Processing of human cytomegalovirus envelope glycoproteins in and egress of cytomegalovirus from human astrocytoma cells

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The synthesis of human cytomegalovirus (HCMV) envelope glycoproteins and the production of infectious HCMV in human astrocytoma and skin fibroblast (SF) cells were analysed. HCMV envelope glycoproteins synthesized in astrocytoma cells had lower Mr's than the same glycoproteins synthesized in SF cells regardless of the strain of HCMV used, showing that the differences observed were due to differences in processing by the host cell and not the strain of HCMV used. HCMV envelope glycoproteins synthesized in astrocytoma cells were found to contain less galactosamine. Moreover, when synthesized in SF cells some HCMV glycoproteins contained a protease-resistant fragment owing to the presence of a cluster of O-linked oligosaccharides on the polypeptide. This fragment was not present when these HCMV glycoproteins were synthesized in astrocytoma cells. These data suggested that HCMV glycoproteins synthesized in astrocytoma cells contain fewer O-linked oligosaccharides. In contrast, other post-translational events such as proteolytic cleavage of the HCMV gB glycoprotein and the formation of disulphide-linked complexes did occur. The virus produced in astrocytoma cells was capable of infecting SF cells, suggesting that complete O-glycosylation is not needed to produce infectious HCMV. However, astrocytoma cells were slow to release virus into the culture medium, suggesting that a fully functional Golgi network is needed for efficient egress of HCMV from the host cell.

Introduction

Human cytomegalovirus (HCMV) is a major cause of birth defects due to congenital infection and a significant cause of morbidity and mortality in immunocompromised patients (Ho, 1982). In the foetus, susceptibility of the developing central nervous system to HCMV is a primary cause of irreversible sequelae such as mental retardation and sensorineural hearing loss (Stagno et al., 1984). HCMV encephalitis has also been observed in AIDS patients, in whom both glial cells and neurons are infected (Wiley et al., 1986). Little is known about the replication of HCMV in cells of nervous tissue origin. In vitro models of HCMV brain cell infection have been established to attempt to gain a better understanding of HCMV replication in brain cells. In vitro, HCMV is capable of replicating in primary brain cells and several cell lines of nervous tissue origin (Wroblewska et al., 1981; Ogura et al., 1986; Kari & Gehrz 1986; Duclos et al., 1989; Poland et al., 1990). Primary brain cells are capable of producing infectious HCMV; however, these cells are slow to release infectious virus, 60 to 70% of infectious HCMV remaining cell-associated 9 to 11 days post-infection (p.i.). In contrast, 60 to 70% of the infectious HCMV made in fibroblast cells is released into the culture medium 9 to 11 days p.i. (Wroblewska et al., 1981). Thus, the egress of HCMV from primary brain cells appears to be impaired relative to that from fibroblast cells.

By using the inhibitors monensin (Johnson & Spear, 1982) and brefeldin A (BFA) (Whealy et al., 1991) and electron microscopy (Jones & Grose, 1988; Severi et al., 1988), it has been established that the Golgi network is important in the egress of herpesviruses from infected cells. Both monensin and BFA can inhibit the processing of herpesvirus glycoproteins in the Golgi network, but not the assembly of enveloped particles. These enveloped particles remained cell-associated and, with monensin, were infectious (Johnson & Spear, 1982).

Since the Golgi network appears to be important to the egress of herpesviruses from the host cell, it seemed possible that brain cells might be slow to release HCMV because of differences in HCMV glycoprotein processing in the Golgi network. To investigate this possibility, we examined the production of infectious HCMV and the synthesis of HCMV envelope glycoproteins in astrocytoma cells. The HCMV envelope glycoproteins analysed in this study were contained in disulphide-linked glycoprotein complexes designated glycoprotein complex I (gC-I) and glycoprotein complex II (gC-II) (Gretch
et al., 1988a). gC-I complexes are composed of multimers of the HCMV gB glycoprotein (Kari et al., 1990b). This HCMV glycoprotein was designated gB because of its similarity to the gB glycoprotein from herpes simplex virus (Cranage et al., 1986). The gB glycoprotein is synthesized as a large precursor glycoprotein which is eventually cleaved into two glycoproteins in the Golgi network of infected fibroblasts (Gretch et al., 1988c; Britt & Vugler, 1989; Kari et al., 1990b). gC-II complexes contain two groups of biochemically and immunologically distinct glycoproteins (Kari et al., 1990a). These glycoproteins have no known homology to other herpesvirus glycoproteins. The HCMV glycoproteins most extensively analysed in this study were those gC-II glycoproteins with $M_r$ of 47000 to 63000 (gp47–63). The gp47–63 glycoproteins are encoded by the HCMV HXLF gene family (Gretch et al., 1988b) and contain the highest content of O-linked oligosaccharides of all the glycoproteins found in gC-I and gC-II (Kari & Gehrz, 1988). This high content of O-linked oligosaccharides made gp47–63 useful for analysing potential differences between glycoprotein processing in the Golgi network of astrocytoma cell lines and skin fibroblast (SF) cells.

