

- system to the developing mouse brain using electroporation: visualization of neuronal migration in the developing cortex. *Neuroscience* 103, 865–872
- 21 Saito, T. and Nakatsuji, N. (2001) Efficient gene transfer into the embryonic mouse brain using *in vivo* electroporation. *Dev. Biol.* 240, 237–246
- 22 Gonzalez-Billault, C. *et al.* (2000) Perinatal lethality of microtubule-associated protein 1B-deficient mice expressing alternative isoforms of the protein at low levels. *Mol. Cell. Neurosci.* 16, 408–421
- 23 Parsons, J.T. (2003) Focal adhesion kinase: the first ten years. *J. Cell Sci.* 116, 1409–1416
- 24 Williamson, R. *et al.* (2002) Rapid tyrosine phosphorylation of neuronal proteins including tau and focal adhesion kinase in response to amyloid-beta peptide exposure: involvement of Src family protein kinases. *J. Neurosci.* 22, 10–20

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Receptors and immune sensors: the complex entry path of human cytomegalovirus

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The ability of human cytomegalovirus (HCMV) to infect an extensive range of cell types has complicated efforts to identify cellular receptors for this significant pathogen. Recent findings demonstrate that epidermal growth factor receptor (EGFR) serves also as a receptor for HCMV. Additional evidence has shown that HCMV entry occurs in concert with immune detection through toll-like receptors. Here, the implications of EGFR activation, the existence of other receptors and the coordination of entry with the innate sensing are discussed.

Human cytomegalovirus (HCMV), a member of the *Herpesviridae* family, is a ubiquitous opportunistic pathogen that has an intimate lifelong relationship with its human host. The manifestations of HCMV disease are diverse, and its clinical presentation is tightly linked to the immune status of the infected patient. A varied array of clinical diseases correlates with the exceptionally broad tropism of this virus [1]. Pathological analysis of infected individuals show that HCMV can infect most of the major cell types including monocyte/macrophages, endothelial cells, epithelial cells, smooth muscle cells, fibroblasts, stromal cells, neuronal cells, neutrophils and hepatocytes [2]. *In vitro*, however, full productive infection is supported in secondary cell strains of fibroblasts and endothelial cells and in fully differentiated myeloid cells. By contrast, the entry of HCMV is promiscuous and the virus is capable of binding, penetrating and initiating replication in virtually all vertebrate cell lines tested [3]. The ability of HCMV to recognize and productively enter such a wide range of cells suggests that HCMV utilizes multiple receptors and/or its entry targets are ubiquitously distributed cell-surface molecules. An example of the latter is heparan sulfate proteoglycans (HSPGs) on the cell surface. HSPGs are expressed on nearly all cells and function to tether the virus to the cell membrane. Although strictly required, HSPGs are not solely sufficient for HCMV infection [4]. Biochemical analysis of HCMV binding to cells clearly

indicates biphasic association properties with two distinct measurable affinities [5]. These findings suggest that other receptors are engaged downstream of HSPGs. Over the past decade, the research on several receptor candidates has advanced. To qualify as a *bona fide* virus receptor, a cell surface molecule must serve several important but related roles in the virus lifecycle. For example, the virus and the receptor must physically interact and this should result in the internalization of virions. This consequence might be a direct effect of the receptor, or the receptor might promote downstream interactions with a co-receptor that triggers fusion and/or internalization of the viral genome. In addition, the expression of the receptor should be required for the gene expression and the replication of the virus in permissive cells. To date, none of the proposed HCMV receptors have met all of these criteria. The possibility that HCMV might utilize multiple redundant receptors to enter cells further compounds the challenge of identifying the molecules that are required for infection. Recently, Wang *et al.* reported that epidermal growth factor receptor (EGFR) serves as a cellular receptor for HCMV [6]. This new finding will be discussed in the context of what is known about HCMV-induced signal transduction pathways, the new knowledge that innate immune sensing occurs simultaneously with virus entry and the possible existence of other HCMV receptors.

