 doi:10.20944/preprints201811.0378.v1

Review

Pathogen at the Gates: HCMV Entry and Cell Tropism.

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ABSTRACT: The past few years have brought substantial progress toward understanding how human cytomegalovirus (HCMV) enters the remarkably wide spectrum of cell types and tissues that the virus is observed to infect. Neuropilin-2 and platelet-derived growth factor receptor alpha (PDGFRα) were identified as receptors, respectively, for the trimeric and pentameric glycoprotein H / glycoprotein L (gH/gL) complexes that in large part govern HCMV cell tropism, while CD90 and CD147 were also found to play roles during entry. X-ray crystal structures for the proximal viral fusogen, glycoprotein B (gB), and for the pentameric gH/gL complex (pentamer) were solved. A novel virion gH complex consisting of gH bound to UL116 instead of gL was described, and findings supporting the existence of a stable complex between gH/gL and gB were reported. Additional work indicates that the pentamer promotes a mode of cell-associated spread that resists antibody neutralization, as opposed to the trimeric gH/gL complex (trimer), which appears to be broadly required for the infectivity of cell-free virions. Finally, viral factors such as UL148 and US16 were identified that can influence the incorporation of the alternative gH/gL complexes into virions. We will review these advances and their implications for understanding HCMV entry and cell tropism.

KEYWORDS: Viral entry; viral glycoproteins; virus receptors; UL148; US16; UL128-131; gB; gO; PDGFRα, integrins; Nrp2; gH/gL; cytomegalovirus; herpesviruses; HCMV; CMV; pentamer; trimer; glycoprotein H; glycoprotein L; glycoprotein O; glycoprotein B; UL128; UL130; UL131A; UL131

HCMV exhibits a broad cell tropism that is reflected in the multifarious tissues and organs in which the virus is observed to cause clinical disease. Despite evidence that the virus alters its gH/gL complexes in a manner that depends on the cell type producing virus [1], the regulation of HCMV cell tropism is poorly understood. This review will focus on HCMV cell tropism as it relates to the viral entry machinery found in the virion envelope. Over the last several years, substantial progress has been made in this area. A number of new cellular receptors have been identified, and in two key examples, the newly identified cellular receptor is matched to a specific gH/gL complex. Furthermore, a new gH complex has been characterized, and a number of viral factors that contribute to strain-specific differences in cell tropism have been described. Although there remain important unresolved questions, these advances provide a new level of clarity for understanding HCMV cell tropism, and suggest new models to explain how HCMV enters cells and spreads within tissues.

Viral envelope glycoprotein complexes and receptors

Before the first complete HCMV genome sequence was published in 1990 [2], three major disulfide-linked viral envelope glycoprotein complexes had been described [3-5]. These complexes, originally designated as gC-I, gC-II, and gC-III, have turned out to play crucial roles in HCMV entry. The viral genes encoding the constituents of each complex are now known, as are the relationships

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to the conserved entry machinery shared among all herpesviruses. Therefore, the complexes are increasingly referred to by the terms shared across the Herpesviridae, e.g., glycoprotein H / glycoprotein L (gH/gL), glycoprotein B (gB), glycoprotein M / glycoprotein N (gM/gN). In certain examples in which a gene product is found only among beta-herpesviruses or is unique to HCMV, the name of that product is often used, e.g. gO (UL74) in the case of gH/gL/gO for trimer, or UL116 in the case of gH/UL116, the newly discovered gH complex with UL116 [6].

gB

gC-I is made up of homotrimers of glycoprotein B (gB), a pan-Herpesviridae conserved glycoprotein that is posited to serve as the proximal mediator of membrane fusion events during viral entry. The three-dimensional structures of post-fusion gB from herpes simplex, HCMV, and the Epstein-Barr virus resemble those of glycoprotein G from the rhabdovirus vesicular stomatitis virus (VSV G), and of gp64 from the *Autographa californica* nuclear polyhedrosis virus, a baculovirus [7,8]. Together, VSV G, gp64, and gB comprise the class III membrane fusogens [9]. Based on inferences from the prefusion structure of VSV G, gB is thought to dramatically rearrange during membrane fusion. The pre-fusion gB is posited to be a relatively flattened conformer, in which the fusion loops are positioned at the base of the homotrimer, close to the viral membrane and hence tucked away from the target membrane and set apart from one another. In the prevailing model, fusion occurs via a transitory intermediate in which the fusion loops reach out to the target membrane [9]. In the post-fusion configuration, three central helices line up at the core of the homotrimer, elongating the structure, causing the fusion loops to cluster closer together at the side of the homotrimer opposite from where they began [10].

