Human cytomegalovirus binding to heparan sulfate proteoglycans on the cell surface and/or entry stimulates the expression of human leukocyte antigen class I

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Human cytomegalovirus (HCMV) is known to down-regulate the expression of human leukocyte antigen (HLA) class I, the process of which involves a subset of virus genes. Infection of human foreskin fibroblast (HFF) cells with UV-inactivated HCMV (UV-HCMV), however, resulted in an increase in HLA class I presentation on the cell surface in the absence of HCMV gene expression. Heparin, which inhibits the interaction of virus particles with cell surface heparan sulfate proteoglycans (HSPGs), blocked the effect of UV-HCMV on HLA class I expression. Pretreatment of cells with heparinase I decreased in a dose-dependent manner the effect of UV-HCMV on HLA class I expression enhancement. Sodium chlorate, which is known to inhibit the sulfation of HSPGs, gave a similar result. Pretreatment of UV-HCMV with trypsin or monoclonal antibody reactive with the envelope glycoprotein gB reduced the increase in HLA class I expression on the HFF cell surface by UV-HCMV. RT–PCR analysis demonstrated that the increase in HLA class I presentation on the HFF cell surface was due to an increase in HLA class I transcription. Thus, binding of HCMV particles to cell surface HSPGs appears to be required for the stimulation of HLA class I expression. It is also possible that virus entry, in addition to binding to HSPGs, may be involved in the stimulation of HLA class I expression, since the UV-HCMV entered the cells and all treatments to block virus binding to HSPGs would necessarily prevent virus entry.

Introduction

Human cytomegalovirus (HCMV) is one of the most prevalent human pathogenic viruses. Although most of the clinical outcomes of HCMV infection are asymptomatic, HCMV can cause a variety of diseases, including cytomegalic inclusion disease, pneumonia and severe neurological anomalies in the infected foetus or neonate. In adults, HCMV can be problematic for immune-compromised patients, such as organ transplant recipients and AIDS patients, due to primary acute infection or reactivation from latency (Britt & Alford, 1996). Latency requires the virus to be persistently present within the body without being destroyed by the host immune system, which comprises the innate and adaptive immune responses. The innate immune response to virus infection is best exemplified by the production of interferon (IFN), whereas the adaptive immune response is responsible for the production of specific antibodies and for destroying virus-infected cells.

Major histocompatibility complex (MHC) antigen molecules are important parts of the adaptive immune response. Therefore, many viruses that establish persistent infections find a way to escape the adaptive immune response by down-regulating the expression of MHC molecules on the infected cell surface (Ploegh, 1998; Miller & Sedmak, 1999; Alcami & Koszinowski, 2000). This process requires the expression of virus genes. For example, HCMV encodes several genes, including US2, US3, US6 and US11, that are responsible for the destruction or impaired transport to the cell surface of the heavy chain of human leukocyte antigen (HLA) class I, a human MHC molecule (Ahn et al., 1996, 1997; Hengel et al., 1996; Jones et al., 1996; Wiertz et al., 1996; Jones & Sun, 1997). The innate immune response is also compromised by HCMV infection. HCMV inhibits IFN-α-stimulated antiviral and immunoregulatory responses by blocking multiple levels of IFN-α signal transduction (Miller et al., 1999). This process also requires HCMV gene expression. The HCMV US28 gene...
is implicated in the depletion of the chemokine RANTES from the infected cell culture (Bodaghi et al., 1998).

On the other hand, in the absence of HCMV gene expression, HCMV infection results in different effects. HCMV stimulates the expression of IFN (Zhu et al., 1998) or IFN-inducible genes (Zhu et al., 1997; Navarro et al., 1998; Boyle et al., 1999) and RANTES production is positively modulated (Michelson et al., 1997). All of these innate immune responses do not require HCMV gene expression and can be mediated by inactivated viruses and, in some cases, dense bodies (DBs) (Pepperl et al., 2000) or purified virion envelope glycoprotein B (gB) (Boyle et al., 1999). Inactivated HCMV stimulates the induction of IFN-responsive genes (Zhu et al., 1997). In these situations, virus particles are recognized by the host cell as foreign antigens. Thus, before the onset of virus gene expression, HCMV can induce a variety of cellular responses by physically interacting with the host cell.