Activation of cell signaling pathways by HCMV

Although the identity of HCMV receptors has remained enigmatic, it has been apparent for many years that HCMV entry into cells is not metabolically silent. Rather, cells respond to HCMV virions by the activation of numerous cell signaling pathways including changes in Ca^{2+} homeostasis, activation of phospholipases C and A2 and increased release of arachidonic acid and its metabolites [7]. All of these changes can be triggered by UV-inactivated virions, suggesting that the structural components of the virus are responsible for activation during virus–cell contact and/or virus entry. Virus–cell contact also results in the activation of transcription

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factors such as *c-fos/jun*, *myc*, *NF-κB*, *SP-1* and mitogen-activated protein kinase (MAP kinase) *ERK1*, *ERK2* and *p38* [8–11]. These virally induced cellular and physiological changes have a profound effect on gene expression in the host cell, because the expression of thousands of transcripts is altered, and most of these changes do not require viral gene expression [12–14]. These data are consistent with the interpretation that HCMV is engaging cellular receptor(s) that activate signal transduction pathways culminating in the reprogramming of cellular transcription.

EGFR is a HCMV receptor

Wang and colleagues [6] recognized that many of the signaling pathways activated by HCMV are similar to those induced by EGF activation of its receptor, EGFR. Furthermore, EGFR is expressed on some HCMV-permissive cells. On the basis of these, they tested the hypothesis that EGFR serves as a receptor for HCMV. The authors found that infecting permissive fibroblasts with HCMV results in the phosphorylation of EGFR, which correlates with the activation of Akt and phosphoinositide 3-kinase and the mobilization of Ca^{2+} . It is not known, however, whether this is an autophosphorylation event or other tyrosine kinases, such as Src, which is known to activate EGFR, are responsible. Wang *et al.* also showed that an EGFR-negative human breast-cancer cell line was resistant to HCMV infection, whereas an EGFR-positive breast-cancer cell line was permissive of viral gene expression. However, the investigators did not provide any direct evidence that EGFR is needed for the promotion of virus–cell fusion or the internalization of virion components to the cytoplasm. Future work is needed to determine whether the engagement of EGFRs by HCMV functions in the entry process or if it directly, or indirectly, triggers signal transduction events that facilitate viral gene expression.

HCMV glycoprotein B interacts with EGFR

The herpesviruses are structurally complex displaying as many as 12 glycoproteins in their envelopes. A variety of evidence points to a crucial role of one of the HCMV glycoproteins, glycoprotein B (gB), in attachment and fusion with the host cell. Analysis of a purified soluble form of gB revealed the nature of specific receptor sites that when occupied renders cells nonpermissive for infection [5]. Moreover, gB independently induces many of the signaling events and most of the transcriptional changes that are activated by HCMV [10,13,15]. Wang *et al.* demonstrated the coimmunoprecipitation of gB and EGFR after the chemical crosslinking of the adsorbed virus and showed that gB was capable of displacing bound EGF. However, the mechanism by which gB binds to EGFR remains unclear. Although gB lacks an EGF-like domain (the interacting domain displayed on natural EGFR ligands), it displays an epitope with some homology to a distinct region of EGF [6]. Alternatively, gB might interact with other cellular receptors that associate with EGFR.

Consequences and caveats of EGFR activation by HCMV

Given the natural role of EGFR as a potent mitogenic signaling receptor, it is important to consider the

consequences of its activation by HCMV. This virus is known to induce cell functions associated with progression through the cell cycle and to co-opt several pathways in an ostensible need to optimize the environment for viral replication events. Wang *et al.* reported that EGFR was cleared from the cell surface after infection – a common consequence of ligand–receptor interactions. In contradiction with this finding, Fairley *et al.* failed to detect either EGFR activation in response to HCMV virions or the clearance of EGFRs from the cell surface. Instead, they, and others, found that HCMV infection is associated with a dramatic reduction in EGFR expression [16,17]. This repression occurred at the transcriptional level and required viral gene expression. This is in contrast to natural EGFR-ligands, which tend to positively regulate EGFR gene expression [18]. Thus, there are some important differences between the effects of HCMV and those of natural EGFR-ligands on EGFR. In general, however, the negative regulation of EGFR by HCMV extends to other growth factor receptors many of which are downregulated in response to HCMV [12–14]. A better understanding of the modulation of mitogenic molecules, such as growth factor receptors, by HCMV might ultimately shed light on the delicate balance maintained by this virus and its host.