HCMV gB, which is encoded by *UL55*, is synthesized as a 160-kD precursor that undergoes furin cleavage in the Golgi, resulting in 116 kD and 55 kD fragments that remain disulfide-linked to each other [11]. In 2015, two crystal structures for HCMV gB ectodomain were published, one at 3.8 Å resolution, and another at 3.6 Å, in which the ectodomain is bound to the Fab portion of a neutralizing antibody [8,12]. A number of cell surface proteins have been reported or implicated as receptors for gB, including the epidermal growth factor receptor (EGFR)[13], the platelet-derived growth factor receptor alpha (PDGFR∝)[14], and, as discussed further below, integrins [15,16]. On the other hand, it has also been suggested that gB functions as a viral fusogen that does not bind cellular receptors [17]. In light of the latter, attempts to visualize interactions between gB and its putative receptors by approaches such as cryo-electron microscopy (cryo-EM) would seem warranted.

gM/gN

gC-II is comprised of a disulfide linked heterodimer of glycoproteins M (gM) and N (gN), which are encoded by *UL100* and *UL73*, respectively [18,19]. gM/gN is the most abundant glycoprotein complex on virions [20,21], and is essential in HCMV, as null mutants are non-viable. The gM/gN complex plays key roles during attachment to host cells, likely by mediating interactions with heparan sulfate proteoglycans on the cell surface [22]. Notably, gM/gN also plays intracellular roles during viral replication that are independent of its roles in attachment [23,24]. gM is an N-glycosylated 48-kD type III transmembrane (TM) glycoprotein with seven predicted TM helices, while gN is single-pass type I TM protein that is extensively O-glycosylated. The unmodified 138 amino acid gN polypeptide in strain AD169 specifies a molecular weight of approximately 18-kD when expressed on its own, however the fully glycosylated (mature) form detected from virion lysates migrates at ~65-kD in sodium dodecyl sulfate polyacrylamide gel electrophoresis [18,19]. Motifs in the gM cytoplasmic tail are required for trafficking during virion assembly [23], and the cytoplasmic tail of gN, which is palmitoylated at two different cysteine residues, is required for secondary envelopment [24]. The gN coding sequence varies remarkably across HCMV strains [25,26], consistent with the observation that gM/gN is an important target for humoral immune responses [27].

The Trimeric gH/gL Complex and Its Receptors

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gC-III, now frequently referred to as "the trimer" or "gH/gL/gO," is a heterotrimeric complex in which the heterodimer of gH (*UL75*) and gL (*UL115*) is disulfide linked to glycoprotein O (gO), a heavily N-glycosylated polypeptide encoded by *UL74* [28-30]. All herpesviruses encode gH/gL complexes, as gH/gL and gB together comprise the "core" herpesvirus membrane fusion machinery. Homologs of gO, in contrast, are found only among betaherpesviruses. The emerging consensus is that gO, in the context of the trimer, is absolutely required for the infectivity of cell-free virions [31,32]. The platelet-derived growth factor receptor alpha (PDGFR∝) was identified in three different independent studies to function as a cellular receptor for the trimer [33-35]. This finding has continued to find support in the literature [36,37].

The latest data suggest that tyrosine kinase activity of PDGFR∝ is dispensable for its role in HCMV entry [34,36]. The severely defective phenotypes observed during infection of fibroblasts lacking PDGFR∝ phenocopy those seen with *gO*-null mutant viruses, with the residual low-level infectivity being pentamer-dependent [31,34-36,38]. Thus, a role for PDGFR∝ in trimer-dependent entry may explain why the trimer is required for cell-free HCMV virions to infect fibroblasts, which ordinarily express PDGFR∝. However, why gO is required for cell-free virions to infect epithelial or endothelial cells remains unclear, since these cell types either do not express PDGFR∝ [34] or express it in only low amounts that are not required for soluble recombinant trimer to bind to cells [39]. Additional hitherto unidentified cellular receptors, or perhaps, receptor-independent roles in membrane fusion may explain why the trimer is required for the infectivity of cell-free virions in cells that lack PDGFR∝.

The Pentameric gH/gL Complex and Its Receptors

In 2005, a second HCMV gH/gL complex, now often referred to as "pentamer," was discovered after the repair of a frame-shift mutation in *UL131 (UL131A)* dramatically expanded the cell tropism of the laboratory-adapted HCMV strain AD169, restoring its infectivity for epithelial and endothelial cells [40,41]. The pentamer is composed of a gH/gL heterodimer bound to a trio of small glycoproteins encoded by *UL128*, *UL130*, and *UL131* (or as some refer to it, *UL131A)* [28,41-43]. The *UL128-131* locus was observed to be (i) unstable during HCMV passage in fibroblasts [44], and (ii) required for infection of leukocytes, dendritic cells, and endothelial cells [45-48]. The latter observations may have also hastened the discovery of the pentamer.