In this study, we hypothesized that HCMV particles may affect the expression of HLA class I on the infected cell surface by binding to surface receptors and/or entering the host cell. We found (i) that UV-inactivated HCMV (UV-HCMV) stimulated the expression of HLA class I and (ii) that the physical interaction between HCMV envelope glycoprotein gB and heparan sulfate proteoglycans (HSPGs) was involved in HLA class I expression.

**Methods**

**Cells and virus.** Human foreskin fibroblast (HFF) cells (passage 8–15) were used in this study. HFF cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% foetal bovine serum (FBS) under 5% CO₂ at 37 °C. When cells reached confluency, the cell monolayer was maintained in DMEM containing 2% FBS. The Towne strain of HCMV was used to infect HFF cells. In order to obtain HCMV stocks, confluent monolayers of HFF cells were infected with HCMV at an m.o.i. of 0.01–0.05 p.f.u. per cell. Infected cells were incubated for 8–10 days in DMEM containing 2% FBS until most of the cells showed extensive cytopathic effects. Cells were then scraped into the culture medium, freeze-thawed three times and spun to remove cell debris. HCMV stocks prepared in this way had titres of 1–5 x 10⁵ p.f.u./ml.

**Inactivation of virus.** Heat-inactivation of HCMV was carried out by incubating the HCMV stock preparation at 56 °C for 30 min. UV-HCMV was prepared by irradiating HCMV with UV at a dose of 2 J/cm²; HCMV inactivated either by heat or by UV, as described above, completely lost infectivity. Serum-neutralization of HCMV was carried out by incubating HCMV for 30 min at 37 °C with an equal volume of pooled serum obtained from individuals who were HCMV sero-positive.

**Reagents.** Heparin, heparinase I and sodium chlorate were purchased from Sigma. Monoclonal antibodies (MAbs) reactive with HCMV pp72, pp65 or gB were obtained from Virogen. MAb W6/32, which is reactive with the common region of classical HLA class I (A, B and C) antigens, was obtained from Serotek.

**Indirect immunofluorescence assay.** HFF cells cultured on cover slips were infected or mock-infected with HCMV (infectious or inactivated). At appropriate times after virus infection, cells were fixed with absolute methanol at −20 °C for 10 min. After rehydration in ice-cold Tris–saline for 5 min, cells were incubated with mouse MAbs at 37 °C for 1 h in a humidified chamber. Cells were then washed three times with Tris–saline and incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin G (IgG) for 45 min. Cover slips were mounted on glass slides and examined under a fluorescence microscope (BX50F-3, Olympus Optical) with an FITC filter. Samples were photographed using a confocal microscope (MRC1024, Bio-Rad Laboratories).

**Flow cytometry.** Cells were harvested by trypsinization and washed with PBS. The number of viable cells was determined using the trypan blue exclusion assay and 10⁵ cells were transferred to a microcentrifuge tube. Cells were collected by centrifuging at 700 r.p.m. for 3 min and resuspending in 90 μl of PBS. Cells were then reacted with 10 μl of FITC-conjugated anti-HLA class I antibody (MAb W6/32) for 30 min at room temperature. Cells were washed with 1 ml of PBS and collected by centrifuging at 700 r.p.m. for 3 min. After resuspending the pellet in 200 μl of PBS, 10⁵ cells were analysed by flow cytometry (FACS Calibur-S, Becton-Dickinson).

