Complementation of a Herpes Simplex Virus Type 1 Vmw110 Deletion Mutant by Human Cytomegalovirus

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SUMMARY

The herpes simplex virus type 1 (HSV-1) mutant dl1403 contains a 2 kb deletion within the sequences encoding the immediate early polypeptide Vmw110. Previous experiments showed that although dl1403 exhibits normal patterns of gene expression following infection at an m.o.i. of 5 p.f.u./cell its growth and plaquing efficiency are impaired in low multiplicity infections, particularly in human foetal lung (HFL) cells. We have now investigated the ability of two other human herpesviruses, varicella-zoster virus (VZV) and human cytomegalovirus (HCMV), to compensate for this defect at low m.o.i. in HFL cells. Co-infection with HCMV resulted in greatly increased plaque numbers and the apparent particle/p.f.u. ratios of dl1403 stocks were reduced to values similar to those exhibited by wild-type HSV-1 stocks. Complementation of dl1403 in low multiplicity infections by HCMV and VZV was also demonstrated by an increased yield of the mutant virus and an increase in synthesis of dl1403 DNA. Ultraviolet irradiation of HCMV abolished its ability to complement dl1403 and the presence of adenovirus 5 had no stimulatory effect on dl1403 DNA replication. When HFL monolayers were infected with dilutions of dl1403 stocks such that no plaques were produced, replication of the mutant virus could be induced by superinfection with HCMV 7 days after the initial infection. These results indicate that a non-lytic interaction between dl1403 and HFL cells is a more likely consequence of a low multiplicity infection than plaque formation.

INTRODUCTION

The herpes simplex virus type 1 (HSV-1) genome contains approximately 70 genes (McGeoch et al., 1988) of which five are defined as belonging to the immediate early (IE) class as a result of their ability to be transcribed in the absence of de novo viral polypeptide synthesis. The products of the IE genes are the polypeptides Vmw175 (ICP4), Vmw110 (ICP0), Vmw68 (ICP22), Vmw63 (ICP27) and Vmw12 (ICP47) (reviewed by Wagner, 1985; Everett, 1987). Two approaches have indicated that IE polypeptides have important roles in regulating the subsequent phases of viral transcription. In short-term transfection experiments the polypeptides Vmw175 and Vmw110 are potent activators of transcription from viral early promoters (Everett, 1984; O'Hare & Hayward, 1985; Gelman & Silverstein, 1985; Quinlan & Knipe, 1985). Vmw63 may also have an augmentary effect in this assay dependent upon the promoter under test (Everett, 1986). The isolation of temperature-sensitive mutants with lesions in Vmw175 and Vmw63 indicates that these polypeptides perform essential replicative functions during lytic infection of tissue culture cells. Studies with these mutants have established that Vmw175 is required for the establishment and maintenance of the early and late phases of transcription (Preston, 1979; Watson & Clements, 1980; Dixon & Schaffer, 1980) and that the function of Vmw63 is required only after the onset of early gene expression and DNA replication (Sacks et al., 1985). In contrast viable mutants with deletions in the genes encoding Vmw110, Vmw68 and Vmw12 have been isolated. Whereas deletion of the Vmw12 gene...
appears to have relatively little effect on virus replication (Longnecker & Roizman, 1986; Umene, 1986; Brown & Harland, 1987), a mutant containing a deletion within the gene encoding Vmw68 exhibits a multiplicity-dependence for growth in certain cell types (Sears et al., 1985).

