Human Cytomegalovirus Modulation of Signal Transduction

A. D. Yurochko

Contents

Abstract An upregulation of cellular signaling pathways is observed in multiple cell types upon human cytomegalovirus (HCMV) infection, suggesting that a global feature of HCMV infection is the activation of the host cell. HCMV initiates and maintains cellular signaling through a multitiered process that is dependent on a series of events: (1) the viral glycoprotein ligand interacts with its cognate receptor, (2) cellular enzymes and viral tegument proteins present in the incoming virion are released and (3) a variety of viral gene products are expressed. Viral-mediated cellular modification has differential outcomes depending on the cell type infected. In permissive cell types, such as diploid fibroblasts, the upregulation of cellular signaling pathways following infection can initiate the viral gene cascade and promote the efficient transcription of multiple viral gene classes. In other cell types, such as endothelial cells and monocytes/macrophages, the upregulation of cellular pathways initiates functional host changes that allow viral spread to multiple organ systems. Together, the modification of signaling processes appears to be part of a thematic

A.D. Yurochko
Department of Microbiology and Immunology, Center for Molecular and Tumor Virology, Feist-Weiller Cancer Center, Louisiana State University Health Sciences Center, 1501 Kings Highway Shreveport, LA 71130-3932, USA
ayuroc@lsuhsc.edu

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strategy deployed by the virus to direct the required functional changes in target cells that ultimately promote viral survival and persistence in the host.

Introduction

HCMV is a species-specific β-herpesvirus found in more than 60% of the human population (Mocarski et al. 2007). HCMV causes severe disease in immunocompromised individuals, where it is a major opportunistic pathogen in AIDS and organ transplant patients, in congenitally infected neonates, and in cancer patients undergoing chemotherapy (see the chapter by W. Britt, this volume). In the immunocompetent host, HCMV causes mononucleosis (see the chapter by W. Britt, this volume) and is associated with chronic human diseases such as atherosclerosis and restenosis (Melnick et al. 1993; Speir et al. 1994; Waldman et al. 1997; Streblow et al. 1999, 2001a) and some forms of cancer (Shen et al. 1993; Cobbs et al. 2002; Soderberg-Naucler 2006).

A hallmark of HCMV infection is a broad cellular tropism in vivo that results in the infection of most host organ tissues (Myerson et al. 1984; Sinzger and Jahn 1996; Mocarski et al. 2007; see the chapter by C. Sinzger et al., this volume). HCMV pathogenesis is a direct result of the infection of host organs and the resulting overt organ disease (Sinzger and Jahn 1996; Mocarski et al. 2007). From an evolutionary standpoint, the ability to infect multiple organs provides the virus access to multiple portals of viral exit and, consequently, allows viral shedding in most human body fluids (Mocarski et al. 2007). Broad cellular tropism necessitates that the virus possess a strategy to productively infect a diverse array of cell types that have unique biochemical features. Regardless of the diversity of cells found in the human host, all cell types utilize cellular signaling pathways as a means of cellular communication and appropriate response to their environment (Cooper and Hausman 2007). Thus, cellular signaling from a general standpoint is a common thread among multiple cell types that, if exploited correctly, would allow HCMV to transcend the differences among cell types. Mechanistically, the exploitation of cellular signaling by the virus provides at least one biological explanation for HCMV's broad tropism in vivo. Certainly viral attachment to an infected cell surface is also a determinant of tropism (see the chapter by C. Sinzger et al., this volume), but because this chapter focuses on the viral modulation of cellular signaling, we will only discuss how cellular signaling can be exploited by the virus to promote persistence and survival in a variety of host cell types. Nevertheless, because we (Yurochko et al. 1995, 1997a; Yurochko and Huang 1999; Bentz and Yurochko, unpublished data) and others (Keay et al. 1995; Boyle et al. 1999; Simmen et al. 2001; Compton et al. 2003; Wang et al. 2003; Boehme et al. 2004; Feire et al. 2004; Wang et al. 2005; Boehme et al. 2006) have strong evidence that viral ligand-mediated signaling is stimulated by the same viral glycoproteins responsible for viral attachment, fusion, and entry (Britt and Mach 1996), it is likely that these two seemingly diverse mechanisms are intimately linked and together provide key control points for the infection of the host. We propose that cellular signaling is a biological aspect exploited by HCMV during infection (from viral entry to