**Methods**

**Cells and virus.** Human SF cells were obtained from foreskins. The astrocytoma cell lines U-373MG and Hs683 were obtained from the ATCC. Stocks of the Toledo and Towne strains of HCMV were obtained by growth in SF cells, as previously described (Kari et al., 1986).

Monoclonal antibodies (MAb). MAbs 15F9 and 27B4 recognized gC-II disulphide-linked glycoprotein complexes and have been previously described (Kari et al., 1990a). Glycoprotein complexes containing the HCMV gB glycoprotein were immunoprecipitated with MAb 41C2 (Lussenhop et al., 1988).

Immunofluorescence and immunoprecipitation of radio-labelled HCMV glycoproteins using MAbs. Infected cells were fixed with cold acetone and methanol (50:50) and processed for indirect immunofluorescence as described (Kari et al., 1990b). Immunoprecipitations were done with [3H]GlcN (Amersham)-labelled HCMV glycoproteins. Briefly, SF or U-373MG cells were scraped from culture flasks in PBS pH 7.5, collected by centrifugation and resuspended in Tris-buffered saline (TBS) (50 mM-Tris-HCl pH 7.5, 10 mM-NaCl, 2 mM-PMSF) containing 10% NP40. Cells were extracted for 1 h with constant mixing. Insoluble material was removed by centrifugation and the supernatant was used for immunoprecipitations. Immunoprecipitations were done using biotinylated MAbs and streptavidin–agarose as described (Gretch et al., 1987).

**Plaque assay.** Cultures were infected at a multiplicity of 3. Virus was allowed to adsorb for 1 h before cultures were rinsed with PBS to remove free virus. Cultures were given fresh medium and, 3 and 7 days p.i., culture medium from infected cultures was removed, centrifuged to remove cell debris and stored in liquid nitrogen. After removal of the culture medium, infected cells were rinsed with PBS and then scraped into 1 ml of PBS. The suspected cells were stored in liquid nitrogen until use, at which time they were frozen and thawed twice to release intracellular virus. Virus titre (p.f.u./ml) was determined as previously described (Lussenhop et al., 1988) using confluent monolayers of SF cells.

**SDS–PAGE and fluorography.** Radioactively labelled HCMV glycoproteins were separated by SDS–PAGE in 9% polyacrylamide gels following the method of Laemmli (1970). Radioactivity in gels was detected by fluorography using EN3HANCE (New England Nuclear).

**Purification of gC-II glycoproteins.** gC-II complexes from SF or U-373MG cells were immunoadfinity-purified using biotinylated MAb 15F9 and streptavidin–agarose as described above. Immunoadfinity-purified gC-II complexes were reduced with DTT and alkylated with iodoacetamide as described (Kari & Gehrz, 1988). Individual gC-II glycoproteins were separated by gel filtration HPLC using coupled TSK 3000 and 4000 columns (Toyo Soda). HPLC was done at a flow rate of 0.3 ml/min using a buffer of 10 mM-Tris–HCl pH 7.8 containing 0.1% SDS. The eluate was monitored for radioactivity.

**Pronase digestion, G-50 chromatography and lectin chromatography.** Pronase digestion and G-50 column chromatography were done as described (Kari & Gehrz, 1988). Agarose–wheatgerm lectin (WGA) and agarose–peanut lectin (PNA) were obtained from United States Biochemical and Helix pomatia lectin–Sepharose (HPA) was obtained from Pharmacia. WGA and PNA affinity chromatography (Kari & Gehrz, 1988) and HPA affinity chromatography (Lundstrom et al., 1987a) were done as described. Briefly, glycopeptides obtained by pronase digestion were adsorbed to 10 ml affinity columns and the columns were washed with 15 column volumes of PBS. Bound glycopeptides were eluted with the appropriate sugars as indicated in Fig. 6.