The preliminary characterization of mutants containing deletions within the Vmw110 gene has recently been reported (Stow & Stow, 1986; Sacks & Schaffer, 1987). These viruses grow poorly at low m.o.i. but exhibit wild-type (wt) patterns of gene expression at high multiplicities. Although not proven, it is probable that the poor growth of the mutant virus at low m.o.i. results from inefficient activation of viral early and/or late promoters. We previously demonstrated that stocks of the deletion mutant dl1403, which contains a 2 kb deletion within both the TR1 and IR1 copies of the Vmw110 gene, exhibit much higher particle/p.f.u. ratios than stocks of the wt parental virus. Furthermore although similar numbers of plaques were obtained when wt HSV-1 was titrated on human foetal lung (HFL) and baby hamster kidney (BHK) cells, dl1403 plaqued with at least 20-fold lower efficiency on HFL cells. It was concluded that replication of dl1403 in cells infected with a low multiplicity of virus (less than one particle per cell) is probably defective and that this results in inefficient formation of plaques (Stow & Stow, 1986). In this communication we demonstrate that this defect in replication and plaque formation can be overcome by co-infection or superinfection with another herpesvirus, human cytomegalovirus (HCMV). This result demonstrates an interaction between two human herpesviruses belonging to different subfamilies, the alpha herpesvirinae (HSV-1) and the beta herpesvirinae (HCMV; Matthews, 1982).

METHODS

Cells. BHK-21 clone 13 cells were grown in Eagle's medium supplemented with 10% calf serum and 10% tryptose phosphate broth, and HFL fibroblasts (Flow Laboratories, 2002) were grown in Eagle's medium supplemented with 10% foetal calf serum.

Viruses. The mutant dl1403 which contains a 2 kb deletion within both the TR1 and IR1 copies of the Vmw110 gene has been previously described (Stow & Stow, 1986). The virus R4 was generated by repair of the dl1403 deletions by marker rescue (Stow & Stow, 1986). Stocks of these viruses were produced in BHK cells and multiplicities of infection were calculated from virus titres on BHK cell monolayers. HCMV strain AD169, provided by Ms J. Russell, was grown and titrated on HFL cells. Ultraviolet irradiation of HCMV was performed as described by Notarianni & Preston (1982) and reduced the titre by a factor of 5 x 10^3. Infections with varicella-zoster virus (VZV) were performed by the addition of trypsinized virus-infected HFL cells (4 x 10^5 cells per 35 mm plastic Petri dish) as previously described (Stow & Davison, 1986). Adenovirus 5 (Ad5) was grown and titrated in HeLa cells.

Complementation assays. Complementation experiments were performed on HFL cell monolayers (10^6 cells) infected with the stated amounts of virus in 35 mm plastic Petri dishes. Forty-five min after virus addition the monolayers were washed twice with Eagle's medium and 2 ml Eagle's medium containing 5% foetal calf serum (EFC5) was added. When HCMV was the complementing virus 10^6 uninfected HFL cells were added in the final overlay to all dishes. Five percent human serum was added to plates on which plaques were to be counted. An incubation temperature of 37 °C was used throughout. Progeny virus was harvested by scraping the cells into the growth medium followed by extensive sonication. Titration of the virus was performed on BHK cell monolayers.

Superinfection assays. HFL cell monolayers were infected with amounts of dl1403 calculated to produce <1 plaque/dish and incubated at 37 °C in EFC5. The dishes were examined for the presence of plaques; those with plaques present were discarded. The medium was replaced with fresh EFC5 4 days post-infection (p.i.). Seven days p.i. the medium was removed and the monolayers were superinfected with 0.5 p.f.u./cell HCMV. Uninfected HFL cells were added to the final overlay and plaques were counted or the progeny titrated as described above.

Analysis of viral DNA. The preparation of DNA from HFL cell monolayers and its analysis by restriction endonuclease digestion, Southern blotting and DNA hybridization were performed as described previously (Stow et al., 1983).