post-entry events) to manipulate a variety of cell types. It is the goal of this chapter to provide an overview of the diverse mechanisms HCMV employs to modulate cellular signaling pathways, as well as a discussion of the likely biological rationale for why the virus may have evolved a strategy to dysregulate host cell signaling pathways following infection.

Signaling Overview

HCMV infection results in a wide range of cellular changes including changes in calcium flux and lipid metabolism, activation of kinase signaling cascades (such as calcium/calmodulin-dependent protein kinases, multiple cell cycle-regulated kinases, the epidermal growth factor receptor (EGFR), the IκB kinase (IKK) cascade, the mammalian target of rapamycin pathway, various members of the mitogen activated protein kinase (MAPK) pathway, the phosphatidylinositol 3-kinase (PI(3)K) pathway, and the src family of kinases), cytoskeletal changes, activation of cellular transcription factors (such as AP-1, ATF/CREB, E2F, NFκ-B, Sp1), the induction of proto-oncogenes and other cellular immediate-early (IE) response genes (reviewed in Albrecht et al. 1990, 1993; Evers et al. 2004; DeMeritt and Yurochko 2006). Signaling-induced changes in infected cells can loosely be grouped into two tiers (Table 1): the first tier represents changes that occur prior to the initiation of viral gene expression and, thus, are mediated by the virion itself; and the second tier represents those changes that occur temporally after the production of viral gene products and, thus, are mediated by proteins from the different temporal gene classes. The virion itself is a potent signaling player as the viral envelope glycoproteins initiate rapid cellular responses upon binding to cognate receptors (reviewed in Evers et al. 2004; DeMeritt and Yurochko 2006).

Table 1 Summary of viral-associated signaling[a]

Modulator	Rapid effects[b]	Delayed effects[b]	Function
Viral glycoproteins	X		Receptor/ligand-mediated signaling
Captured cellular enzymes	X	?	Activation of signaling pathways
Tegument proteins[c]	X	?	Activation of signaling pathways/ cell cycle regulation
Other viral gene products[d]	–	X	Activation of signaling pathways/ cell cycle regulation

[a] Individual gene products are discussed in the text

[b] Signaling induced upon HCMV infection can loosely be grouped into the products that regulate rapid responses (beginning within minutes of infection) and are caused by modulators associated with the virion vs those products that regulate effects later in infection (or delayed compared to the rapid effects) and are caused by viral gene products de novo synthesized following infection

[c] Tegument proteins or tegument-associated virion proteins are included together

[d] Other viral gene products in this table represent those gene products that are synthesized de novo in the infected cell and are not attributed to virion mediated signaling