**RT–PCR.** Total cellular RNA was extracted from 10⁶ HFF cells (mock-, HCMV- or UV-HCMV-infected) using the RNeasy RNA Extraction kit (Qiagen), according to the manufacturer’s protocol. The amount of extracted RNA was measured and cDNA was synthesized from 2 μg of extracted RNA using the Omniscript Reverse Transcription kit (Qiagen). Briefly, the reaction mixture containing 0.5 mM of each dNTP, 1 μM oligo(dT) primer, 20 U RNase inhibitor, 4 U Omniscript reverse transcriptase (RT) and 2 μg of sample RNA in 20 μl RT buffer was incubated at 37 °C for 60 min. The RT was inactivated by incubating at 93 °C for 5 min and cooling on ice for 10 min. PCR was performed in 50 μl Taq buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl and 2 mM MgCl₂) containing 1.5 μl of template cDNA, as prepared above, 0.3 μM of the HLA class I primer pair (forward, 5’-GATTTCCCCCAGCCGCGAG, and reverse, 5’-CTGCGAGTGTGTCATCTC), 0.2 mM of each dNTP and 5 μl Taq polymerase, based on the method described by Johnson et al. (2000). The reaction was incubated in a Primus96 Plus thermocycler (MWG Biotech) using the hot-start method of 1 cycle at 94 °C for 1 s, followed by 28 cycles at 60 °C for 30 s, 72 °C for 1 min and 94 °C for 30 s and then 1 cycle at 72 °C for 10 min. PCR products were analysed by 1% agarose gel electrophoresis. Gels were stained with ethidium bromide, visualized by a short-wave UV transilluminator and photographed.

**Results**

**UV-HCMV stimulated the cell surface expression of HLA class I**

HFF cells were infected with HCMV and the expression of HLA class I on the surface of virus-infected cells was analysed by flow cytometry using MAb W6/32. The mean fluorescence (MF) of mock-infected HFF cells was 130. The HFF cell population was divided into three subpopulations: one with a low level of fluorescence, one with a high level of fluorescence and one with a mid-range level of fluorescence [Fig. 1a (i)]. The upper and lower limits were determined so that most of the cells (95%) in the control set could fall between the limits. Cells with a high level of fluorescence express increased amounts of
HCMV and HLA-I expression

Fig. 1. UV-HCMV stimulates the presentation of HLA class I molecules on the cell surface. (i) For flow cytometry, HFF cells were infected with either infectious HCMV or inactivated HCMV at an m.o.i. of 3 p.f.u. per cell or equivalent. At 24 h post-infection, $10^5$ cells were collected and reacted with FITC-conjugated anti-HLA class I antibody (MAb W6/32) for 30 min at room temperature. Cells were washed with PBS and collected by centrifugation. After resuspending the cell pellet in PBS, cells were analysed by flow cytometry. (ii, iii) For the immunofluorescence assays, HFF cells were grown on cover slips, infected with HCMV, fixed with ice-cold methanol and incubated with mouse MAbs reactive with HCMV immediate-early gene product pp72 or lower matrix protein pp65 for 45 min at $37\,^\circ\mathrm{C}$. After washing with PBS, cells were reacted with FITC-conjugated sheep antibody to mouse IgG for 30 min at $37\,^\circ\mathrm{C}$.

HLA class I and cells with a low level of fluorescence express decreased amounts of HLA class I. As expected, HCMV infection resulted in a decrease of cell surface HLA class I [Fig. 1b (i)]. Although the MF decreased slightly from 130 to 119, the fraction of HFF cells with a low level of fluorescence increased from 2-5 to 30%. When HFF cells were infected with UV-HCMV, however, the expression of HLA class I increased (MF = 233) and the percentage of HFF cells expressing a high level of fluorescence increased to 30-7% [Fig. 1c (i)]. The immunofluorescence assay using the MAb reactive with the HCMV major immediate-early gene product pp72, MAb anti-pp72, indicated that HCMV gene expression was observed in HCMV-infected HFF cells, but not in HFF cells infected with UV-HCMV [Fig. 1b, c (ii)]. Penetration of virus particles into the cells, however, was observed with infectious HCMV or UV-HCMV when infected cells were stained at 2 h after virus infection with the MAb reactive with the HCMV lower matrix protein pp65, MAb anti-pp65 [Fig. 1b, c (iii)].
The purified virion component of UV-HCMV was responsible for the increase in HLA class I expression. UV-HCMV stock was centrifuged at 20,000 g for 1 h at 4 °C. The virus pellet and virus-free supernatant were harvested separately and used to infect HFF cells. The virus-free supernatant did not contain infectivity as it failed to produce HCMV plaques when inoculated onto HFF cell monolayers. Flow cytometry using MAb W6/32 was performed 24 h later. (a) Mock-infected cells, (b) UV-HCMV-infected cells, (c) cells infected with the supernatant fraction of UV-HCMV, (d) cells infected with the pellet fraction of UV-HCMV and (e) cells infected with reconstituted UV-HCMV containing the supernatant and pellet fractions were analysed.