RESULTS

Complementation of dl1403 by HCMV

We previously showed that the particle/p.f.u. ratios of stocks of the Vmw110 deletion mutant dl1403 were much higher than those of wt HSV-1 stocks, and that the mutant virus plaqued with
Complementation of an HSV-1 mutant by HCMV

Table 1. Effect of HCMV on dl1403 plaquing*

<table>
<thead>
<tr>
<th>Virus dilution</th>
<th>HFL dl1403</th>
<th>HFL R4</th>
<th>BHK dl1403</th>
<th>BHK R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^-4</td>
<td>40, 34</td>
<td></td>
<td>301, 309</td>
<td></td>
</tr>
<tr>
<td>10^-5</td>
<td>0, 0</td>
<td></td>
<td>26, 27</td>
<td></td>
</tr>
<tr>
<td>10^-6</td>
<td>0, 0</td>
<td></td>
<td>1, 0</td>
<td></td>
</tr>
<tr>
<td>10^-7</td>
<td>67, 65</td>
<td></td>
<td>8, 9</td>
<td></td>
</tr>
</tbody>
</table>

Apparent titre:

<table>
<thead>
<tr>
<th>Particle/p.f.u. ratio</th>
<th>HFL dl1403</th>
<th>HFL R4</th>
<th>BHK dl1403</th>
<th>BHK R4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3·7 x 10^6</td>
<td>6·6 x 10^6</td>
<td>2·6 x 10^7</td>
<td>1·3 x 10^8</td>
</tr>
<tr>
<td></td>
<td>1·2 x 10^4</td>
<td>69</td>
<td>1·8 x 10^3</td>
<td>3·5 x 10^2</td>
</tr>
</tbody>
</table>

* Duplicate HFL or BHK cell monolayers were infected with 0·1 ml of the indicated dilutions of dl1403 or R4 either in the absence or presence of 0·5 p.f.u./cell HCMV. Plaques were counted 2 days p.i.

† TMTC, Too many to count.

‡ Calculated from plates containing between 20 and 200 plaques (p.f.u./ml).

§ Calculated from particle counts and the above titres. Infection with the 10^-5 dilutions of dl1403 or R4 represents the addition of 4·5 x 10^4 or 1·5 x 10^4 virus particles/10^6 cells respectively.

an efficiency at least 20-fold lower on HFL than BHK cells. These results indicated that at low m.o.i. the ability of dl1403 particles to initiate plaque formation, particularly in HFL cells, was greatly reduced (Stow & Stow, 1986). To determine whether HCMV could compensate for this defect, dl1403 and a derivative, R4, in which the deleted sequences had been restored by marker rescue, were titrated on HFL and BHK cell monolayers either in the presence of 0·5 p.f.u./cell HCMV or in its absence. Because HCMV produces an extensive c.p.e. within 2 days of infection of HFL cells, uninfected cells were added to the final overlay on each HFL dish. The monolayers were incubated at 37 °C for 2 days, fixed and stained with Giemsa and the plaques were counted. The results are presented in Table 1.

The rescued control virus, R4, produced similar numbers of plaques on BHK and HFL cells in either the presence or absence of HCMV. In contrast the presence of HCMV resulted in a five-fold increase in the number of plaques on BHK monolayers infected with a 10^-5 dilution of dl1403, and a greater than 100-fold increase in the apparent titre of dl1403 on HFL cells. HCMV alone takes 10 to 12 days to produce plaques on HFL cells and is unable to replicate in BHK cells (J. C. M. Macnab, personal communication). The non-linear relationship between dl1403 dilution and plaque number on BHK and HFL cells in the absence of HCMV is reproducibly observed and presumably occurs because the phenotypic effect of the deletion is largely overcome upon increasing the m.o.i. (Stow & Stow, 1986).

To confirm that dl1403 replication in HFL cells was enhanced in the presence of HCMV a yield experiment was performed and the virus progeny were titrated on BHK cell monolayers. Table 2 shows that when dl1403 was present at a low m.o.i. (10^-5 or 10^-4 p.f.u./cell, calculated from the titre of the stock on BHK cells) the addition of HCMV, as expected, resulted in significant increases in the virus yield. The addition of HCMV to dishes receiving a higher multiplicity of dl1403 (1 p.f.u./cell) or to cells infected with high or low multiplicities of R4 or wt HSV-1 had relatively little effect on the final yield.