Activation of innate immunity during HCMV entry: role of toll-like receptors (TLRs)

The transcriptional profiling studies of HCMV revealed that the genes most strongly induced are interferon-stimulated genes, interferon β itself and inflammatory cytokines, all of which are indicators of host innate immunity [12–14]. TLRs are ancient conserved pathogen-sensors, which are now well appreciated as the activators of signal transduction pathways that lead to the expression of antimicrobial/antiviral genes and the induction of inflammatory cytokines [19]. Until recently, the role of TLRs in the recognition of viruses was unknown; now, however, innate sensing of the members of the herpesvirus, retrovirus and paramyxovirus families by TLRs has been demonstrated [20–23]. In particular, TLR2 recognizes HCMV in a replication-independent manner. The stimulation of TLR2 results in the activation of *NF-κB* and the production of inflammatory cytokines [20]. The common theme arising from the studies of viral systems is that a viral envelope glycoprotein is the specific molecular trigger for TLR responsiveness without a need for viral gene expression. These studies directly point to an as yet unknown ability of the host to detect viruses during entry but before the onset of any replication events. The innate sensing of viruses by TLRs does not appear to play any

Table 1. Glycoprotein complexes on HCMV envelope with known roles in virus entry

Envelope complex	Cellular binding partner	Role in entry
gM/gN	HSPGs	Tethering
gB	EGFR	Docking, signaling
	$\beta 1$ Integrins	Fusion, internalization, signaling
	TLR2	Activation of innate immunity
GH/gL/gO	Unknown	Fusion