When HFF cells were infected with HCMV inactivated by heat (Fig. 1d) or neutralized by pooled sero-positive serum (Fig. 1e), the expression of HLA class I on the cell surface was not significantly affected. Neither HCMV gene expression nor binding of HCMV particles to the cell surface and penetrating into the cells was observed in HFF cells infected with HCMV inactivated by either heat or serum.

Since the virus stock contained both virus particles and cell lysate, we tested if the enhancement of HLA class I expression was mediated, at least in part, by soluble factors present in the cell lysate. UV-HCMV stock preparation was spun and the pellet containing the virus particles was separated from the supernatant containing the cell lysate. Cell lysate (supernatant) alone did not affect the expression of HLA class I and the purified UV-HCMV preparation free from the cell lysate stimulated the HLA class I expression on HFF cells to a level similar to that seen with HFF cells infected with UV-HCMV stock (Fig. 2). Thus, it appears that the enhancement of HLA class I expression was due to the virus particles. In order to demonstrate further the effect of UV-HCMV particles on HLA class I expression, HFF cells were infected with different amounts of UV-HCMV and the expression of HLA class I on the surface of HFF cells was examined. As shown in Fig. 3, the enhancement of HLA class I expression was dependent on the amount of UV-HCMV used for infections. Further experiments were performed with UV-HCMV at an m.o.i. of 3, unless otherwise stated.

**Involvement of HCMV gB**

The data shown above suggest that the physical interaction of UV-HCMV and HFF cells might be responsible for the stimulation of HLA class I expression on the cell surface. Previous studies have proposed that the initial interaction of HCMV with permissive cells occurs between viral envelope glycoproteins and HSPGs on the cell surface. To determine whether HCMV glycoproteins were involved in the stimulation of HLA class I expression by UV-HCMV, UV-HCMV was incubated with trypsin for 10 min to remove its envelope glycoproteins. Trypsin treatment inhibited the increase in HLA class I expression by UV-HCMV in a dose-dependent manner (Fig. 4a). Next, MAb anti-gB was used to block the specific interaction of gB and HFF cells. Purified UV-HCMV was resuspended in serum-free media and incubated with serial dilutions of MAb anti-gB for 1h. As a control, the same amount of non-specific isotype control antibody (in this experiment mouse IgG1) was used in parallel. Non-specific antibody did not affect the expression of HLA class I on HFF cells infected with UV-HCMV. On the other hand, MAb anti-
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Fig. 4. Involvement of gB in the stimulation of HLA class I expression by UV-HCMV. Purified UV-HCMV particles (20,000 g for 1 h at 4°C) were pre-incubated with (a) trypsin for 10 min or (b) mouse MAb anti-gB or mouse IgG1 (as an isotype control) for 1 h. Trypsin was inactivated and HFF cells were infected with UV-HCMV and the surface expression of HLA class I was examined 24 h later using MAb W6/32. Filled bars show the per cent increase in MF in mock-infected, treated cells measured against untreated cells. Open bars indicate the per cent increase in MF in UV-HCMV-infected, treated cells measured against mock-infected, treated cells.

gB reduced the effect of UV-HCMV on the increase in HLA class I expression in a dose-dependent manner (Fig. 4b).