The virus progeny from cells co-infected with HCMV and a low multiplicity of dl1403 exhibited the characteristic plaque morphology of the deletion mutant on BHK cells (Stow & Stow, 1986). In addition the DNA of virus from 12 plaques was analysed with restriction endonuclease BamHI and produced profiles indistinguishable (with the exception of the variably sized BamHI k joint fragment) from the previously published patterns for dl1403. Similarly, infections of BHK cells with virus from these plaques resulted in the low burst size characteristic of dl1403. The results therefore demonstrate that the replication of dl1403 in cells infected with a low multiplicity of virus can be complemented by HCMV and that the progeny
Table 2. Effect of HCMV on the yield of dl1403

<table>
<thead>
<tr>
<th>Virus II</th>
<th>Titre (p.f.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dl1403</td>
<td>1 × 10⁶</td>
</tr>
<tr>
<td>dl1403</td>
<td>1 × 10⁷</td>
</tr>
<tr>
<td>wt HSV-1</td>
<td>2.5 × 10⁵</td>
</tr>
<tr>
<td>wt HSV-1</td>
<td>1.5 × 10⁶</td>
</tr>
<tr>
<td>dl1403</td>
<td>1 × 10⁷</td>
</tr>
<tr>
<td>dl1403</td>
<td>1 × 10⁷</td>
</tr>
<tr>
<td>wt HSV-1</td>
<td>2.5 × 10⁶</td>
</tr>
<tr>
<td>wt HSV-1</td>
<td>2.5 × 10⁶</td>
</tr>
</tbody>
</table>

* Monolayers of HFL cells were infected with dl1403, R4 or wt HSV-1 at the indicated m.o.i. (p.f.u./cell, calculated from virus titres on BHK cells) in either the absence or presence of 0.5 p.f.u./cell HCMV. Virus was harvested 24 h.p.i. and titrated on BHK cell monolayers. The dl1403 inocula used for the 10⁻³ and 10⁻⁴ p.f.u./cell infections produced 1.8 x 10⁶ and 1.8 x 10⁶ virus particles/10⁶ cells. The R4 10⁻⁴ p.f.u./cell and wt HSV-1 10⁻³ p.f.u./cell infections represent 2.5 x 10⁶ and 5.1 x 10⁴ virus particles/10⁶ cells.

Table 3. Effect of u.v. irradiation of HCMV on dl1403 plaquing

<table>
<thead>
<tr>
<th>Dilution of dl1403</th>
<th>No HCMV</th>
<th>U.v.-irradiated HCMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻³</td>
<td>112</td>
<td>TMTC†</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>0</td>
<td>160</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Apparent titre (p.f.u./ml)</td>
<td>1.1 x 10⁶</td>
<td>1.5 x 10⁸</td>
</tr>
</tbody>
</table>

* Monolayers of HFL cells were infected with 0.1 ml of the indicated dilutions of dl1403 in the presence or absence of HCMV or in the presence of an equivalent amount of u.v.-irradiated HCMV. Infection with the 10⁻³ dilution of dl1403 represents the addition of 4.5 x 10⁶ virus particles/10⁶ cells. Plaques formed after incubation for 2 days at 37 °C were counted and the apparent titres calculated.

† TMTC, Too many to count.

Complementation of dl1403 by VZV and Ad5

The abilities of the human alphaherpesvirus VZV, and of Ad5 to complement dl1403 in HFL cells infected with a low multiplicity of virus were also examined. Because these viruses cause extensive c.p.e. within 2 days p.i. it was not possible to observe directly an increase in dl1403 plaque numbers. Complementation could, however, be examined at the level of dl1403 DNA.
Complementation of an HSV-1 mutant by HCMV