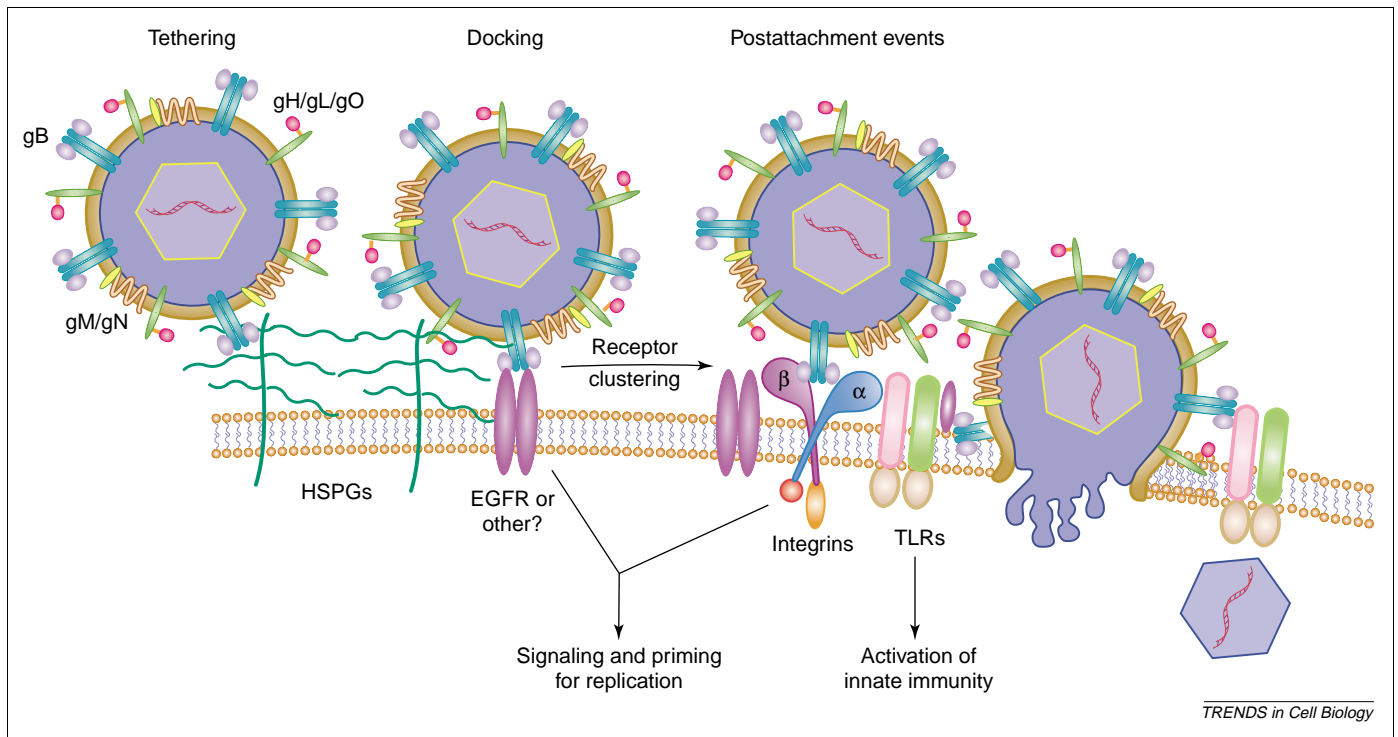


Figure 1. Working model for human cytomegalovirus (HCMV) entry into cells. HCMV initially attaches in a tethering step to heparan sulfate proteoglycans (HSPGs) through gM/gN and/or gB glycoproteins. In a stable docking step, gB interacts with the epidermal growth factor receptor (EGFR) on many HCMV-permissive cell types or with as yet unidentified receptors in hematopoietic cells. Other interactions between the glycoproteins of the HCMV envelope and cellular integrins promote receptor clustering. At least one of these interactions triggers fusion that leads to the internalization of virion components. Signal transduction events are initiated through EGFRs and/or integrins and these events are hypothesized to prime and facilitate downstream steps in the virus lifecycle such as nuclear translocation of the capsid and efficient viral gene expression. Toll-like receptors (TLRs) detect an HCMV-displayed pathogen-associated molecular pattern during the entry of the virus leading to distinct signaling events and the activation of innate immunity.

direct role in the entry events. However, TLRs and their respective envelope glycoprotein-trigger generally can be coimmunoprecipitated indicating a physical association between the two molecules and suggesting that entry and innate sensing events are somehow coordinated.

Overview of the HCMV-entry pathway

The findings of Wang *et al.* permit an update on the current model for HCMV entry and its possible coordination with innate sensing (Figure 1). A summary of glycoprotein complexes on the HCMV envelope and their known roles in the entry pathway is shown in Table 1. HCMV infection begins with an initial low-affinity tethering interaction with HSPGs. Two glycoprotein complexes of the envelope have heparin-binding ability. The gM/gN heterodimer and gB both adhere to heparin affinity columns and contain consensus heparin-binding sites. The binding of virions to HSPGs is rapidly followed by their stable docking to EGFRs. This receptor, however, although expressed on many HCMV-permissive cells, is not expressed on hematopoietic cells, such as monocyte/macrophages, dendritic cells and neutrophils [24], all of which are efficiently infected by HCMV and/or function to harbor particles and disseminate the virus *in vivo*. This strongly argues for the existence of other HCMV receptors in addition to EGFR. Other growth factor receptors are strong candidates for this role if the virus has a specific requirement for the signaling events that these receptors activate. Ultimately, the virus fuses with the plasma membrane depositing virion components in the cytoplasm. The heterotrimeric