Glycoprotein-mediated signaling is not the only tool in the virion arsenal, as the virion has evolved a mechanism to capture cellular signal modifying enzymes (Michelson et al. 1996; Gallina et al. 1999; Nogalski et al. 2007), which can be dumped directly into the cytosol following viral entry into host cells. In addition, like all herpesviruses, HCMV has a large number of tegument proteins that modulate cellular signaling (Mocarski et al. 2007). Lastly, viral gene products synthesized following infection can also manipulate host cellular responses (some examples include IE proteins that alter the cell cycle and regulate apoptosis (reviewed in Castillo and Kowalik 2004; Andoniou and Degli-Esposti 2006) or those viral gene products that mimic cellular cytokine/chemokine signaling receptors including US28 (reviewed in Streblow et al. 2001b; Stropes and Miller 2004; van Cleef et al. 2006), a viral G protein-coupled receptor (GPCR), and UL144 (Benedict et al. 1999; Poole et al. 2006), a tumor necrosis factor-like receptor. Together, it is evident that HCMV possesses an array of signal-modifying capabilities that are deployed over a temporal range during the infection process. The likely outcome of this viral-mediated signaling is currently under debate. We suggest the viral-mediated cellular modification is required for multiple critical steps in the viral infection cycle and that the viral-directed signaling can have different outcomes in different cell types. In fibroblasts, for example, the initial signaling seen following receptor/ligand engagement is reported to promote viral entry (Wang et al. 2003; Feire et al. 2004; Wang et al. 2005) and then productive infection by promoting efficient gene transcription (Caposio et al. 2004; DeMeritt et al. 2004, 2006; DeMeritt and Yurochko 2006). In other cell types such as endothelial cells (Bentz et al. 2006) and monocytes (Smith et al. 2004b, 2007), viral-mediated signaling can stimulate the functional changes in these cells required for hematogenous dissemination of the virus. Below we provide a more detailed overview of these different viral-directed steps controlling signaling.

Receptor/Ligand-Mediated Signaling: Viral Glycoproteins

Envelope glycoproteins play an essential role in viral attachment and entry (Britt and Mach 1996; Mocarski et al. 2007; see the chapter by M.K. Isaacson et al., this volume). From a signaling standpoint, these molecules are logical players in the rapid manipulation of the host cell because they are the first viral molecules to contact a target cell. Although HCMV encodes a number of envelope glycoproteins (Britt and Mach 1996; Mocarski et al. 2007), glycoprotein B (gB/UL55; Britt and Mach 1996) and glycoprotein H (gH/UL75 and its associated partners gL/UL115, gO/UL74, and the UL131-UL128 loci; Britt and Mach 1996; Hahn et al. 2004; Wang and Shenk 2005a, b; Patrone et al. 2007) are the glycoproteins documented to be bona fide signaling molecules (Keay et al. 1995; Yurochko et al. 1997a; Boyle et al. 1999; Yurochko and Huang 1999; Simmen et al. 2001; Compton et al. 2003; Wang et al. 2003, 2005; Boehme et al. 2004, 2006; Feire et al. 2004). The gH complex was originally shown to stimulate calcium flux (Keay et al. 1995), while we

have demonstrated that both gB and gH stimulate the activation of the cellular transcription factors, NFκ-B and Sp1 (Yurochko et al. 1997a; Yurochko and Huang 1999). Other studies confirmed and expanded these results (Boyle et al. 1999; Simmen et al. 2001; Wang et al. 2003, 2005; Boehme et al. 2004, 2006) and together determined that HCMV fires cellular signal transduction pathways via the actions of the major viral glycoproteins, gB and gH. Viral glycoprotein-mediated signaling occurs in multiple cell types (fibroblasts, monocytes, endothelial cells, etc.), suggesting that the capacity to induce cellular signaling is part of a central theme in the viral infection strategy.

The recent identification of several cellular receptors for HCMV attachment/entry that are found on multiple cell types supports this proposal: HCMV glycoproteins were recently shown to interact with the epidermal growth factor receptor (EGFR; Wang et al. 2003, 2005), integrins ($\alpha_2\beta_1$, $\alpha_6\beta_1$, $\alpha_V\beta_3$; Feire et al. 2004; Wang et al. 2005), and toll-like receptor 2 (TLR2; Compton et al. 2003; Boehme et al. 2006). From a signaling standpoint, the engagement of these receptors by the virus makes sense, as each receptor is biochemically integrated with the signaling machinery. EGFR dimerizes upon ligand binding and then directs downstream signaling events via the action of its intrinsic tyrosine kinase (Wang et al. 2003, 2005). Integrins do not possess intrinsic kinase activity; however, upon their engagement they interact with members of the Src family of tyrosine kinases to modulate downstream signaling events (Wang et al. 2003, 2005). Finally, like all TLRs, TLR2 is part of a signaling network involving a cascade of players (Compton et al. 2003; Boehme et al. 2006).