**Determination of hexosamine content.** Hexosamine content was determined as previously described (Kari & Gehrz, 1988).

**Results**

**Analysis of infected cells for gC-II glycoproteins by immunofluorescence, and for production and release of infectious HCMV**

The intracellular localization of gC-I and gC-II glycoproteins was determined by indirect immunofluorescence using fixed permeabilized cells. Cells were infected with HCMV Towne strain at a multiplicity of 3 and were fixed 3 days p.i. with cold acetone:methanol (50:50, v/v). Cultures were processed for indirect immunofluorescence using MAbs specific for gC-II glycoproteins. When this was done, a bright spot of perinuclear immunofluorescence was detected in many U373, Hs683 and SF cells (Fig. 1a, b and c). The staining detected was typical of proteins localized to the Golgi network (Donaldson et al., 1990). Similar results were obtained with MAbs specific for gC-I (data not shown).

Production of infectious virus was determined for Hs683, U-373MG and SF cells as described in Methods. The number of infectious units generated by each cell type was determined by plaque assay using SF cells. At 3 days p.i., 2% of the infectious virus was found in the culture medium from infected U-373MG cells and 23%
HCMV glycoprotein synthesis

by 7 days p.i. (Fig. 1d). With Hs683 cells, 2% was found in the culture medium at 3 days p.i., but this increased to 41% by 7 days p.i. (Fig. 1e). With SF cells, 42% of the infectious HCMV had been released from the cells at 3 days p.i. and 88% by 7 days p.i. (Fig. 1f). These data show that HCMV was released most efficiently from SF cells, less efficiently from Hs683 cells and least efficiently from U-373MG cells. SF cells also produced more virus in total than either astrocytoma cell line, but the virus produced by astrocytoma cells was capable of infecting SF cells.

Analysis of HCMV gB synthesis in astrocytoma cells

Since drugs that interfere with herpesvirus glycoprotein processing in the Golgi network have also been shown to inhibit their egress from the infected cell (Johnson & Spear, 1982; Whealy et al., 1991), we compared the HCMV glycoproteins synthesized in SF and U-373MG cells to determine whether there was any difference in how astrocytoma cells process HCMV glycoproteins. U-373MG cells were used because they were the slowest to release HCMV into the culture medium. Studies were initiated by analysis of the HCMV gB glycoproteins. To do this, infected cells were incubated with [3H]GlCN to label glycoproteins. Infected cells were harvested and extracted with NP40, insoluble material was removed by centrifugation and the supernatant was used for immunoprecipitations using a gB-specific MAb. The M_r of the gB glycoproteins synthesized in SF and U-373MG cells and the disulphide-linked complexes formed by gB were compared by SDS-PAGE. When isolated from SF cells the most abundant disulphide-linked complexes had M_r of 150000 and 250000 (Fig. 2a, lane 1); a minor component with an M_r of 130000 was also detected (Fig. 2a, lane 1). This component was not isolated from extracellular virus (Kari et al., 1990b) and may be a precursor to the higher M_r complexes. When isolated from U-373MG cells, complexes were obtained which had M_r less than 150000, as were complexes with an M_r of 250000 (Fig. 2a, lane 2). The component with an M_r of 130000 was also detected. The amounts of the 150000 and 250000 M_r complexes were lower relative to the 130000 M_r component, suggesting that these complexes were slower to form in U-373MG cells.

After reduction of disulphide bonds in complexes obtained from SF cells, three glycoproteins were detected in SDS-polyacrylamide gels (Fig. 2b, lane 1). These glycoproteins had M_r of 52000 (gp52), 93000 to 130000 (gp93–130) and 158000 (gp158); gp158 is a precursor which generates gp52 and gp93-130 after proteolysis.

