Restricted Replication of Herpes Simplex Virus Type 1 in Murine Embryonal Carcinoma Cells

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(Accepted 28 October 1986)

SUMMARY

Herpes simplex virus type 1 (HSV-1) has a broad host range but the KOS strain of HSV-1 did not replicate efficiently in murine embryonal carcinoma (EC) cells. The yield of infectious HSV-1 from EC cells was 100- to 1000-fold lower than that from fibroblast cell lines of mouse, monkey or human origin. The thymidine kinase (TK) gene of HSV-1 is expressed early during the infectious cycle. The levels of TK mRNA and of TK activity in infected EC cells were only two- to threefold lower than levels from infected fibroblast cells. Infected EC cells supported replication of about half as much HSV-1 DNA as did fibroblast cells. The reduced yield of infectious virus was consistent with a paucity of virions in infected EC cells examined by electron microscopy, suggesting a major block late during the HSV-1 infectious cycle. We isolated a variant strain of HSV-1, called KOSEC, which replicated as efficiently in EC cells as in mouse fibroblasts. KOSEC infected EC and fibroblast cells, synthesized more TK mRNA, more TK enzyme, and more HSV-1 DNA than did the same cells infected with the KOS strain. Both HSV-1 strains induced similar levels of synthesis of gD, an early viral glycoprotein. By co-infection of EC cells with the KOS and KOSEC virus, both the elevated virus yield and the elevated TK synthesis seen in KOSEC-infected cells appeared to be recessive. Apparently a viral mutation that affects expression of some early viral functions can also overcome the EC cell restriction to HSV-1 replication.

INTRODUCTION

To investigate the means by which cellular genes are regulated during embryonic development, investigators have made use of well characterized viral systems as probes for the different mechanisms of gene expression utilized in different cell types (Kelly & Condamine, 1982; Maltzman & Levine, 1981). The pluripotential mouse embryonal carcinoma (EC) cell is notable because infection of these cells by polyoma virus results in no virus replication (Swartzendruber & Lehman, 1975) and because no synthesis of polyoma early or late proteins occurs in infected cells (Dandolo et al., 1983; Fujimura et al., 1981a, b). Mutant polyoma viruses (Fujimura et al., 1981a; Katinka et al., 1980, 1981; Sekikawa & Levine, 1981; Vasseur et al., 1980) able to grow in EC cells carry alterations in a non-coding region of the viral genome comprising the enhancer (deVilliers & Schaffner, 1981; Tyndall et al., 1981) of early gene transcription. This suggests that EC cells differ from fibroblast cells in that EC cells do not efficiently transcribe viral genes whose regulation is controlled by enhancers. Direct evidence

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for an EC cell component interacting with viral enhancers has been obtained (Gorman et al., 1985). It may be possible, therefore, to use the EC cells as a means of identifying those genes in other viruses which are regulated by enhancers.

Herpes simplex virus type 1 (HSV-1) has a complex infectious cycle (Spear & Roizman, 1980). Since it is likely that expression of some HSV-1 genes is regulated by enhancers, we reasoned that HSV-1 replication in EC cells may be arrested at a stage of the infectious cycle when these gene products are required. We found that the KOS strain of HSV-1 replicated poorly in mouse EC cells. Viral DNA replication occurred efficiently in infected EC cells suggesting that the restriction in viral replication occurred late in the infectious cycle. A mutant virus was selected by its ability to replicate efficiently in EC cells. We describe some of the characteristics of the restricted viral replication cycle and of the mutant virus strain.

METHODS

Cells and media. Most of the studies described below involved the use of O1A1 cells (McBurney et al., 1982), a thioguanine-resistant and ouabain-resistant clone of the P19 line of EC cells (McBurney & Rogers, 1982). These cells are developmentally pluripotential, euploid, and derived from the C3H/He strain of mice. The F9 (Bernstine et al., 1973) and OC1581 (McBurney, 1976) lines of EC cells are of strain 129/J origin whereas C86S1 cells are from C3H/He strain mice. P19(TK-) is a thymidine kinase-deficient (TK-) clone of P19 cells selected for resistance to 100 µg/ml 5-bromodeoxyuridine.