Mechanistically, it has been documented that gB and gH are responsible for the engagement of the various cellular receptors (EGFR, the integrins, and TLR2) and that, through this receptor/ligand interaction, they rapidly activate signal transduction pathways (Wang et al. 2003, 2005; Boehme et al. 2006). Wang et al. have reported that gB interacts with EGFR and gH interacts with cellular integrins (Wang et al. 2003, 2005), demonstrating that individual receptor/ligand events are controlled by different viral gene products. gB and gH can also interact with TLR2 (Boehme et al. 2006), while gB may additionally interact with cellular integrins (Feire et al. 2004). All three receptors appear to be present on most cell types, suggesting an evolutionarily conserved mechanism may exist for viral binding and receptor engagement during infection of multiple cell types. This possibility is supported by work showing that EGFR and/or integrins are central determinants of signaling and/or attachment/entry in fibroblasts (Wang et al. 2003, 2005), cytotrophoblasts (Maidji E et al. 2007), endothelial cells (Bentz and Yurochko 2008) and monocytes (Yurochko et al. 1992; Chan et al., unpublished data). Nevertheless, the role these receptors play remains controversial, as it was recently reported that EGFR was not required for attachment and signaling on some fibroblast, epithelial and endothelial cell lines (Isaacson et al. 2007). Thus, it remains unclear if all three receptors are utilized on all cell types infected or if different combinations are utilized depending on the cell type. Overall, these findings suggest the following general model (discussed in more detail below): gB and gH binding to cellular receptors initiates the activation of multiple downstream players including the focal

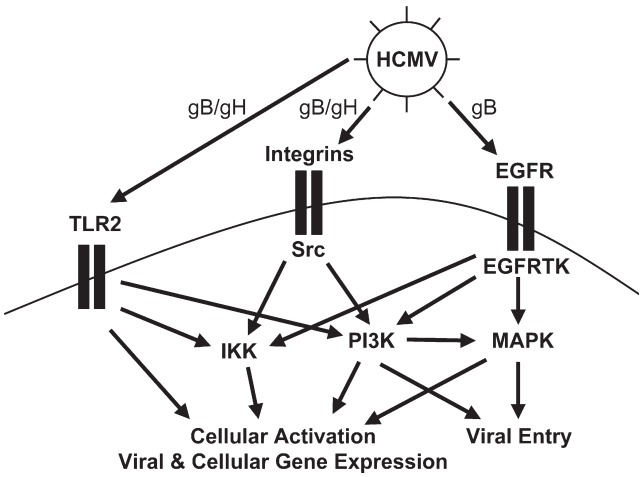

Fig. 1 HCMV binding to cognate receptors initiates signaling cascades. Binding of the envelope glycoproteins, gB and gH, to the cellular receptors, EGFR, integrins and TLR2 begin the outside-in signaling process observed in cells following infection. These known HCMV receptors are integrated with cellular signal transduction pathways; thus viral ligand engagement is the stimulus to fire downstream signaling processes. The initial receptor/ligand-directed signaling modulates a number of pathways, of which a few examples are shown in the drawing. The consequences of this outside-in signaling modulated by the viral glycoproteins include viral entry, cellular activation and transcriptional regulation of cellular and viral genes

adhesion kinase (FAK), the IKK cascade, the MAPK pathway, and the PI(3)K pathway to promote both viral entry and cellular changes such as the activation of NFκ-B and other transcription factors required for the transactivation of key cellular and/or viral genes (Fig. 1).

