adsorption, extract was readded and replenished every 24 hr. At 2, 24, 48, and 72 hr postinfection, samples were harvested and assayed for infectious virus. (□) Cells with HEF extract; (▲) cells with 333-8-9 extract; (○) cells with 333-8-9, T-1 extract.

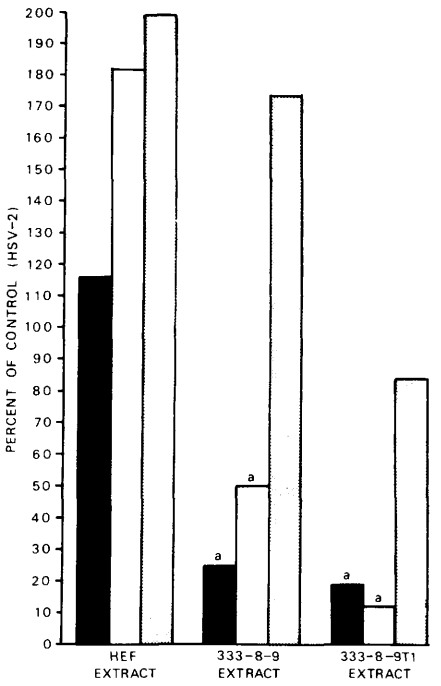

FIG. 2. Detection of inhibitory activity in untreated and heat-treated extracts. Normal HEF cells were exposed to untreated extracts or extracts heated at 37 or 56° for 30 min. After 24 hr, the cells were infected with 10^2 PFU of HSV-2. Plaques were counted 48 hr postinfection. (■) Cells with untreated extract; (□) cells with extract heated at 37°; (▦) cells with extract heated at 56°. Each transformed cell extract was compared to an HEF cell extract under the same temperature conditions by the Student's t test. a Indicates $P > 0.01$

growth of vaccinia virus is also not inhibited by transformed cell extracts (data not shown). The fact that neither VSV nor vaccinia is inhibited by the transformed cell extracts indicates that the extracts are not toxic to viruses.

All experiments were performed at least twice with similar results. The plaque reduction assays, in particular, were repeated four times. The data presented are representative, since different batches of extracts gave levels of inhibition ranging from 60 to 99%. The variability may have been due to the physiological state of the cells when the extract was prepared.

We have previously demonstrated (6) that the HSV-2-transformed cells are resistant to superinfection by HSV-1 and HSV-2 and that the replication cycle of HSV is probably blocked after penetration. The results presented here are consistent with that report. A soluble inhibitor present in HSV-2-transformed cell extracts reduces the plaque formation and growth of HSV in normal cells. The inhibitor probably works intracellularly since the extract can be washed from the cells before infection without precluding inhibition. If this inhibitor acts within the infected or transformed cell to prevent virus production, it is possible that the function of this inhibitor is to regulate the viral genome and, consequently, maintain transformation.

Summary. Extracts of HSV-2-transformed hamster cells inhibit plaque formation of HSV-1 and HSV-2 in normal hamster cells. Extracts also inhibit replication of HSV. The inhibitor is a soluble component of the cells, and probably does not act directly on extracellular virus. The inhibitor is not a classicial interferon since the inhibition is temperature-sensitive and the replication of VSV is not impaired.

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