RAP1 is a mouse fibroblast cell line derived from a differentiated culture of O1A1 cells (Bell et al., 1986). LTK- (Kit et al., 1963) and 143 (Rhim et al., 1975) are TK- mouse and human fibroblast cell lines respectively obtained from Dr S. Bacchetti (McMaster University, Hamilton, Ontario, Canada). Vero cells were purchased from Microbiological Associates (Walkersville, Md., U.S.A.). Both RAP1 and LTK- cells are fibroblasts from strain C3H/He which supported the replication of HSV-1 to similar degrees. These fibroblast lines were used interchangeably as fibroblast controls in our experiments.

The medium used to culture all cells was alpha-MEM (Flow Laboratories) supplemented with 2.5% foetal bovine serum and 7.5% calf serum (Animal Health Laboratories, Toronto, Ontario, Canada). Aggregates of EC cells are developmentally pluripotential, euploid, and derived from the F9 (Bernstine et al., 1973) and OC1581 (McBurney, 1976) lines of EC cells are of strain 129/J origin whereas C86S1 cells are from C3H/He strain mice. P19(TK-) is a thymidine kinase-deficient (TK-) clone of P19 cells selected for resistance to 100 µg/ml 5-bromodeoxyuridine.

The medium used to culture all cells was alpha-MEM (Flow Laboratories) supplemented with 2.5% foetal bovine serum and 7.5% calf serum (Animal Health Laboratories, Toronto, Ontario, Canada). Aggregates of EC cells were induced to differentiate in the presence of 1 µM DMSO (Sigma) or 10-7 M-retinoic acid (Sigma) as previously described (Jones-Villeneuve et al., 1982; McBurney et al., 1982).

Viruses and infectivity assays. The KOS strain of HSV-1 (Smith, 1964) used throughout this study was obtained from Dr W. E. Rawls (McMaster University). Viral stocks were prepared in monolayers of Vero cells as described previously (Campione-Piccardo et al., 1979). Cloning or plaque purification of the virus was done by limiting dilution on Vero cells.

For virus infection, 104 cells in 1.0 ml were mixed in suspension with virus at 37 °C for 60 min. The infected cells were washed in fresh medium, plated in tissue culture plastic dishes (105 cells per 60 mm dish). To determine the cell-associated fraction of the progeny virus, the cells were scraped off the plastic surfaces, washed with fresh medium, resuspended in 1 ml of medium at 0 °C and sonicated for 60 s using a Fisher-sonic dismembrator model 300 at maximum output. Cell debris was removed by centrifugation at 1000 g and the supernatant was divided into samples and stored at -70 °C. Titrations were performed on Vero cells as previously described (Campione-Piccardo et al., 1979).

Two variants of the KOS strain of HSV-1 were isolated and plaque-purified. The KOS61 strain was isolated following growth of the KOS virus on O1A1 cells (see Results). The KOS(PA+) strain was selected for its ability to replicate in Vero cells in the presence of 100 µglml phosphonoacetic acid (PA) (Hay & Subak-Sharpe, 1976). In this concentration of PA, the KOS and the KOS61 strains of HSV-1 do not form plaques on Vero cells.

Molecular hybridization procedures. Dot blots of RNA and DNA were carried out on nitrocellulose filters by the selective retention NaI method (Bresser et al., 1983a, b). Control experiments using RNase or DNase pre-digestion of the cell extracts confirmed that the retained nucleic acid was either RNA or DNA as expected. Filters were probed with DNA from nick-translated pX-I (obtained from Dr J. R. Smiley, McMaster University), a pBR322 plasmid carrying the 3.4 kb BamHI fragment of HSV-1 DNA containing the TK gene (Enquist et al., 1979). Following hybridization and washing (Maniatis et al., 1982), the filters were exposed to XAR-5 X-ray film (Kodak) with intensifier screens at -70 °C.