Fig. 1. Immunofluorescence micrographs of HCMV Towne strain-infected U-373MG (a) and Hs683 (b) astrocytoma cells and SF cells (c). Cells were fixed with cold acetone: methanol (50:50) and prepared for indirect immunofluorescence using the gC-II-specific MAb 27B4. Bar markers represent 25 μM. (d, e, f) Virus production in U-373MG, Hs683 and SF cells, respectively. Bars represent the mean plus and minus the standard deviation of virus production in six separate cultures. C, Cell; S, supernatant.
Fig. 2. U-373MG and SF cells were infected with the Towne strain of HCMV. Glycoproteins were labelled with [3H]GlcN. At 7 days p.i., infected cells were collected, extracted with 1% NP40 in TBS, and insoluble material was removed by centrifugation. Extracts were immunoprecipitated with gB-specific MAb 41C2. (a) gC-I complexes containing gB examined by SDS-PAGE under non-reducing conditions. Lane 1, gC-I complexes obtained from SF cells; lane 2, gC-I complexes obtained from U-373MG cells. (b) gC-I complexes examined by SDS-PAGE under reducing conditions. Lane 1, gC-I glycoproteins synthesized in SF cells; lane 2, gC-I glycoproteins synthesized in U-373MG cells. Mrs are indicated.

(Kari et al., 1990b). Three glycoproteins were also detected in SDS–polyacrylamide gels after reduction of complexes obtained from U-373MG cells (Fig. 2b, lane 2). A 52000 Mr glycoprotein was detected, but instead of gp93–130 a 80000 to 93000 Mr glycoprotein was detected. Thus, although gB was synthesized, cleaved and assembled into disulphide-linked complexes in U-373MG cells, the Mrs of some of the glycoproteins were different.

Analysis of gC-II synthesis in astrocytoma cells

gC-II complexes were isolated and examined as described above. Disulphide-linked gC-II complexes were obtained from both SF and U-373MG cells (Fig. 3a, lanes 1 and 2), but there were differences in the Mrs of the most heavily labelled complexes. Those from SF cells had Mrs of 67000 to 93000, whereas those from U-373MG cells had Mrs of 63000 to 90000 (Fig. 3a, lanes 1 and 2).

gC-II complexes were also reduced prior to SDS–PAGE to examine individual glycoproteins. When synthesized in SF cells and labelled with [3H]GlcN, the most heavily labelled gC-II glycoproteins had Mrs of 47000 to 63000 (gp47–63) (Fig. 3b, lane 1). When synthesized in U-373MG cells, glycoproteins contained in gC-II complexes had lower Mrs than the same glycoproteins synthesized in SF cells (Fig. 3b, lanes 1 and 2). In particular, the glycoproteins from SF cells designated gp47–63 had Mrs of 33000 to 50000 in U-373MG cells. Another glycoprotein, which had an Mr of 90000 when synthesized in SF cells, was observed to have an Mr of 70000 when synthesized in U-373MG cells (Fig. 3b, lanes 1 and 2).

The experiments with gC-II were also done with the Towne strain of HCMV and similar results were obtained (data not shown), suggesting that the differences observed were due to differences in cell processing of HCMV glycoproteins and not the strain of HCMV used.

Analysis of GalN incorporation into gp47–63 synthesized in SF and U-373MG cells

When synthesized in SF cells, gp47–63 contains a high content of O-linked oligosaccharides (Kari & Gehrz, 1988). Furthermore, by using monensin to prevent post-translational processing in the Golgi network of SF cells, in which O-glycosylation occurs, the Mr of gp47–63 was reduced by 20000 (Gretch et al., 1988b). This decrease was similar to that observed when gp47–63 glycoproteins were synthesized in U-373MG cells. Taken together, these data suggest that the glycoproteins which had lower Mrs when synthesized in U-373MG cells may have contained fewer O-linked oligosaccharides. Owing to this, we analysed gC-II gp47–63 for their O-linked oligosaccharide content by comparing the hexosamine content of gC-II glycoproteins obtained from infected SF and U-373MG cells because the amount of GalN present would be an indication of the level of O-glycosylation.
HCMV glycoprotein synthesis

Infected cells were incubated with \(^{3}H\)GlcN. Under the conditions of these experiments, both GlcN and GalN are labelled because UDP-GlcNAc can be converted into UDP-GalNAc by the action of a 4-epimerase (Schachter & Roden, 1973). gC-II complexes were immunoaffinity-purified from extracts of infected SF or U-373MG cells. The purified gC-II complexes were reduced and alkylated, and individual gC-II glycoproteins were purified by gel filtration HPLC. Purified glycoproteins were digested with pronase to obtain water-soluble glycopeptides. These peptides were hydrolysed with 4 m-HCl to obtain free hexosamines, which were separated by thin-layer chromatography. When this was done, gC-II glycoproteins obtained from HCMV Towne- or Toledo-infected SF cells contained approximately twice as much radioactive GalN as gC-II glycoproteins obtained from HCMV Towne- or Toledo-infected U-373MG cells (Fig. 4). These data indicated that gC-II glycoproteins synthesized in SF cells contain more O-linked oligosaccharides than gC-II glycoproteins synthesized in U-373MG cells.