Captured Cellular Enzymes

The virion has long been known to harbor enzymatic activity (Mar et al. 1981), although the nature of this signaling potential has been unresolved. The signaling potential present in the virion imparts the virus with another mechanism to rapidly mediate distinct cellular changes following infection. Two distinct signaling capabilities are present in the virion: (1) HCMV captures cellular enzymes that directly modify the host cell signaling capabilities following viral fusion (discussed in this section) and (2) tegument proteins found in the mature virion can directly modulate host cell biochemical pathways (discussed in the next section).

The virion contains at least four distinct functional enzyme activities of host cell origin (Michelson et al. 1996; Gallina et al. 1999; Nogalski et al. 2007). A recent mass spectrometry analysis of the HCMV proteome revealed that additional cellular

modulators may exist in the virion (Varnum et al. 2004). Michelson et al. first showed that HCMV virions contain serine/threonine protein phosphatase activity due to the cellular protein phosphatases PP1 and PP2A (Michelson et al. 1996). This work provided key evidence that HCMV captures cellular enzymes capable of manipulating phosphorylation. Kinases are also present in the HCMV virion. Gallina et al. showed that HCMV possess serine/threonine kinase activity due to the cellular kinase, (polo-like kinase 1 (Plk1; Gallina et al. 1999)). Plk1 was shown to interact with the major tegument protein, UL83/pp65, identifying a mechanism in which cellular products could be captured by the virus during maturation through a specific interaction with viral tegument proteins. We identified a second serine/threonine kinase, casein kinase II (CKII), that is also incorporated into the mature virion (Nogalski et al. 2007). The virion CKII possesses potent IκB kinase activity and promotes the efficient transactivation of the major IE promoter (MIEP). Why would the virus have evolved a mechanism to capture cellular enzymes? Reversible phosphorylation via the reciprocal action of kinases and phosphatases is an effective and rapid mechanism for modulating cellular function (Arena et al. 2005); thus this biochemical process is an attractive target for a virus that needs to rapidly modulate the host cell for viral infection, survival and persistence. The release of captured enzymes may allow an increase in the local concentration of those enzymes in the viral microenvironment (Nogalski et al. 2007). It is also possible the virion-associated enzymes have a different subcellular localization and thus potentially different targets (Gallina et al. 1999). Additionally, because the virus infects multiple cell types with different biological characteristics, the evolution of multiple mechanisms to drive the rapid activation of the cell may ensure sufficient and appropriate activation of each cell type following infection.

Tegument Protein-Mediated Signaling

HCMV possesses a number of tegument proteins that are able to modulate the host cell, although many tegument proteins do not have identified functions (Mocarski et al. 2007). Because another chapter will cover tegument proteins in detail (see the chapter by R. Kalejta, this volume), the signaling potential of select tegument proteins will only briefly be summarized. UL83, the major tegument protein, has been shown to block the antiviral response through the inhibition of the cellular transcription factors NFκ-B and interferon regulatory factor 1 (Browne and Shenk 2003). Other tegument proteins including UL82 (Schierling et al. 2004; Cantrell and Bresnahan 2006a; Saffert and Kalejta 2006), UL35 (Schierling et al. 2004), US24 (Feng et al. 2006) and UL26 (Stamminger et al. 2002; Munger et al. 2006) can also influence the early events involved with MIEP transactivation and IE gene expression. Tegument proteins also alter the cell cycle (reviewed in Kalejta and Shenk 2002; Kalejta 2004; Mocarski et al. 2007). For example, UL82 promotes cell cycle progression through the degradation of Rb family members (Kalejta et al. 2003; Kalejta and Shenk 2003a, 2003b), while UL69 blocks cell cycle progression

by arresting cells in the G_1 phase of the cell cycle (Lu and Shenk 1999). Functionally, UL82 has also been shown to interact with the cellular protein hDaxx resulting in IE gene transcription and viral replication (Cantrell and Bresnahan 2006a, 2006b; Saffert and Kalejta 2006; Hwang and Kalejta 2007; see the chapter by R. Kalejta, this volume).