Virus-induced proteins. The synthesis of the major virus-induced proteins was assessed by exposing cultures to medium containing 2% foetal bovine serum and [3S]methionine (30 µCi/ml; 2 Ci/mmol) for 4 h following infection of cells at a multiplicity of 3. Cultures were washed with ice-cold phosphate-buffered saline and scraped into RIPA buffer (1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium lauryl sulphate, 3 mm-PMSE, 200 mm-NaCl, 12.5 mm-Tris-HCl, pH 7.0) (Bell et al., 1984). Extracts were incubated on ice for 30 min, vortexed, and centrifuged for 10 min at 4 °C and 13000 g. Samples were electrophoresed in 15% polyacrylamide gels containing SDS (Laemmlli, 1970).
For immunoprecipitation of the gD glycoprotein, labelled lysates of 10^6 cells in 500 µl of RIPA buffer were centrifuged for 10 min at 4 °C and 13000 g and the supernatants were mixed with 10 µl of ascites fluid containing the 18betaB3 monoclonal antibody (Balachandran et al., 1982) and 40 µl of 10% Protein A-Sepharose beads (Pharmacia). These mixtures were gently agitated at 4 °C for 4 h then centrifuged at 13000 g for 5 min and washed four times in RIPA buffer. Samples were resolved on 15% SDS-polyacrylamide gels.

Assays of TK activity were performed as described by Campione-Piccardo & Rawls (1981). The monospecific rabbit antiserum used to inactivate HSV-1 TK was kindly provided by Dr W. Summers and has been previously characterized (Smiley et al., 1980).

Electron microscopy. Infected cells were scraped from the plate, washed in 0.1 M-phosphate buffer pH 7.3 and fixed for 30 min at room temperature in 2.0% glutaraldehyde in 0.1 M-phosphate buffer pH 7.3. Samples were gently centrifuged to concentrate cells and post-fixed in phosphate-buffered 1.0% osmium tetroxide for 1.5 h. Cells were subsequently dehydrated in a graded ethanol series and acetone, and embedded in Epon-Araldite (Polysciences, Niles, Ill., U.S.A.). Thin sections (60 to 70 nm) were cut using an LKB Ultratome III, stained with uranyl acetate and lead citrate, and examined with a Zeiss 10C electron microscope at 60 keV.

RESULTS

Replication of HSV-1 in EC cells

To determine whether the KOS strain of HSV-1 replicates efficiently in EC cells, we compared the yields of infectious virus during a single infectious cycle (m.o.i. 3) in the O1A1 line of EC cells derived from C3H strain mice, in mouse (LTK-) fibroblast cells also of C3H strain origin, and in monkey (Vero) cells. The yield of cell-associated infectious virus from O1A1 cells was 100- to 1000-fold lower than in the other two cell types (Fig. 1). Following the eclipse phase, there was a small increase in the number of infectious particles from EC cultures, indicating that some viral replication did take place. At 20 h post-infection there appeared to be a complete cytopathic effect within these O1A1 cultures suggesting that all cells had been infected but that each yielded relatively few mature virions.

Preliminary experiments indicated that EC and Vero cells adsorbed infectious HSV-1 virions with equal efficiency. To determine whether the low yield of virus from infected EC cells was due to premature release of progeny virus, the yield of HSV-1 in the culture medium from infected cells was assayed. The medium from infected EC cultures contained less than 1% of the infectious virus in the culture medium of infected LTK- and Vero cells.

We tested a variety of other mouse EC lines (F9, C86S1, OC15S1) for their ability to support the replication of the KOS strain of HSV-1 and all yielded virus titres 100- to 1000-fold lower than those produced by Vero and LTK- cells (data not shown).

The O1A1 line of EC cells can be induced to differentiate when aggregated and exposed to DMSO (McBurney et al., 1982) or retinoic acid (RA) (Jones-Villeneuve et al., 1982). Such differentiated cultures yielded titres of HSV-1 10- to 100-fold higher than those of the undifferentiated O1A1 cells. To determine when differentiating cultures of O1A1 cells become capable of supporting efficient replication of HSV-1, cultures were exposed to RA (10^-7 M) and, at daily intervals, replicate samples of cells were infected with HSV-1 and the yield of virus was assessed 24 h later. The O1A1 cells inefficiently supported replication of HSV-1 during the first 5 days following RA exposure (Fig. 2) but by 9 days the virus yields were similar to those obtained from infected LTK- fibroblast cell cultures. In RA-treated O1A1 cultures, cells become morphologically differentiated by 5 to 6 days (Jones-Villeneuve et al., 1983). The result reported in Fig. 2 suggests that the ability to support efficient replication of HSV-1 occurs relatively late during the differentiation of EC cells.