Table 4. Complementation of dl1403 by VZV*

<table>
<thead>
<tr>
<th>Virus I</th>
<th>Virus II</th>
<th>M.o.i</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(p.f.u./cell)</td>
<td>(p.f.u./ml)</td>
</tr>
<tr>
<td>MI</td>
<td>dl1403</td>
<td>1</td>
<td>7.5 x 10^5</td>
</tr>
<tr>
<td>VZV</td>
<td>dl1403</td>
<td>1</td>
<td>2.0 x 10^5</td>
</tr>
<tr>
<td>MI</td>
<td>dl1403</td>
<td>0.001</td>
<td>1.9 x 10^4</td>
</tr>
<tr>
<td>VZV</td>
<td>dl1403</td>
<td>0.001</td>
<td>2.1 x 10^4</td>
</tr>
<tr>
<td>MI</td>
<td>wt HSV-1</td>
<td>1</td>
<td>4.9 x 10^7</td>
</tr>
<tr>
<td>VZV</td>
<td>wt HSV-1</td>
<td>1</td>
<td>5.9 x 10^7</td>
</tr>
<tr>
<td>MI</td>
<td>wt HSV-1</td>
<td>0.001</td>
<td>4.0 x 10^7</td>
</tr>
<tr>
<td>VZV</td>
<td>wt HSV-1</td>
<td>0.001</td>
<td>1.2 x 10^6</td>
</tr>
<tr>
<td>MI</td>
<td>-</td>
<td>-</td>
<td>&lt;10</td>
</tr>
<tr>
<td>VZV</td>
<td>-</td>
<td>-</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

* Monolayers of HFL cells were mock-infected (MI) or infected with VZV. Two days later they were superinfected at the indicated m.o.i. with dl1403 or wt HSV-1 (calculated from titres on BHK cells). An m.o.i. of 0.001 represents the addition of 1.8 x 10^6 dl1403 or 5.1 x 10^4 wt HSV-1 virus particles/10^6 cells. Twenty-four hours post-superinfection the virus was harvested and titrated on BHK cell monolayers.

synthesis, and in the case of VZV which does not produce c.p.e. on BHK cells, by titrating the yields of progeny virus on these cells.

Table 4 shows the results of a yield experiment with VZV. HFL cells were either mock-infected or infected by the addition of VZV-infected cells. Two days p.i., when the infected monolayers exhibited approximately 40% c.p.e., superinfections with dl1403 or wt HSV-1 were performed. After a further 2 days the plates were harvested and virus progeny was titrated on BHK cell monolayers. The yields of dl1403 and wt HSV-1 from plates superinfected with 1 p.f.u./cell were reduced 3.5- and eightfold respectively in the presence of VZV. Similarly VZV also reduced the yield from cells infected with 0.001 p.f.u./cell wt HSV-1. In contrast the presence of VZV resulted in an approximately 10-fold increase in yield from cells superinfected with 0.001 p.f.u./cell dl1403. VZV, like HCMV, is therefore capable of complementing the growth of dl1403 following a low multiplicity infection.

To examine the effect of VZV at the level of dl1403 genome replication a similar experiment was performed with the exception that total cellular DNA was prepared from the infected HFL cells. Samples of DNA were cleaved with PstI plus SstI, the fragments were separated by electrophoresis through an agarose gel, transferred to nitrocellulose and hybridized to 32P-labelled plasmid pJR3 DNA which contains the PstI plus SstI fragment specifying the Vmw110 gene. As previously shown this fragment detects a single band generated from within both TRL and IRE and which is 2 kb smaller in dl1403 than wt HSV-1 DNA (Stow & Stow, 1986). Fig. 1(a) shows that the effect of VZV on viral DNA synthesis paralleled its effect on virus yield (Table 4). In cells infected with 1 p.f.u./cell wt HSV-1 or dl1403, or with 0.001 p.f.u./cell wt HSV-1, VZV had an inhibitory effect, but in contrast it greatly stimulated dl1403 DNA synthesis in cells superinfected with 0.001 p.f.u./cell of the mutant virus.