Other Viral Gene Products That Modulate Signaling

Once viral gene transcription begins, HCMV increases its repertoire of signaling molecules. For example, the major IE genes (IE1-72/UL123 and IE2/UL122) have been shown to interact with a multitude of transcription factors to increase transcription of required viral and cellular genes (reviewed in DeMeritt and Yurochko 2006; Mocarski et al. 2007), as well as interact with cell cycle regulators such as p53, pRB, p107 and others to modulate the cell cycle (reviewed in Kalejta and Shenk 2002; Castillo and Kowalik 2004). IE1-72 has also been reported to contain intrinsic kinase activity and to activate cells through the targeted phosphorylation of members of the E2F family of transcription factors (Pajovic et al. 1997). In addition, IE1-72 and IE2-86 (Zhu et al. 1995) along with the other IE genes, UL36 (viral inhibitor of caspase activation; Skaletskaya et al. 2001; McCormick et al. 2003) and UL37×1 (viral mitochondrial inhibitor of apoptosis; Goldmacher et al. 1999; McCormick et al. 2003; Reboredo et al. 2004), can modulate various survival pathways and provide protection from apoptosis (for additional information see Andoniou and Degli-Esposti 2006). HCMV also encodes other proteins with distinct signaling capabilities such as UL97, a viral kinase that plays a critical role during viral infection through its ability to phosphorylate cellular and viral substrates (Prichard et al. 2005); four putative GPCRs (US27, US28, UL33 and UL78) that have been shown to bind chemokines, activate G proteins in a manner similar to traditional GPCRs, mediate calcium flux, activate various kinases (MAPKs, Src, and FAK) and modulate smooth muscle cell migration (reviewed in Streblow et al. 2001b; Stropes and Miller 2004; van Cleef et al. 2006); and a TNF-like receptor, UL144 that activates NFκ-B through a TRAF6-dependent signaling cascade (Poole et al. 2006).

Biological Rationale for Modulation of Host Cell Signaling

There is little doubt that HCMV binding and/or infection of multiple cell types induces a sequence of signaling events (more detail provided in DeMeritt and Yurochko 2006), of which key points have been discussed briefly above. The question that remains is why the virus has evolved an elaborate strategy involving a multitiered approach to activate host target cells? The available evidence suggests the viral-induced signaling serves to promote multiple steps required for an

efficient infection cycle. In human diploid fibroblasts, gB and gH stimulate signal transduction pathways required for viral entry (Wang et al. 2003, 2005; Feire et al. 2004), demonstrating that rapid signaling serves initially to stimulate entry. The same pathways required for this essential first step in the infection process (the activation of the EGFR kinase and Src via binding to EGFR and the integrins, respectively) also rapidly induce transcription factors such as NFκ-B. In our model, this induction is required for efficient transactivation of the MIEP and the production of viral IE gene products (DeMeritt et al. 2004), as well as the later viral gene classes (DeMeritt et al. 2006). It is likely that this facet of the viral biology, the activation of required host cell factors (transcription factors, cell cycle regulators, etc.) through the targeted specific activation of signal transduction pathways, is repeated for other specific pathways documented to be activated during infection of target cells. For example, additional transcription factors such as Sp1 are also induced following viral binding to promote the transactivation of the MIEP (Isomura et al. 2005; Yurochko et al. 1997a, 1997b). Because other signaling players such as the virion-associated CKII (Nogalski et al. 2007) and various tegument proteins (Romanowski et al. 1997; Stamminger et al. 2002; Schierling et al. 2004; Cantrell and Bresnahan 2006a; Feng et al. 2006; Munger et al. 2006; Saffert and Kalejta 2006) also promote the efficient expression of the IE gene products, it appears that multiple signaling pathways, although biochemically distinct, coordinate their efforts to focus on a single goal for the virus such as the upregulation of the MIEP and the initiation of the viral gene cascade. Other steps in the viral infection cycle are also essential to the infection process; thus it is likely that additional viral-mediated signaling pathways converge on a common molecular outcome to benefit the virus. An example is the role various tegument proteins and IE gene products play in ensuring that the required cellular replicative enzymes are available for viral replication (Castillo and Kowalik 2004).