Macromolecular synthesis in HSV-1-infected cells

To identify the stage of the HSV-1 infectious cycle that was involved in the reduced viral yield in EC cells, we first investigated the expression of the TK gene of HSV-1, an early (or beta class) gene (Spear & Roizman, 1980). We infected Vero, RAP1 and O1A1 cells, and at various times after infection, the cells were solubilized and RNA or DNA was selectively retained in dot blots on nitrocellulose filters (Bresser et al., 1983a, b). The filters were probed with the 3.4 kb BamHI fragment of HSV-1 DNA containing the viral TK gene. The radioactivity associated with each
Reduced yield of HSV-1 following infection of mouse EC cells. Cultures of Vero (■), LTK− (○) and O1A1 (●) cells were infected in suspension with the KOS strain of HSV-1 at an m.o.i. of 3. At intervals after infection the cells were harvested, sonicated, and the cell-associated virus was titrated by plaque formation on monolayers of Vero cells.

Differentiating cultures of EC cells become permissive for HSV-1 replication late during drug-induced differentiation. Cultures of O1A1 cells (●) were grown in the presence of 10−7 M-RA and at intervals after RA addition one of these cultures was removed from the drug and infected with HSV-1 at m.o.i. 3. Twenty h after infection the cell-associated virus was titrated on monolayers of Vero cells. As a control for possible effects of RA, LTK− cells were treated in the same way. RA induced differentiation of O1A1 cells into cells with a fibroblast morphology (Jones-Villeneuve et al., 1982) but had no effect on LTK− cell morphology.

dot was determined in a scintillation counter. The rate of accumulation of TK mRNA in EC cells was about 30% that of the other two cell lines (Fig. 3a). The TK enzyme activities were assayed in infected cultures of TK-deficient human 143, mouse fibroblast LTK−, and EC P19(TK−) cells. Viral TK activity appeared in infected EC cells and was about half that of the two other cell lines (Fig. 3c). These results indicate that transcription and translation of one of the early genes of HSV-1 occur in EC cells at rates reduced by only two- to threefold.

The replication of viral DNA was assessed by probing DNA dot blots with the 3.4 kb fragment of HSV-1 DNA. Extensive replication of HSV-1 DNA was detected in infected EC cells. The amount of viral DNA in EC cells was approximately half that of the other two cell lines (Fig. 3b).

Electron microscopy of Vero cells at 20 h post-infection (m.o.i. 3) revealed large amounts of electron-dense fibrillar material aggregated near the inner surface of the nuclear envelope (Fig. 4a, c). Assembly of virus particles appeared to take place near the sites of this dense material because large numbers of intranuclear virus capsids were present. Many of these capsids contained dense cores (Fig. 4c). These infected Vero cells also contained focal proliferation of the nuclear envelope, a characteristic of HSV-1-infected cells (Fig. 4d). Intact viruses were
Restricted growth of HSV-1 in EC cells

Fig. 3. HSV-1-infected EC cells synthesize viral TK mRNA, TK enzyme activity, and viral DNA at almost normal rates. (a) Cultures of Vero (■), RAP1 (▲) and O1A1 (●) cells were infected with HSV-1 at m.o.i. 3. At 3 h intervals thereafter, samples containing 10⁶ cells were removed, dissolved in NaI (Bresser et al., 1983a, b), and filtered through nitrocellulose membranes under conditions that allow for specific retention of mRNA. The membrane was probed with nick-translated 32P-labelled pX-1 plasmid which contains the cloned HSV-1 TK (Enquist et al., 1979). The radioactivity associated with each spot was assessed by liquid scintillation counting. Ribonuclease treatment of cell extracts completely abolished all signals, indicating that the radioactivity was associated with RNA. (b) The same cell extracts used for the experiment described in (a) were filtered through nitrocellulose under conditions allowing for the specific retention of DNA. This filter was probed with the same nick-translated plasmid and the radioactivity associated with each spot determined by scintillation counting. (c) Cultures of human 143(TK⁻) (▲), LTK⁻ (▲) and P19(TK⁻) (○) cells were infected with HSV-1 at m.o.i. 3 and at 2 h intervals aliquots of cells were harvested and assayed for TK activity (Campionepiccardo & Rawls, 1981). The activities measured were of HSV-1 origin because they were inhibited by more than 80% by an antibody specific to the HSV-1 TK (see Fig. 7).