Involvement of HSPGs on the HFF cell surface

HCMV envelope glycoproteins have been known to interact with HSPGs on the cell surface and heparin is expected to competitively inhibit the effect of UV-HCMV on HLA class I expression. Heparin alone did not affect HLA class I expression on mock-infected cells. On the other hand, the levels of HLA class I on the surface of HFF cells infected with UV-HCMV were significantly decreased in a dose-dependent manner following treatment with soluble heparin (Fig. 5a). The increase in HLA class I expression on the cell surface by UV-HCMV was almost completely blocked by heparin, even at 100 µg/ml, the highest concentration tested in this study. With lower amounts of UV-HCMV (m.o.i. of 1), almost 90% inhibition was observed when 10 µg/ml of heparin was used (data not shown). The immunofluorescence assay using MAb anti-pp65 demonstrated that heparin prevented UV-HCMV from binding to the HFF cell surface and penetrating into the cells (Fig. 5b). Heparin seemed to block the HLA class I-enhancing activity of UV-HCMV by physically hindering the interaction of the virus particle with cellular HSPGs.

Further experiments were carried out to exploit the involvement of HSPGs in the effect of UV-HCMV on HLA class I expression. Treatment of HFF cells with heparinase I for 1 h prior to virus infection to remove cellular HSPGs diminished the effect of UV-HCMV on cell surface HLA class I expression in a dose-dependent manner (Fig. 6a). Heparinase I at the concentrations used in this study did not significantly affect the expression of HLA class I on uninfected cells. Sodium chlorate is known to affect the sulfation of HSPGs. At a concentration of 50 or 100 mM, sodium chlorate slightly increased the expression HLA class I on HFF cells. The expression of HLA class I on HFF cells infected with UV-HCMV, however, was decreased in a concentration-dependent manner when HFF cells were treated with sodium chlorate for 72 h prior to virus infection (Fig. 6b).
Involvement of HSPGs in the stimulation of HLA class I expression by UV-HCMV. HFF cells were incubated with (a) heparinase I for 10 min or (b) sodium chlorate for 72 h prior to UV-HCMV infection. Cells were washed and infected with UV-HCMV at an m.o.i. of 3 p.f.u. per cell. The surface expression of HLA class I was examined at 24 h after infection using MAb W6/32. Filled bars indicate the per cent increase in MF of mock-infected, treated cells measured against untreated cells. Open bars indicate the per cent increase in MF of UV-HCMV-infected, treated cells measured against treated, mock-infected cells. Definition of heparinase unit: according to the manufacture, 600–800 units of heparinase (Sigma) is equivalent to 1 IU. Therefore, 10 units/ml, the highest concentration used in this study, is about 12.5–16 mIU/ml.

Stimulation of HLA class I gene transcription by HCMV infection

The molecular basis for the increase in HLA class I expression on the surface of HFF cells infected with UV-HCMV, as described above, was examined by RT–PCR using a primer pair specific for the HLA class I common region (Johnson et al., 2000). Analysis of the RT–PCR products after 28 cycles of amplification demonstrated that the transcription of HLA class I in HFF cells was stimulated by infection with infectious HCMV or UV-HCMV (Fig. 7, lanes 2 and 3). On the other hand, treatment with heparin (100 µg/ml) blocked the HCMV- or UV-HCMV-induced increase in HLA class I gene transcription to a level similar to that seen in mock-infected cells (Fig. 7, lanes 4–6). Therefore, the increase in HLA class I expression on the surface of UV-HCMV-infected HFF cells appears to result from the stimulation of HLA class I gene transcription.