The effect of co-infection with HCMV or Ad5 on dl1403 DNA synthesis was similarly examined. HFL cells were infected with 0.001 p.f.u./cell dl1403 or wt HSV-1 either alone or in the presence of HCMV or Ad5, and total cellular DNA was analysed. Fig. 1(b) shows that Ad5 and HCMV both caused a slight reduction in the yield of wt HSV-1 DNA. HCMV greatly stimulated the synthesis of dl1403 DNA confirming its ability to complement the mutant virus, but the presence of Ad5 resulted in a reduction in the yield of dl1403 DNA. Ad5, in contrast to HCMV and VZV, is therefore unable to stimulate dl1403 replication in cells infected with a low multiplicity of the mutant virus.

Activation of dl1403 by superinfection with HCMV

The results presented in Table 1 indicate that it is possible to infect HFL cell monolayers with amounts of dl1403 such that even though no plaques appear on control plates significant numbers are produced in the presence of complementing HCMV. Plaques fail to appear and
Fig. 1. Effect of VZV, HCMV and Ad5 on dl1403 DNA synthesis. (a) HFL cells were initially mock-infected (−) or infected with VZV (+). After 2 days the cells were superinfected with wt HSV-1 at 0.001 (lanes 3) or 1 (lanes 4) p.f.u./cell or dl1403 at 1 (lanes 1) or 0.001 (lanes 2) p.f.u./cell or were mock-superinfected (lanes 5). Twenty-four h later DNA was prepared and samples recovered from 2 × 10^5 cells cleaved with *PstI* plus *SstI*. The fragments were separated in a 0.8% agarose gel, transferred to nitrocellulose and hybridized to nick-translated plasmid pJR3. An autoradiograph of the washed filter is shown. A and B indicate the *PstI* plus *StuI* fragments detected by the probe and originating from dl1403 and wt HSV-1 genomes respectively. (b) HFL cells were infected as follows: lane 1, dl1403 only; lane 2, dl1403 and HCMV; lane 3, dl1403 and Ad5; lane 4, wt HSV-1 only; lane 5, wt HSV-1 and HCMV; lane 6, wt HSV-1 and Ad5; lane 7, mock-infected; lane 8, HCMV only; lane 9, Ad5 only, (dl1403, 0.001 p.f.u./cell; wt HSV-1, 0.001 p.f.u./cell; HCMV, 0.5 p.f.u./cell; Ad5, 2 p.f.u./cell). DNA was prepared and analysed as in (a). The mark alongside position B in lane 1 is a blemish on the autoradiograph. In both (a) and (b) 0.001 p.f.u./cell represents the addition of 1.8 × 10^6 dl1403 particles or 5.1 × 10^4 wt HSV-1 particles per 10^6 cells.
Complementation of an HSV-1 mutant by HCMV

Table 5. Effect of HCMV superinfection on dl1403 plaquing*

<table>
<thead>
<tr>
<th>dl1403 dilution</th>
<th>Co-infection HCMV</th>
<th>MI</th>
<th>Infection 7 days p.i. HCMV</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^{-4.5}</td>
<td>286</td>
<td>0</td>
<td>66</td>
<td>0</td>
</tr>
<tr>
<td>10^{-5}</td>
<td>140</td>
<td>0</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>10^{-5.5}</td>
<td>54</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>10^{-6}</td>
<td>18</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

* HFL cell monolayers were infected with 0.1 ml of the indicated dilutions of dl1403 (infection with the 10^{-5} dilution represents the addition of 4.5 x 10^4 virus particles/10^6 cells). Co-infected plates simultaneously received either HCMV (0.5 p.f.u./cell) or uninfected medium (MI). Replicate plates were similarly either infected with HCMV or mock-infected 7 days p.i. with dl1403. Plaques formed after incubation at 37°C were counted.

Virus progeny remains undetectable by titration even if incubation of the former plates is continued for as long as 10 days. Superinfection experiments were therefore performed to determine whether HCMV could reactivate dl1403 from such plates.

