Primary chimpanzee skin fibroblast cells are fully permissive for human cytomegalovirus replication

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Cytomegaloviruses generally display a host range restricted to differentiated cell types from the species they infect. For human cytomegalovirus (HCMV) this has meant that with few exceptions tissue culture systems have relied on the use of primary foreskin fibroblast (HF) cells or primary human embryonic lung cells to study gene expression and virus replication functions. We have observed that primary skin fibroblast (CF) cells derived from the chimpanzee (Pan troglodytes) support the replication of a laboratory strain (Towne) of HCMV. The kinetics of gene expression of the Towne strain grown in CF or HF cells appeared to be equivalent. Titres of progeny virions grown in CF cells appeared to be reduced 10-fold relative to those of virus grown in HF cells. In contrast, replication of the Towne virus was not supported by growth in WES cells (ATCC no. CRL 1609), a chimpanzee skin fibroblast cell line transformed by an adenovirus 12–simian virus 40 hybrid. This study shows that HCMV is less parochial in its host range than previously thought.

Human cytomegalovirus (HCMV) is a herpesvirus which has long been recognized as a pathogen in humans, but was not isolated until human cells could be grown in culture (for a review see Ho, 1991). Progress in applying the full power of recombinant DNA-based technology to the study of this medically important virus has been hampered by the limited types of cells fully permissive for virus replication. In general, high titre yields of laboratory virus strains can only be obtained by infection of primary diploid fibroblast cells (LaFemina & Hayward, 1988; Kari et al., 1992; Wroblewska et al., 1981). Nevertheless, in an examination of a panel of human cell lines for the ability to sustain productive HCMV replication, Smith (1986) has shown that a human epithelial cell is as able to support HCMV replication as fibroblast cells. These data suggest that ploidy, along with the extent of differentiation, may be the predictive factors in determining cell susceptibility to HCMV. This latter observation is consistent with an earlier demonstration that the state of cellular differentiation is critical in allowing HCMV replication (Gönczöl et al., 1984). LaFemina & Hayward (1988) have surveyed a wide variety of primary, secondary and established cell lines for their ability to support HCMV replication. They demonstrated that the expression of the major immediate early (IE) gene, IE68, is variable in several transformed cell lines following infection. They were unable to detect HCMV DNA replication in fibroblasts of rodent or simian origin. Simian and murine CMV exhibit a broader host range and provide exceptions to the species specificity seen for HCMV (LaFemina & Hayward, 1988; Swinkels et al., 1984).

A group-common antigen has been defined for primate cytomegaloviruses (Weiner & Gibson, 1981), and cross-reactivity of human and chimpanzee CMV antigens has been observed by using immunofluorescence methods (Swinkels et al., 1984). In the course of studies examining the proliferative response of lymphocytes isolated from chimpanzees, we observed proliferation in response to antigens presented by HCMV. This immunological cross-reactivity prompted us to investigate whether primary chimpanzee fibroblasts would permit the replication of HCMV following infection.

We chose to infect fully permissive human foreskin fibroblasts (HF), chimpanzee fibroblasts (CF) derived from cells taken in a skin punch biopsy and WES cells (ATCC no. CRL 1609), a chimpanzee skin fibroblast cell line transformed by an adenovirus 12–simian virus 40 (SV40) hybrid (Rhim et al., 1981). Cells were infected with the Towne strain as described previously (Spaete & Mocarski, 1985), fixed in acetone and stained using immunofluorescence methods described previously (Pachl et al., 1987). Fig. 1 shows the results obtained when the three cell types were stained with murine monoclonal antibody (MAb) CH160-5, specific for
Fig. 1. Immunofluorescence analysis of the expression of the HCMV major IE gene in HF, CF and WES cells. Chamber slides were seeded with HF (a), CF (b) or WES cells (c), and infected at high multiplicity with HCMV strain Towne. At 24 h p.i. infected cells were fixed and stained with MAb CH 160-5 and fluorescein-labelled goat F(ab')2 anti-mouse IgG.

HCMV IE68 (L. Pereira, unpublished results). The anti-IE68 MAb reacted strongly with the infected cell nuclei of all three cell types. Therefore, HCMV can infect and express the major IE protein in primary CF cells and even in transformed chimpanzee skin fibroblasts.

To examine the full range of gene expression in the chimpanzee cells, HF, CF and WES cells were infected with HCMV at a multiplicity of 1 and metabolically labelled with [35S]methionine at 72 and 120 h post-infection (p.i.) as previously described (Pachl et al., 1987). The labelled infected cell protein (ICP) profiles are shown in Fig. 2 and reveal HCMV-specific ICPs in infected HF cells as expected (lanes 2 and 4) (compare with the protein profiles of mock-infected HF cells, lanes 1 and 3), but also show similar ICP bands in infected CF cells at both times (lanes 6 and 8) (compare with their mock-infected counterparts, lanes 5 and 7). In contrast, the protein profiles of infected WES cells (lanes 10 and 12) do not differ from the protein profiles of mock-infected WES cells (lanes 9 and 11). These results suggest that CF cells are capable of supporting the replication of HCMV but that WES cells do not support full HCMV gene expression.