envelope glycoprotein complex, gH/gL/gO, and gB are required for membrane fusion. The current model also poses a central role for cellular integrins in the promotion of virus internalization by HCMV. Cellular integrins are known to serve as co-receptors for many viruses, including another herpesvirus, Kaposi sarcoma-associated herpesvirus, and interaction with integrins is required for viral internalization [25,26]. Integrins are also known to interact with EGFR and synergize with signal transduction pathways [27–29]. The gB glycoprotein interacts with certain $\beta 1$ -containing integrins through a novel disintegrin domain (A. Feire, H. Koss and T. Compton unpublished). In these studies, an interaction with integrins was required for the internalization of tegument proteins suggesting that it plays a direct role in the entry process. Thus, integrins probably serve as co-receptors in the HCMV entry pathway. At some point in the entry process, however, a host immune sensor, TLR2, detects a pathogen-associated molecular pattern displayed on HCMV and activates host innate defenses. Intriguingly, integrins also associate with TLR2 [30].

Concluding remarks – coordination of entry and innate immune activation

The findings of Wang *et al.* provide new and important insights into the intricate interactions between HCMV and host cells. One possibility is that entry receptors (EGFR, integrins and accessory signaling molecules) and innate immunity machinery (TLR2, its membrane-associated partners, cytoplasmic adaptors and signaling machinery)

coalesce into specialized membrane microdomains with integrins playing central ligating role. The concentration of all these receptors on the cell surface into a defined platform might facilitate cell-signaling events, some of which are optimal for replication and others that are clearly hostile to the virus. Future work will focus on the precise role of EGFR in HCMV infection, the identification of other HCMV receptors on the cell surface and the coordination of entry with host defenses.