occasionally observed within the cytoplasm and extracellular space. By contrast, the HSV-1-infected EC cells contained very few virus particles and few intranuclear areas of condensed material (Fig. 4b). Few of the intranuclear viral capsids contained dense cores. There was no proliferation of the nuclear envelope and enveloped virus particles were rarely found within the EC cytoplasm. Multiple samples and serial sections confirmed that this paucity of virus was not a sectioning or sampling artefact.

Isolation of the KOSEC mutant virus

We set out to isolate a mutant of the KOS strain of HSV-1 able to replicate efficiently in EC cells. To enrich for such virus, a culture of O1A1 cells was infected at an m.o.i. of 0.01 and the virus was harvested from the culture at 48 h post-infection. Virus was passaged five times in this way. After the third passage, the virus yield started to increase and after the fifth passage the virus was cloned and called KOSEC. Stocks of KOSEC were prepared in Vero cells. The KOS and KOSEC strains were compared for virus yield during one infectious cycle in Vero, mouse RAP1 fibroblast, and O1A1 cells. The results (Fig. 5) show that both KOS and KOSEC viruses replicated equally well in Vero and mouse fibroblast cells (Fig. 5a, b), but that the KOSEC strain yielded 10 times more infectious particles than the KOS strain following infection of O1A1 cells (Fig. 5c).

Electron microscopy indicated that the KOSEC-infected O1A1 cells contained more maturing virions and more electron-dense viral material in their nuclei than did EC cells similarly infected with KOS HSV-1 (data not shown). Some of the KOSEC capsids contained dense cores whereas such cores were rare in capsids in KOS-infected cells. In both cases, few enveloped virions were found in the cytoplasm, indicating that maturing virus particles pass rapidly through the cytoplasm into the extracellular space.
Macromolecular synthesis in KOSEC-infected cells

We determined the levels of TK mRNA and of viral DNA in Vero and O1A1 cells infected with either KOS or KOSEC virus (Fig. 6). The TK mRNA was first detected at 6 h post-infection in both cell types infected with either virus. However, the KOSEC-infected cells accumulated TK mRNA in increasing amounts until, by 12 to 15 h post-infection, the levels of TK mRNA were five- to tenfold higher than those in the KOS-infected cells. This enhanced synthesis of TK mRNA from KOSEC occurred in both cell types.
Restricted growth of HSV-1 in EC cells

Fig. 5. The KOSEC strain of HSV-1 replicates efficiently in EC cells. Cultures of Vero (a), RAP1 (b) and O1A1 (c) cells were infected with the KOS (○) or the KOSEC (●) strains of HSV-1 at m.o.i. 3. At intervals post-infection, samples of infected cells were harvested and the cell-associated virus was titrated in monolayers of Vero cells.

Fig. 6. KOSEC-infected EC cells synthesize more TK mRNA and more viral DNA than do KOS-infected cells. Cultures of Vero (lanes a to d) and O1A1 cells (lanes e to h) were infected with the KOSEC (lanes a, c, e, g) or the KOS (lanes b, d, f, h) strains of HSV-1 at m.o.i. 3. At 3 h intervals, samples of $10^5$ cells were harvested, dissolved in NaI (Bresser et al., 1983a, b) and spotted onto nitrocellulose filters under conditions allowing for specific retention of DNA (lanes a, b, e, f) or RNA (lanes c, d, g, h). Filters were probed with nick-translated $^{32}$P-labelled pX-1 plasmid DNA (Cheng & Praszkier, 1982), washed, and exposed to X-ray film.