The protein lysates prepared at 120 h p.i. were subjected to radioimmunoprecipitation with the glycoprotein B (gB)-specific MAb 15D8 (Rasmussen et al., 1985) to confirm that HCMV-specific gene products were being synthesized. Fig. 3 shows that MAb 15D8 precipitated the gp130 precursor of gB from lysates prepared from infected HF and CF cells (lanes 2 and 4) but not from the mock-infected control lysates (lanes 1 and 3). Longer exposure of this autoradiograph revealed that gB was proteolytically processed in both HF and CF cells (Spaete et al., 1988) (data not shown). Radioimmunoprecipitation with MAb 15D8 of lysates prepared from mock-infected or HCMV-infected WES cells showed no specific precipitated products (data not shown). This result suggests that the ICP bands seen in...
Fig. 3. Radioimmunoprecipitation of mock-infected or HCMV-infected cell lysates with a MAb directed to gB. HF cells (lanes 1 and 2) or CF cells (lanes 3 and 4) were mock-infected (lanes 1 and 3) or infected (lanes 2 and 4), labelled as described in the legend to Fig. 2 and at 72 h p.i. lysates were radioimmunoprecipitated with gB-specific MAb 15D8 using methods described previously (Pachl et al., 1987). Protein Mr standards are shown to the left.

1 2 3 4

200K 97K 68K 43K

the fibroblast cell profiles shown in Fig. 2 are HCMV-specific gene products. Furthermore, CF cells support the expression of a late gene product such as gB.

Having demonstrated that HCMV-infected CF cells are capable of late gene expression, production of infectious virus was tested in CF cells and compared with virus yields from permissive HF cells. Cells were infected at a multiplicity of 5 and the number of p.f.u. was determined at 1, 2, 3, 5, 7 and 9 days p.i. by plaque assay on HF cell monolayers. As shown in Fig. 4, overall virus yields from cell-free and cell-associated virus stocks prepared from CF cells were about 10-fold lower than those from stocks prepared from supernatants or lysates of infected HF cells. The amount of free or cell-associated infectious virus did not differ for either infected cell type. The c.p.e. associated with HCMV infection of CF cells became apparent faster than that associated with infection of HF cells. Consequently, by 7 days p.i. the CF cells had degenerated to a point where the virus yield was produced by fewer cells. At 9 days p.i. very few intact CF cells remained compared with intact infected HF cells (titres for CF cells not shown). The size of plaque produced by Towne virus on CF cells was smaller than that on HF cells (data not shown). This experiment shows that CF cells are fully permissive for HCMV replication.

The lymphoproliferative response of lymphocytes isolated from chimpanzees when presented with HCMV antigens prompted a consideration of the close phylogenetic relationship between the Pongidae (chimpanzee, gorilla and orang-utan) and humans (De Grouchy, 1987), and suggested that chimpanzee cells might be permissive for HCMV infection. Our experiments have demonstrated that primary skin fibroblast cultures derived from the chimpanzee are fully permissive for HCMV infection. The karyotypes of humans and chimpanzees differ by only a few well characterized chromosome rearrangements with the karyotypic consequence being a reduction in diploid chromosome number from 48 in the Pongidae to 46 in man (De Grouchy, 1987). Because the euchromatin is so highly conserved between the species, perhaps it should not be surprising that CF cells support HCMV replication. Although the titres of infectious virus released from infected CF cells were lower than those released from HF cells, this may be a reflection of a more robust c.p.e. associated with HCMV infection of CF cells. Towne virus stocks prepared from infected HF cells formed plaques on CF cells with roughly equivalent efficiency, although the plaquing efficiency on CF cells was slightly lower (about 80% of the efficiency observed on HF cells; data not shown). Finally, the size of plaque produced by the Towne virus on CF cells was much smaller than the typical Towne virus plaque on HF cells.
All of these factors may have contributed to the reduced production of virus from infected CF cells.

The adenovirus 12–SV40 hybrid used to generate the WES cells has not been well characterized. Neither adenovirus 12 nor SV40 particles can be demonstrated in WES cells examined by electron microscopy (Rhims et al., 1981). Both adenovirus and SV40 T antigen expression can be detected but the expression of other viral antigens cannot. WES cells have been shown to be of chimpanzee origin by karyological analysis, although the chromosome number is not available.

The non-permissiveness of transformed WES cells is not surprising. LaFemina & Hayward (1988) have shown previously that HP cells transformed by T antigen are not capable of IE68 expression following infection. Others have demonstrated restricted IE gene expression in human lymphocytes and monocytes due to influences other than that of T antigens (Rice et al., 1984). Our observation that IE68 can be synthesized in WES cells leads us to consider the role of host transcription factors and the transcription factor E2F should be examined in this regard (Cao et al., 1992; Faha et al., 1992).

Finally, we do not know what relevance the growth of HCMV in cultured chimpanzee fibroblasts has for predicting the susceptibility of the chimpanzee to infection by the human virus. The species specificity exhibited by HCMV has inhibited the development of vaccine strategies designed to prevent the disease associated with infection. However, it might be difficult to find a seronegative chimpanzee owing to the high rate of seropositivity reported for chimpanzees (Swinkels et al., 1992).

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References


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