Groups of four HFL plates were infected with serial 3.16-fold (0.5 log_{10}) dilutions of a stock of dl1403. Two plates from each group were either co-infected with HCMV or mock-infected and the number of plaques present after 2 days was counted. The remaining plates were incubated at 37°C. Seven days p.i. they were microscopically examined to ensure that no dl1403 plaques were present, and then either infected with HCMV or mock-infected. The number of plaques present on these plates was scored 3 days after superinfection (the extra day was allowed because dl1403 produces rather small, slowly-spreading plaques following infection of 'old' HFL monolayers).

The results are presented in Table 5. The ability of co-infected HCMV to complement dl1403 plaque formation is again apparent (a 10^{-4} dilution of dl1403 produced three plaques per HFL monolayer in the absence of HCMV). Even when the addition of HCMV was delayed for 7 days, significant numbers of plaques were obtained. The number of plaques observed on the plates which initially received the 10^{-4.5} and 10^{-5} dilutions of dl1403 were approximately 20% of the numbers obtained when dl1403 and HCMV were added simultaneously to the cells. The genomes of viruses recovered from plaques produced following superinfection with HCMV were indistinguishable, by restriction endonuclease analysis, from those of the input dl1403.

The addition of 2% human serum, containing antibodies able to neutralize HSV-1 infectivity, to the growth medium for the first 24 h after infection with dl1403, or trypsinization and replating of the HFL cells 24 h after initial infection, did not significantly alter the number of plaques obtained when monolayers were superinfected 7 days p.i. with HCMV, eliminating the possibility that plaques result from the presence of residual dl1403 inoculum. In a similar experiment HFL cells were maintained in medium containing 1 μg/ml acyclovir from the time of infection with dl1403 to inhibit any possible low level chronic infection. After 7 days the acyclovir was washed from the cells and 2 h later the monolayers were superinfected with HCMV. Similar numbers of plaques were obtained following superinfection of the acyclovir-treated plates and control plates which had not been exposed to the drug (data not shown).

The results therefore demonstrate that dl1403 genomes can reside in HFL cells for at least 1 week without giving rise to progeny virus, remaining in a state from which virus replication can nevertheless be induced by superinfection with HCMV.

DISCUSSION

Our previous characterization of the HSV-1 Vmw110 deletion mutant dl1403 demonstrated that although the Vmw110 polypeptide was not absolutely essential for virus replication, the ability of dl1403 virions to produce plaques was reduced in comparison with wt HSV-1, and that this difference was particularly marked in HFL cells (Stow & Stow, 1986). In this paper we show that in low m.o.i. infections with dl1403 the presence of co-infecting HCMV greatly increased both the number of plaques obtained and the yield of mutant virus. VZV, but not Ad5, was also able to complement the replication of dl1403 at low m.o.i.
In the presence of HCMV the observed particle/p.f.u. ratios for dl1403 stocks were decreased
to values similar to those obtained for stocks of wt HSV-1 (Table 1). This suggests that
inefficient plaque formation by dl1403 is unlikely to result from a defect in virion structure, but
rather to be a consequence of a block during replication following infection with a low
multiplicity of the mutant virus (e.g. less than one particle/cell). The observation that dl1403
progeny are not detected when monolayers are infected with amounts of virus capable of
producing plaques only in the presence of co-infecting HCMV (Table 2) excludes the possibility
that the mutant replicates in initially infected cells but fails to spread to form a plaque.