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References

- Alford, C.A. and Britt, W.J. (1993) Cytomegalovirus. In *Human Herpesviruses* (Roizman, B., Whitley, R. and Lopez, C., eds), pp. 227–255, Raven Press
- Jarvis, M.A. and Nelson, J.A. (2002) Human cytomegalovirus persistence and latency in endothelial cells and macrophages. *Curr. Opin. Microbiol.* 5, 403–407
- Nowlin, D.M. *et al.* (1991) Expression of a human cytomegalovirus receptor correlates with infectibility of cells. *J. Virol.* 65, 3114–3121
- Compton, T. *et al.* (1993) Initiation of human cytomegalovirus infection requires initial interaction with cell surface heparan sulfate. *Virology* 193, 834–841
- Boyle, K.A. and Compton, T. (1998) Receptor-binding properties of a soluble form of human cytomegalovirus glycoprotein B. *J. Virol.* 72, 1826–1833
- Wang, X. *et al.* (2003) Epidermal growth factor receptor is a cellular receptor for human cytomegalovirus. *Nature* 424, 456–461
- Fortunato, E.A. *et al.* (2000) Exploitation of cellular signaling and regulatory pathways by human cytomegalovirus. *Trends Microbiol.* 8, 111–119
- Kowalik, T.F. *et al.* (1993) Multiple mechanisms are implicated in the regulation of NF-kappa B activity during human cytomegalovirus infection. *Proc. Natl. Acad. Sci. U. S. A.* 90, 1107–1111
- Yurochko, A.D. *et al.* (1995) Human cytomegalovirus upregulates NF-kappa B activity by transactivating the NF-kappa B p105/p50 and p65 promoters. *J. Virol.* 69, 5391–5400
- Boyle, K.A. *et al.* (1999) Engagement of the cellular receptor for glycoprotein B of human cytomegalovirus activates the interferon-responsive pathway. *Mol. Cell. Biol.* 19, 3607–3613
- Boldogh, I. *et al.* (1991) Transcriptional activation of cellular oncogenes fos, jun, and myc by human cytomegalovirus. *J. Virol.* 65, 1568–1571
- Browne, E.P. *et al.* (2001) Altered cellular mRNA levels in human cytomegalovirus-infected fibroblasts: viral block to the accumulation of antiviral mRNAs. *J. Virol.* 75, 12319–12330
- Simmen, K.A. *et al.* (2001) Global modulation of cellular transcription by human cytomegalovirus is initiated by viral glycoprotein B. *Proc. Natl. Acad. Sci. U. S. A.* 98, 7140–7145
- Zhu, H. *et al.* (1998) Cellular gene expression altered by human cytomegalovirus: global monitoring with oligonucleotide arrays. *Proc. Natl. Acad. Sci. U. S. A.* 95, 14470–14475
- Yurochko, A.D. *et al.* (1997) The human cytomegalovirus UL55 (gB) and UL75 (gH) glycoprotein ligands initiate the rapid activation of Sp1 and NF-kappaB during infection. *J. Virol.* 71, 5051–5059
- Beutler, T. *et al.* (2003) Downregulation of the epidermal growth factor receptor by human cytomegalovirus infection in human fetal lung fibroblasts. *Am. J. Respir. Cell Mol. Biol.* 28, 86–94
- Fairley, J.A. *et al.* (2002) Human cytomegalovirus infection inhibits epidermal growth factor (EGF) signalling by targeting EGF receptors. *J. Gen. Virol.* 83, 2803–2810
- Clark, A.J. *et al.* (1985) Epidermal growth factor regulates the expression of its own receptor. *Proc. Natl. Acad. Sci. U. S. A.* 82, 8374–8378
- Akira, S. (2001) Toll-like receptors and innate immunity. *Adv. Immunol.* 78, 1–56
- Compton, T. *et al.* (2003) Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-like receptor 2. *J. Virol.* 77, 4588–4596
- Haynes, L.M. *et al.* (2001) Involvement of toll-like receptor 4 in innate immunity to respiratory syncytial virus. *J. Virol.* 75, 10730–10737
- Kurt-Jones, E.A. *et al.* (2000) Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat. Immunol.* 1, 398–401
- Rassa, J.C. *et al.* (2002) Murine retroviruses activate B cells via interaction with toll-like receptor 4. *Proc. Natl. Acad. Sci. U. S. A.* 99, 2281–2286
- Ewald, J.A. *et al.* (2003) Ligand- and kinase activity-independent cell survival mediated by the epidermal growth factor receptor expressed in 32D cells. *Exp. Cell Res.* 282, 121–131
- Akula, S.M. *et al.* (2002) Integrin alpha3beta1 (CD 49c/29) is a cellular receptor for Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) entry into the target cells. *Cell* 108, 407–419
- Nemerow, G.R. (2000) Cell receptors involved in adenovirus entry. *Virology* 274, 1–4
- Li, J. *et al.* (1999) Integrin-mediated migration of murine B82L fibroblasts is dependent on the expression of an intact epidermal growth factor receptor. *J. Biol. Chem.* 274, 11209–11219
- Miyamoto, S. *et al.* (1996) Integrins can collaborate with growth factors for phosphorylation of receptor tyrosine kinases and MAP kinase activation: roles of integrin aggregation and occupancy of receptors. *J. Cell Biol.* 135, 1633–1642
- Schneller, M. *et al.* (1997) Alphavbeta3 integrin associates with activated insulin and PDGFbeta receptors and potentiates the biological activity of PDGF. *EMBO J.* 16, 5600–5607
- Ogawa, T. *et al.* (2002) Bacterial fimbriae activate human peripheral blood monocytes utilizing TLR2, CD14 and CD11a/CD18 as cellular receptors. *Eur. J. Immunol.* 32, 2543–2550

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Bcl-w(edding) with mitochondria

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Bcl-2-family members (Bcl-2, Bax, Bcl-w and Bcl-x_L) are crucial integrators of signals for cell survival and death; the pro- or antiapoptotic activities of these proteins are

regulated by their subcellular localization. Bcl-2 directly inserts into the membranes, where it acts; however, Bax requires a stimulus-dependent translocation from an inactive cytosolic to an active membrane-inserted state. Recently, a novel mechanism is described for the

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