Different cell types have distinct signaling capabilities, and even the same signal transduction pathway can have divergent downstream consequences in different cell types. Thus, we hypothesize that the viral regulation of signaling pathways will have different outcomes in cells such as endothelial cells and monocytes, which are critical cells for in vivo infection. We recently provided evidence for a unique two-pronged strategy for hematogenous dissemination involving endothelial cells and monocytes: (1) HCMV directly infects vascular endothelial cells (see references within Bentz et al. 2006; Mocarski et al. 2007; C. Sinzger et al., this volume), which in turn promotes naïve monocyte transendothelial migration and viral transfer to these migrating monocytes (Bentz et al. 2006), and (2) HCMV directly infects peripheral blood monocytes in order to promote their transendothelial migration (Smith et al. 2004a). Following transendothelial migration, both pools of infected monocytes differentiate into pro-inflammatory macrophages permissive for the replication of the original input virus, even though the original undifferentiated monocyte was not permissive for viral replication at the time of infection. The virus initiates these functional changes in endothelial cells and monocytes through the binding of viral glycoproteins to EGFR and cellular integrins and the resulting modulation of downstream signaling cascades such as the PI(3)K and NFκ-B

pathways (Smith et al. 2004b; Bentz et al. 2006; Bentz and Yurochko 2008; Chan et al., unpublished data; Smith et al. 2007). Thus, these signal transduction pathways do not initially drive viral gene expression in these cell types, but instead induce cellular changes required for motility and firm adhesion to endothelial cells and transendothelial migration, suggesting that the biological rationale for the activation of these pathways is to modulate functional changes in cells of the vasculature that favor viral spread to and persistence within host organs. The role EGFR and integrins play in entry and attachment of endothelial cells and monocytes is not clear, although we have data that rapid signaling occurs through these receptors in both cell types (Bentz and Yurochko 2008; Chan et al., unpublished data), similar to that seen in fibroblasts (Wang et al. 2003, 2005; Feire et al. 2004), suggesting that these receptors are globally relevant to infection of multiple cell types. Overall, we propose that viral-induced signaling creates distinct cell-type-specific signaling signatures such that viral infection proceeds appropriately in each cell type (Fig. 2).