Pronase sensitivity of gC-II group I glycoproteins

We have shown previously that when gp47-63 is digested with pronase, a large fragment is obtained which is resistant to further proteolysis (Kari & Gehrz, 1988). The resistance of this fragment to proteolysis is due to the presence of a large number of O-linked oligosaccharides. If fewer hydroxyamino acids were O-
Figs. 6. The [3H]GlcN-labelled glycopeptides in the peaks near the $V_t$

near the void volume ($V_v$) of the G-50 column were collected and the glycopeptides in these

fractions were analysed by lectin affinity chromatography. HPA (a and b), PNA (c and d) and WGA (e and f) columns were used, which bind the glycopeptides Ser-GalNAc, Ser-GalNAc-Gal and Ser-GalNAc-Gal-NANA/GlcNAc, respectively. Bound glycopeptides were eluted with 0-1 M-GalNAc (a and b), 0-05 M-Gal (c and d) or 0-1 M-GlcNAc (e and f). (a), (c) and (e) are the radioactivity profiles obtained with glycopeptides from infected SF cells; (b), (d) and (f) are the profiles obtained with glycopeptides from infected U-373MG cells.

Herpes simplex virus gC-1 synthesized in C1300 neuroblastoma cells has been reported to contain O-linked oligosaccharides consisting of the monosaccharide GalNAc O-linked to hydroxamino acids. In contrast, gC-1 synthesized in green monkey kidney cells contains O-linked oligosaccharides which are tri- and tetrasaccharides (Lundstrom et al., 1987b). The differences in glycosylation of gC-1 in these two cell types were characterized using lectins (Lundstrom et al., 1987a b). Since smaller O-linked polysaccharides could account for part of the reduction in $M_t$ of gC-II gp47-63 glycoproteins in infected U-373MG cells, we used lectin columns to characterize the structure of the polysaccharides present in the $V_t$ peaks in G-50 chromatography. The glycopeptides in the $V_t$ peak most probably contained O-linked oligosaccharides because of their small $M_t$ and because they contained a large amount of GalN (data not shown). Three lectins (HPA, PNA and WGA) were used: HPA is specific for terminal N-acetylgalactosamine (Hammarstrom et al., 1977; Goldstein & Hayes, 1978); PNA has a narrow specificity for the terminal disaccharide sequence Gal(1-3)GalNAc (Goldstein & Hayes, 1978, Mansson & Olofsson, 1983); WGA has affinity for non-reducing terminal sialic acid and GlcNAc residues (Peters et al., 1979). Analysis of gC-II glycopeptides from SF or U-373MG cells showed that they have very little affinity for HPA lectin (Fig. 6a and b), suggesting that little free GalNAc O-linked to hydroxamino acids is present. In contrast, gC-II glycopeptides from infected U-373MG or SF cells have affinity for PNA and WGA lectins (Fig. 6c to f). The greatest percentage of radioactive glycopeptides obtained from either type of cell bound to WGA. These data suggest that the same oligosaccharide structures were present in glycopeptides obtained from either type of cell. Therefore, the $M_t$ differences were not due to the synthesis of smaller O-linked oligosaccharides in U-373MG cells.

Discussion

The functions of the Golgi network in the assembly and egress of herpesviruses have been studied by examining the effects of drugs such as monensin and BFA on these processes (Johnson & Spear, 1982; Whealy et al., 1991). Although these drugs have proved useful, they interfere with all processing events in the Golgi network. For example, monensin prevents the late processing of oligosaccharides on the HCMV gB glycoprotein, but also prevents the proteolytic cleavage of gB into gp52 and gp93-130 (Gretch et al., 1988c). Interference with several processing events simultaneously makes interpre-
glycosylation. We have shown previously that among disulphide-linked glycoprotein complexes gC-I and gC-II, the proteolytic cleavage of gB and the assembly of gC-I and gC-II glycoproteins, gC-II gp47-63 glycoproteins are the most heavily O-glycosylated (Kari & Gehrz, 1988). In the present study, we have shown that when these glycoproteins are synthesized in U-373MG cells, they have lower \( M_r \)s because fewer hydroxyamino acids are O-glycosylated. This conclusion is based on several observations. First, the decrease in the \( M_r \) of gp47-63 synthesized in U-373MG cells as compared to SF cells is similar to that detected when monensin was used to prevent O-glycosylation in the Golgi network of SF cells (Gretch et al., 1988b). Second, gC-II gp47-63 glycoproteins synthesized in U-373MG cells contain less \[^3H\]GalN than the same glycoproteins synthesized in SF cells. Third, gC-II gp47-63 glycoproteins synthesized in U-373MG cells lack a pronase-resistant fragment. This suggests that the hydroxyamino acids which were O-glycosylated are not clustered, but dispersed on the peptide backbone. One function that has been associated with oligosaccharides attached to proteins is protection against proteolysis (Rademacher et al., 1988). This may be one of the functions of the O-linked oligosaccharides on gC-II gp47-63 glycoproteins.