To determine whether the KOSEC-infected cells synthesized elevated levels of the TK protein, the TK activities present in infected TK$^-$ cells were assessed. KOSEC-infected EC cells contained two to three times more TK activity than KOS-infected cells (Fig. 7a). The TK activity was viral in origin because antiserum to HSV-1 TK was able to abolish more than 80% of the activity (Fig. 7b).
Fig. 7. KOSEC-infected EC cells produce more TK enzyme activity than do KOS-infected EC cells. (a) Cultures of P19(TK-) cells were infected with the KOS (○) or the KOSEC (△) strains of HSV-1 at m.o.i. 3. At various times after infection, cells were harvested and extracts were assayed for TK enzyme activity (Campione-Piccardo & Rawls, 1981). (b) Extracts from KOSEC-infected P19(TK-) cells were prepared as in (a) and incubated with preimmune (○) or immune anti-TK antiserum (△) before assay of TK activity.

Fig. 8. The HSV-1 virion glycoprotein, gD, is synthesized at the same rates in KOS- and KOSEC-infected cells. Vero (lane a), RAP1 (lanes b, c), and O1A1 (lanes d, e) cells were infected at m.o.i. 3 with the KOS (lanes a, b, d) or the KOSEC (lanes c, e) strains of HSV-1 and cultures were labelled for 4 h with [35S]methionine starting at 20 h post-infection. Cell extracts were immunoprecipitated with 18betaB3 (Balachandran et al., 1982) which reacts with gD. Immunoprecipitated material was resolved on 15% SDS-polyacrylamide gels. Mol. wt. standards are indicated.

Another virus-encoded protein synthesized both early and late during infection is the gD glycoprotein of HSV-1 which is recognized by the monoclonal antibody 18betaB3 (Balachandran et al., 1982). [35S]Methionine-labelled proteins from KOS- and KOSEC-infected cultures were immunoprecipitated with the 18betaB3 antibody and the precipitates were analysed by SDS-PAGE. The infected Vero and O1A1 cell samples contained a single protein of about 55 000 mol. wt., whereas the mouse fibroblast cells yielded two protein bands of higher intensity (Fig. 8). Balachandran et al. (1982) have previously noted that rodent cells synthesize higher levels of gD than do monkey cells. The KOS and KOSEC strains yielded similar amounts of gD in EC and mouse fibroblasts, indicating that this viral protein was not synthesized at elevated rates in KOSEC-infected cells.

The amount of HSV-1 DNA in KOS- and KOSEC-infected cells was measured by the dot blot procedure. The latter cells synthesized five to ten times more DNA than did cells infected with the KOS strain, and this enhanced DNA replication also occurred in both Vero and O1A1 cells (Fig. 6).

To assess the synthesis of the major late proteins, O1A1 and RAP1 cells were infected with the KOSEC or KOS viruses and pulse-labelled for 4 h with [35S]methionine starting at 9, 16 and 20 h post-infection. The labelled proteins were separated by SDS-PAGE and representative autoradiograms are shown in Fig. 9 and 10. These gels of high acrylamide concentration (15%) did not resolve all of the high molecular weight viral proteins. Some of the virus-induced protein bands were unique to each cell type probably due to differences in post-translational
Restricted growth of HSV-1 in EC cells

Fig. 9. Shutdown of host cell protein synthesis and induction of viral protein synthesis occurs earlier in KOSEC-infected cells than in KOS-infected cells. EC cells and mouse fibroblasts were infected with KOSEC or KOS and exposed to [35S]methionine-containing medium for 4 h starting at 9 h or at 16 h post-infection. Lane (a), uninfected O1A1 cells; lane (b), KOSEC-infected O1A1 labelled at 9 h; lane (c), KOSEC-infected RAP1 cells labelled at 9 h; lane (d), KOSEC-infected O1A1 labelled at 16 h; lane (e), KOSEC-infected RAP1 labelled at 16 h; lane (f), KOS-infected O1A1 labelled at 9 h; lane (g), KOS-infected RAP1 labelled at 9 h; lane (h), KOS-infected O1A1 labelled at 16 h; lane (i), KOS-infected RAP1 labelled at 16 h. Mol. wt. standards are indicated.
Fig. 10. The major late proteins from KOS- and KOSEC-infected cells are similar in infected O1A1 and RAP1 cells. Cells were infected as described for Fig. 9 and labelled with $[^{35}S]$methionine for 4 h, from 20 to 24 h post-infection. The proteins were electrophoresed as described in Fig. 9. Lane (a), uninfected O1A1 cells; lane (b), KOS-infected Vero cells; lane (c), KOS-infected O1A1 cells; lane (d), KOSEC-infected O1A1 cells; lane (e), KOS-infected RAP1 fibroblasts; lane (f), KOSEC-infected RAP1 fibroblasts. Mol. wt. standards are indicated.