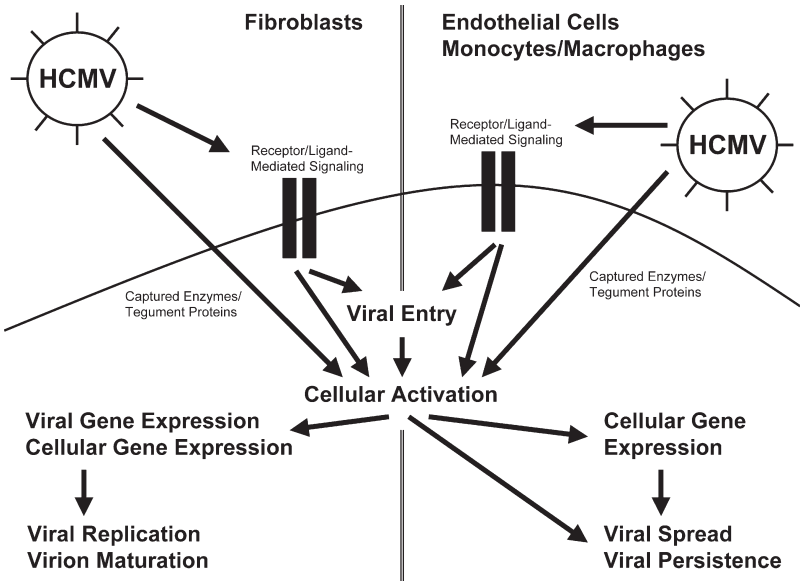

Fig. 2 Potential biological outcome of the viral-mediated signaling. Although unresolved, it is likely that the initially receptor/viral-ligand-mediated signaling promotes viral entry into target cells, regardless of cell type. This same receptor/ligand-mediated signaling also activates multiple biochemical pathways in target cells; both common pathways and cell-type-specific pathways are activated. The other potential mechanisms discussed in this review such as the cellular enzymes and tegument proteins that come in with the virion, as well as various synthesized viral gene products, also play a critical role in cellular modification. The net outcome of the viral-mediated signaling appears to vary depending on the cell type: for example, as represented in this drawing, productive infection is promoted in fibroblasts, while long-term persistence and survival of the virus is promoted in endothelial cells and monocytes/macrophages. Note: monocytes are not

Role of Signaling in Pathogenesis

Aberrant signaling and transcription factor regulation is associated with a multitude of diseases that including birth defects, cancer, and chronic inflammatory diseases such as cardiovascular disease (Kim et al. 2006). Cell cycle abnormalities are equally associated with diseases such as cancer and cardiovascular disease (Castillo and Kowalik 2004; Bentz and Yurochko 2008). Because these same diseases are associated with or caused by HCMV infection, modulation of multiple signaling transduction pathways, although beneficial to the virus, may be a molecular mechanism tying HCMV infection to the onset or severity of viral-mediated disease (reviewed in Evers et al. 2004; DeMeritt and Yurochko 2006; Soderberg-Naucler 2006). Certainly more work is needed to understand the possible direct role that viral-mediated cellular activation has on the infected host. It is also likely that these viral-manipulated cellular pathways required for viral pathogenesis may serve as new therapeutic targets for antiviral agents.

Final Thoughts

Together, it appears that HCMV has evolved a strategy for viral infection, survival, and persistence within the host that involves a complex biochemical manipulation of the host. Because of the possibility of severe effects on the host of unchecked signaling, HCMV as an evolutionarily ancient virus may also have evolved a strategy to mitigate the pathological consequences of this signaling strategy. For example, a recent report shows that HCMV through the UL83/pp65 tegument protein downregulates NFκ-B activity (Browne and Shenk 2003). Although this report runs counter to the data showing that NFκ-B activity is required for viral gene expression (Caposio et al. 2004, 2007; DeMeritt et al. 2004, 2006; Nogalski et al. 2007), if one considers that the virus must walk a fine line when activating a cell between those changes required for viral infection and the activation of cellular antiviral/host defense pathways and/or pathogenic consequences, these divergent results may represent two sides of the same coin. Perhaps this is why other reports have shown that NFκ-B activation negatively regulates or at least does not upregulate MIEP activity (Benedict et al. 2004; Isomura et al. 2004; Eickhoff and Cotten 2005; Gustems et al. 2006) and that for example the viral gene product, IE2p86, can act as a negative regulator of some NFκ-B-dependent cellular promoters (Taylor and Bresnahan 2006a, 2006b; Gealy et al. 2007). Using this example as a model, we argue that

Fig. 2 (continued) productive for viral replication following primary infection, but in response to the viral-mediated signaling, as represented in the drawing, they differentiate into macrophages that support viral replication (Smith et al. 2004a), thus both monocytes and their differentiated counterparts, macrophages, are critical for viral spread and persistence

HCMV needs to activate threshold levels of NFκ-B to initiate gene transcription (cellular and/or viral), but because high levels of this host factor are detrimental to the virus (generation of antiviral responses) and the host (pathogenic consequences), the virus has a mechanism to balance and moderate this transcription factor, or in a more general sense cellular signaling pathways; the virus thus walks a fine line by activating the factors necessary to allow productive infection and life-long persistence within the host with only minimal pathological consequences.

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