Discussion

Our study demonstrates for the first time that physical interaction between HCMV particles and cellular HSPGs stimulates the expression of HLA class I molecules on the cell surface. It is well-known that infection of cells with HCMV results in the down-regulation of HLA class I presentation on the infected cell surface (Wiertz et al., 1997; Hengel et al., 1998; Alcami & Koszinowski, 2000). A subset of virus genes are involved in this process. Initial interaction of HCMV particles with cell surface molecules such as HSPGs, however, appears to trigger the opposite pathway, resulting in the stimulation of HLA class I presentation on the cell surface. This process does not require HCMV gene expression, since UV-HCMV was able to stimulate HLA class I expression on the infected cell surface. If HCMV genes are expressed, the pathway leading to the down-regulation of HLA class I will be activated. Our data from RT–PCR analysis support the above view. HCMV infection appeared to stimulate the transcription of HLA class I genes from HFF cells, as suggested by the RT–PCR results. The expression of HCMV genes, such as US3, inhibited the processing and/or transport of HLA class I molecules to the surface of HCMV-infected cells (Ahn et al., 1996, 1997; Hengel et al., 1996; Jones et al., 1996; Wiertz et al., 1996; Jones & Sun, 1997). Thus, although the overall level of HLA class I gene transcription was stimulated by HCMV infection, the HLA class I molecules presented on the surface of HCMV-infected cells decreased in quantity, as shown by the flow cytometry data. On the other hand, UV-HCMV was able to stimulate HLA class I gene transcription, but was not able to inhibit the presentation of HLA class I molecules on the infected cell surface, since none of the HCMV genes could be expressed.

Contact of HCMV particles with host cells has been
reported to induce immediate-early cellular responses (Fortunato et al., 2000b). These include the rapid influx of calcium from the extracellular medium into the cell and transcription of proto-oncogenes, such as c-fos, c-jun and c-myc (Albrecht et al., 1991). Generation of reactive oxygen intermediates is stimulated by the physical contact of HCMV particles, which leads to the activation of transcription factor NF-κB (Speir et al., 1996). All these immediate-early cellular responses appear to be a prerequisite for HCMV replication, since increased calcium and activation of NF-κB are thought to stimulate HCMV immediate-early gene expression (Kang et al., 1993; Speir et al., 1996). Recently, chromosome 1q42 breakage (Fortunato et al., 2000a), expression of IFN-inducible genes (Navarro et al., 1998; Boyle et al., 1999) and induction of the chemokine RANTES (Michelson et al., 1997) were reported to result from HCMV infection. Each of these cellular responses does not require HCMV gene expression and can be mediated by inactivated virus or purified virion gB (Boyle et al., 1999). Interestingly, although the initial physical contact with HCMV particles stimulates several IFN-inducible genes as the infection progresses, virus gene expression results in the suppression of IFN signalling (Miller et al., 1999). While the induction of RANTES requires only virus contact with cells, the depletion of RANTES requires HCMV US28 gene expression (Bodaghi et al., 1998). Therefore, some of the immediate-early cellular responses that do not require virus gene expression but need physical contact with virus particles are counteracted by subsequent virus gene expression. Our study adds another line to a growing list of immediate-early cellular responses to HCMV infection that do not require virus gene expression.

The physical interaction between HCMV particles and the cell surface appears to require at least two components: viral envelope glycoproteins and HSPGs on the surface of infected cells. It has been known for some time that HSPGs are envelope glycoproteins and HSPGs on the surface of infected cell surface appears to require at least two components: viral and HSPGs seem to be involved in the pathway leading to the stimulation of HLA class I by interaction with HCMV particles. Although HCMV binding to the cell surface HSPGs is the initial interaction between the virus and the host cell, HSPGs alone are believed to be insufficient (Pietropaolo & Compton, 1997). Several cellular proteins on the cell surface, such as the 92.5 kDa phosphoprotein, CD13 or annexin II, would function downstream of the HSPG–virus interaction (Keay et al., 1989; Soderberg et al., 1993; Wright et al., 1994). It is possible that components on the cell surface other than HSPGs may be involved in the stimulation of HLA class I expression by UV-HCMV.