modifications of these proteins resulting from differences in host cell species and cell type (Balachandran et al., 1982). Nevertheless, proteins bands from cells infected with either the KOS or the KOSEC virus were virtually identical for a given cell type (Fig. 10). It seems unlikely, therefore, that an absolute block in the synthesis of any of the major late proteins could account for the differences in virus yield between differentiated and undifferentiated cells or between KOS- and KOSEC-infected cells.

The rates at which host protein synthesis was shut down and virus-induced proteins appeared were faster in KOSEC- than in KOS-infected cells. This is consistent with the elevated synthesis of early proteins by the KOSEC virus. It is also interesting to note that the appearance of virus-
Co-infection of KOSEC and KOS strains of HSV-1 indicates that the KOSEC strain carries a recessive mutation(s). (a) Cultures of O1A1 cells were infected at m.o.i. 3 with KOS(PAr) or the KOSEC strain of HSV-1 (open bars) and the virus yields were titrated on Vero cells. A parallel culture was co-infected with both viruses (m.o.i. 3 for each virus) and the yields of viruses were titrated on Vero cells in the presence or absence of PA to determine the proportion of KOS(PAr) and KOSEC(PAr) virus yields (stippled bars). (b) Cultures of P19(TK^-) cells were infected at m.o.i. 3 with the KOS strain (first open bar), the KOSEC strain (second open bar), or both strains of HSV-1 (stippled bar). At 7 h post-infection the TK activity in cell extracts was determined.

Specific proteins in KOS-infected O1A1 (Fig. 9, lanes f and h) was slower than in KOS-infected fibroblasts (lanes g and i), but the appearance of viral proteins in both cell types was similar (and faster) in KOSEC-infected cells (lanes b to e).

Co-infection of EC cells with KOSEC and KOS viruses

To determine whether the mutation(s) in the KOSEC virus was able to complement the defect in the KOS virus, we performed experiments in which the two virus strains were used to co-infect the same EC cells. In the first experiment (Fig. 11a), EC cells were infected with the KOSEC strain along with a variant of the KOS strain, called KOS(PAr), which carried a mutation in the DNA polymerase gene making that virus resistant to phosphonoacetic acid (PA^r). Following infection (m.o.i. 3 for each virus), either alone or together, the yield of virus was assessed in the presence and absence of PA. The KOSEC(PAr) yield was more than tenfold higher than the yield of KOS(PAr^r) when EC cells were infected with one virus strain only. Following the mixed infection, both viruses replicated at the low level characteristic of the parental KOS strain. Thus the elevated viral yield from the KOSEC strain behaved as a recessive characteristic.

In a second co-infection experiment (Fig. 11b), TK activity was measured following co-infection of EC cells with KOS and KOSEC. The TK activity in co-infected cells was as low as in KOS-infected cells, indicating that the enhanced synthesis of TK in KOSEC-infected cells also behaved as a recessive characteristic.

DISCUSSION

EC cells infected with the KOS strain of HSV-1 yielded 100- to 1000-fold fewer infectious virus particles than infected fibroblast cells. EC-derived differentiated cells supported the efficient replication of HSV-1, but the ability to replicate HSV-1 appeared relatively late during the course of drug-induced differentiation. Infected EC cells synthesized viral TK, gD and
DNA at levels 30 to 100\% those of infected fibroblast cells but very few intranuclear viral components were evident by electron microscopy, suggesting that the major restriction to HSV-1 replication in EC cells occurred at a late stage in the infectious cycle.

It has been reported that an unspecified strain of HSV-1 efficiently replicated in the F9 line of EC cells (Dutko & Oldstone, 1981; Oldstone et al., 1980). In our experiments the KOS strain of HSV-1 did not replicate efficiently in any of the EC cells tested including the F9 line. The apparent discrepancy between these reports and our results may be due to the different abilities of different HSV-1 strains to replicate in EC cells. For example, different wild-type and mutant strains of polyoma virus replicate differently in EC cells (Georges et al., 1982; Levine, 1982).