Complementation by HCMV was observed in cells both permissive (HFL) and non-
permissive (BHK) for this virus. As in many other non-permissive cell types, HCMV infection
of BHK cells is blocked at an early stage and the major IE polypeptide accumulates (LaFemina
& Hayward, 1988; J. C. M. Macnab, personal communication) suggesting that the expression of
an HCMV IE polypeptide could be responsible for the observed complementation. This idea is
attractive because, like Vmw110, HCMV IE gene products have been shown to function as
transcriptional activators in transfection experiments (Everett, 1984; Pizzorno et al., 1988).
There is no obvious amino acid sequence homology, however, between Vmw110 and the
predicted HCMV IE polypeptides, and in cells in which dl1403 is also present it is possible that
HCMV expression may proceed beyond the IE stage. Some degree of specificity is however apparent because co-infection with Ad5, in
contrast to VZV and HCMV, did not result in increased synthesis of dl1403 DNA.

To determine whether complementation by VZV and HCMV is gene specific (and if so to
identify the gene) it will be necessary to express their genes in isolation. To date we have been
unable to complement dl1403 in HFL cells using transfected DNA, although this may be a
consequence of the low transfection efficiencies obtained with these cells. In addition if intact
dl1403 DNA is used in transfections the conditions of low multiplicity infections (i.e. less than
one viral genome per cell) cannot be easily reproduced. Another possible approach may be to
examine the growth of dl1403 in cell lines expressing specific VZV and HCMV gene products.
However, the relatively inefficient complementation of dl1403 by cell lines containing the
HSV-1 Vmw110 gene (Stow & Stow, 1986; Sacks & Schaffer, 1987) suggests that this could
also be difficult.

Although we have not identified specific VZV or HCMV gene products responsible for the
complementation of dl1403 replication, it is clear that both VZV, which like HSV-1 is a member
of the subfamily *alpha*herpesvirinae, and HCMV (subfamily *beta*herpesvirinae) interact
genetically with the mutant. Few other instances of genetic interactions between different
herpesviruses have been documented. Felser et al. (1987, 1988) demonstrated that VZV but not
HCMV could complement the growth of an HSV-1 ts mutant with a lesion in the Vmw175 gene
and that complementation resulted from the expression of the VZV homologue of HSV-1
Vmw175. An example of an interaction between HSV and HCMV is provided by *in vitro* model
systems for HSV latency in which various treatments of infected cells enable infecting HSV to be
converted to a latent state. Subsequent to the establishment of latency, the latent virus can be
reactivated by superinfection not only with wt or mutant HSV, but also with HCMV (Colberg-
Poley et al., 1981; Wigdahl et al., 1982; Russell & Preston, 1986). The observation that dl1403 is
defective as a reactivating virus in this system (Russell et al., 1987) provides further evidence
that HCMV gene products are able to substitute for a defective Vmw110 polypeptide.

Experiments in which dl1403-infected monolayers were superinfected 7 days p.i. with HCMV
revealed that the mutant was able to persist in a non-replicating state and that its replication
Complementation of an HSV-1 mutant by HCMV

could be induced by HCMV. This situation bears some similarities to the in vitro latency system described above, but it should be emphasized that we did not treat the monolayers in order to inhibit viral replication, and that the cells were fully permissive for infection by wt HSV-1. A possible explanation is that blocking the entry of HSV into the lytic cycle, as a result of either appropriate treatment of infected cells or the presence of a viral mutation which precludes the expression of genes required for lytic growth, may be sufficient to allow other viral and/or cellular products to convert the genomes to a stable latent form. It is interesting to note that no viral gene product essential for the establishment of latent infection in vitro has been identified, and that the viral factors necessary (if any) include at most four of the IE polypeptides, the early polypeptide Vmw136 and possibly components of the virion (Russell et al., 1987). If a block to lytic infection is sufficient for establishment of latency it might be expected that mutants other than dl1403 would similarly persist in tissue culture cells and be recovered by superinfection. One requirement for this may be that the level of mutant virus gene expression should not prove toxic to the cell.

Irrespective of the events which lead to a non-lytic interaction between dl1403 and HFL cells it is clear from the number of plaques obtained after superinfection with HCMV that at a low multiplicity the entry of dl1403 into a 'latent' state is a much more common event than a productive infection and as such is a relevant topic for further study.

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