The virion components for initial binding to HSPGs have been characterized to be gB and/or glycoprotein complex II (gC-II), which are present on the envelope of the virus (Kari & Gehrz, 1992; Compton et al., 1993; Boyle & Compton, 1998). Treatment of virus with trypsin degrades the protein moiety of the envelope glycoproteins and we found that brief treatment of UV-HCMV with trypsin reduced the HLA class I-enhancing activity of UV-HCMV in a concentration-dependent manner. Furthermore, MAb anti-gB blocked the effect of UV-HCMV on HLA class I expression. Therefore, the molecules involved in an interaction with HSPGs on HFF cells appear to include envelope glycoproteins such as gB. If HSPGs are not the sole molecules involved in the stimulation of HLA class I expression by UV-HCMV, as suggested above, it is worthwhile to note that an undefined non-heparin component in the absence of HSPGs could be bound to gB (Boyle & Compton, 1998). It is not clear whether HCMV gB is the sole glycoprotein involved in the stimulation of HLA class I expression on HFF cells by HCMV. Further studies are needed to identify all of the viral and cellular components that are required for the physical interaction of HCMV and HFF cells leading to the increase in cell surface HLA class I expression.

It has been taken for granted or customary to say that certain effects are due to virus binding to cellular receptors if the effects are observed with inactivated viruses, DBs or non-infectious enveloped viruses (NIEPs), or if the effects are inhibited by blocking the interaction of the virus with cellular receptors. For example, Yurochko & Huang (1999) proposed that the expression of immunoregulatory genes, such as interleukin-1β, is induced by HCMV binding to human monocytes, since this stimulation was blocked by treatment with neutralizing anti-gB or anti-gH antibodies. However, inactivated viruses, such as UV-HCMV, or DBs can enter the host cell in a manner similar to normal infectious virus (Pepperl et al., 2000). The presence of virion components in the cells infected with UV-HCMV, NIEPs or DBs may raise the possible role of virion components in the stimulation of HLA class I gene expression or other initial signal transduction cascades induced by HCMV (Fortunato et al., 2000b). This possibility can be supported further by the reports that suggest that certain virion components, such as the tegument protein pp71 (Homer et al., 1999; Bresnahan & Shenk, 2000) or the lower matrix protein pp65 (Gallina et al., 1999), can modulate the functions of heterologous genes. Thus, the stimulation of HLA class I expression could result from, at least in part, the virion components present inside the host cell. Further studies are needed to elucidate the relative role of virus binding (interaction between envelope glycoproteins such as gB and HSPGs) and entry in stimulating the expression of HLA class I molecules. Whatever the mechanism is, it is clear that the
stimulation of HLA class I expression does not require HCMV gene expression.

What is the significance of the stimulation of cell surface HLA class I expression on HCMV-infected cells? Viruses, although regarded as living with active genetic entities once inside the host cell, are seen at first sight as foreign antigens. Binding of virus particles to cellular receptor molecules such as HSPGs may serve as a signal for the host cell to recognize the infecting virus. Host cells naturally respond to infecting viruses by activating defense mechanisms, such as the stimulation of IFN-inducible genes (Navarro et al., 1998; Boyle et al., 1999; Zhu et al., 1998) or enhancing the expression of HLA class I molecules, as suggested in this study. Furthermore, HCMV-infected cells secrete soluble factors, including IFN-β, into the extracellular medium before dying from the virus infection (Lee et al., 2001). Host cells are now equipped with antiviral defense systems, which provides additional meaning for the development and application of a HCMV vaccine. An attenuated, live vaccine has been studied extensively and an improved strain may result from genetic manipulation. An immunogenic subunit vaccine, such as the HCMV gB vaccine, is currently undergoing clinical trials to determine if the antibodies alone will be protective (Plotkin, 1999). Although inactivated or subunit vaccines would be expected to be less effective than live, attenuated vaccines in stimulating specific immune responses, they are expected, as our data suggests, to augment the immune response by stimulating the expression of HLA class I. Further studies are merited to elucidate the components and mechanisms necessary for the stimulation of HLA class I expression by the interaction of HCMV with cell surface HSPGs as well as to understand the clinical importance of HCMV in the defense against virus infection.

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References


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