We isolated a mutant strain of HSV-1 (the KOSEC strain) which replicates as efficiently in EC cells as in mouse fibroblast cells. In EC as well as fibroblast cells infected with the KOSEC virus, we found elevated levels of synthesis of one early (beta) gene product (TK) but normal levels of another (gD). Viral DNA replication was also elevated. Both unique phenotypes of the mutant strain, elevated virus yield and elevated levels of synthesis of viral TK and DNA, appeared to be genetically recessive characteristics in co-infection experiments. The gene(s) harbouring the mutation(s) in the KOSEC strain has not yet been identified, but the characteristics of this viral strain suggest that the affected gene may be one whose gene product is required for suppressing transcription of some beta genes.

If the ability of KOSEC to replicate in EC cells is related to the enhanced synthesis of viral TK mRNA and DNA, it is not immediately obvious how elevated synthesis of early viral proteins could increase the efficiency of virus replication in EC cells where the block appears to occur late during the viral infectious cycle. However, there is at least one example of a host range mutant of polyoma virus in which the mutation is present within an early polyoma gene, but the block to virus replication appears late in the infectious cycle during virion assembly (Garcea & Benjamin, 1983). Apparently the defect in polyoma viral assembly resulted from the inability of the mutant early gene product to induce appropriate post-translational modifications of a late viral protein.

Although HSV-1 has a very broad host range, there have been a number of reports of restricted replication of HSV-1 in primary cultures of certain cell types and in certain cell lines. In many cases (Lesso et al., 1983; Levine et al., 1980; Linnnavuori & Hovi, 1983; Tucker & Docherty, 1975; Yura & Sato, 1983) the reduced viral yield appeared to result from a block or restriction early in the infectious cycle which severely reduced the amounts of viral DNA replication and of late protein synthesis. In other cases, such as infection of certain Chinese hamster cell lines (Campadelli-Fiume et al., 1980) and human (Daniels et al., 1978) and mouse (Stevens & Cook, 1971) peripheral blood monocytes, the infectious cycle proceeded normally and the reduced virus yield resulted from inefficient viral maturation. These latter observations resembled what we observed in EC cells.

It is interesting to note that an HSV-1-permissive cell became restrictive following adenovirus transformation (Tucker & Docherty, 1975). The adenovirus early gene E1A is important in cell transformation induced by adenovirus. Apparently EC cells express a cellular gene with E1A-like activity (Imperiale et al., 1984) and it is possible that this activity may contribute to the restricted HSV-1 replication in the adenovirus-transformed cells and in EC cells because the E1A proteins can increase (Imperiale et al., 1985) or decrease (Borrelli et al., 1984) the expression of viral genes.

Many DNA viruses replicate inefficiently in EC cells but efficiently in their differentiated derivatives. For polyoma virus (Swartzendruber & Lehman, 1975) and human (Gonczol et al., 1984) and mouse (Dutko & Oldstone, 1981) cytomegaloviruses, the viral replication is completely blocked in EC cells and neither viral proteins nor DNA is synthesized. For adenovirus (Cheng & Praszker, 1982; Kelly & Boccara, 1976) and HSV-1, synthesis of many viral macromolecules occurs but few infectious particles emerge from infected EC cells. It has been possible to isolate mutant polyoma virus HSV-1 strains able to overcome the blocks to viral replication. It may be possible to use these viral mutants to identify the cellular functions responsible for the restriction of viral replication.
Restricted growth of HSV-1 in EC cells

We thank Dr W. E. Rawls for the KOS strain of HSV-1 and for the 18betaB3 antibody, Dr J. R. Smiley for the pX-1 plasmid, Dr S. Bacchetti for the LTK- and 143 cells, and Dr W. Summers for anti-TK antibody. This work was supported by grants from the Medical Research Council of Canada and the National Cancer Institute of Canada. J.C.-P. was supported by an MRCC post-doctoral fellowship and M.W.McB. is a research associate of the NCIC.

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(Received 23 April 1986)