As stated above, when processed in SF cells the HCMV gB glycoprotein precursor is cleaved into gp93-130 and gp52. When synthesized in U-373MG cells, gp93-130 had a lower \( M_r \); in contrast, gp52 had the same \( M_r \) regardless of the type of cell infected. Previous analysis of these glycoproteins has shown that gp93-130 contains detectable amounts of O-linked oligosaccharides whereas gp52 does not (Britt & Vugler, 1989; Kari et al., 1990b). These results suggest that the decrease in \( M_r \) of gp93-130 is in part due to lack of O-glycosylation of this glycoprotein.

Even though several major HCMV envelope glycoproteins were not completely O-glycosylated in U-373MG cells, the virus produced in these cells was still infectious in SF cells. Thus, complete O-glycosylation is not needed to produce infectious HCMV. Infectious HCMV was also released into the culture medium from U-373MG cells, suggesting that complete O-glycosylation is not an absolute requirement for release of HCMV from infected cells. However, unlike SF cells most of the infections HCMV produced in U-373MG cells was found to be cell-associated. This result is very similar to that obtained when monensin is used to prevent the processing of HSV-1 glycoproteins in the Golgi network of infected cells, i.e. infectious virus is produced, but most of this virus remains cell-associated (Johnson & Spear, 1982). These results suggest that a fully functional Golgi network is needed for complete processing of herpesvirus glycoproteins and efficient egress of herpesviruses from the host cell.

There are several possible reasons why HCMV envelope glycoproteins are under-glycosylated in U-373MG cells. First, there may be other factors that cause transport failure of HCMV through the Golgi network of U-373MG cells, causing a decrease in the O-glycosylation of the envelope glycoproteins. Alternatively, the Golgi network of HCMV-infected U-373MG cells may O-glycosylate glycoproteins differently from HCMV-infected SF cells. For example, the inability of U-373MG cells to O-glycosylate HCMV glycoproteins completely could involve a number of different enzymes needed for O-glycosylation. The signals or amino acid sequences that identify O-glycosylation sites are not well-defined. However, in general the amino acid sequence of a protein and the capacity of a cell to glycosylate a protein determine which potential glycosylation sites are used and the structure of the polysaccharides at these sites (Datema et al., 1987). It is likely that accessibility of specific serine or threonine residues to the N-acetylgalactosaminyl transferase plays a major role in determining whether these residues become O-glycosylated. The inability of U-373MG cells to O-glycosylate HCMV glycoproteins completely may reflect differences in the accessibility of serine or threonine residues to glycosyltransferases in SF and U-373MG cells. Another possibility is that the level of N-acetylgalactosaminyl transferase activity is too low in U-373MG cells to accommodate the synthesis of HCMV glycoproteins. It has been demonstrated that HCMV can trigger or increase the activity of several glycosyltransferases in fibroblasts and teratocarcinoma cells (Andrews et al., 1989). It may be that HCMV can increase the activity of N-acetylgalactosaminyl transferase in SF cells but not in U-373MG cells. Without an increase in the level of enzyme activity, the amount of HCMV glycoproteins synthesized in an infected U-373MG cell may exceed the capacity of the cell to glycosylate these glycoproteins fully.

We would like to thank Nancy Lussenhop and Curt Nelson for technical assistance. This work was supported by a grant from the Helen Lang Trust, Biomedical Research Institute of Children’s Hospital, and grant PO 1 HD 19937 from the National Institute of Child Health and Human Development.

References


(Received 13 March 1991; Accepted 11 October 1991)