In 2015, a group from GSK Vaccines further defined the assembly of the pentamer. These investigators identified that the cysteine at amino acid position 144 (Cys144) of the gL polypeptide chain forms a disulfide bond to either Cys162 of UL128 or Cys351 of gO [28]. This finding explains why the two gH/gL complexes are mutually exclusive. The same study provided low resolution cryo-EM images of recombinant pentamer and trimer bound to gH antibodies. Similar cryo-EM approaches were taken to characterize neutralizing antibody binding sites [49].

In 2017, x-ray crystal structures for the pentamer bound to two different neutralizing antibodies were reported at 3.0 Å and 5.9 Å [43]. Several aspects of the gH domain structure closely resemble Epstein Barr virus (EBV) gH, while the overall structure is nonetheless described as an intermediate between the "rod-like" conformation of herpes simplex virus-2 gH/gL and the "boot-like" one seen for EBV gH/gL. Two disulfide bonds connect the N-termini of gH and gL to each other: gH Cys59 to gL Cys54, and gH Cys95 to gL Cys47. As predicted from the literature [44,45,50-53], UL128, UL130 and a C-terminal region of gL adopt chemokine folds; of the CC type for gL and UL128, and of the C- type for UL130. This observation suggests that the ancestral cytomegalovirus "pirated" host chemokine genes on multiple occasions. Of course, integration of chemokines into the viral cell entry machinery could provide receptor binding and signaling properties that could be immediately advantageous to the virus, although some of these features may ultimately be lost or modified during evolution.

Another striking aspect of the pentamer structure is how UL128 connects to gL. A ~40 amino acid region of UL128, comprising residues 123 to 162 of the primary sequence, forms a surprisingly long (~50 Å) flexible linker that stretches across UL130 and UL131 to reach gL, at which point it makes three alpha helical turns and presents Cys162 to form its disulfide linkage to Cys144 of gL [43]. It is

 doi:10.20944/preprints201811.0378.v1

fascinating to consider in what order the subunits of pentamer must assemble for UL128 to adopt this peculiar final conformation.

Earlier this year, neuropilin-2 (Nrp2) was convincingly identified as a functional cell entry receptor for the pentamer [37]. To identify Nrp2, the study made use of a high-throughput "avidity-based extracellular interaction screen" (AVEXIS), in which recombinant single-pass transmembrane proteins were monitored *in vitro* for interactions with recombinant trimer and pentamer. After identifying a high-affinity interaction between Nrp2 and pentamer, the investigators demonstrated that Nrp2 is essential for pentamer-dependent HCMV infection of endothelial and epithelial cells. The screen also identified interactions of trimer and pentamer with other cellular molecules that may represent additional receptors. For trimer, the additional hits included transforming growth factor beta receptor type 3 (TGFβRIII) and neuregulin-2 (NRG2). For pentamer, the additional high-affinity interaction hits were thrombomodulin (THBD), leukocyte immunoglobulin-like receptor subfamily B member 3 (LILRB3) the immunoglobulin alpha Fc receptor (FCAR). Another hit for pentamer, though of lower affinity, was CD46. Although the biological relevance for these other hits remains to be established, it seems likely that at least some of the molecules will turn out to play roles during natural infection.

Additional Receptors

CD147 was recently shown to be required for pentamer-dependent entry into epithelial cells, however, in a manner that does not involve a direct interaction with the pentamer [54]. Entry of Lujo virus, an arenavirus, requires Nrp2 as a surface receptor but also requires CD63, a tetraspannin protein, as an intracellular factor for entry [55]. Notably, another tetraspannin, CD151, was also recently reported to play roles during HCMV entry [56]. By analogy, it seems plausible that Nrp2 functions as the proximal cell surface receptor for the pentamer, while other molecules, such as CD147 or CD151, are required as co-receptors that act later— perhaps at a post-internalization step, during entry.

Another cellular molecule recently implicated as an HCMV receptor is THY-1 (CD90), which reportedly interacts with both gH and gB [57,58]. THY-1 engages $\alpha_v\beta_3$ integrins and recruits the signaling adaptor molecule paxillin during signaling. The $\alpha_v\beta_3$ integrins reportedly function gH-dependent co-receptors during entry [59], and paxillin has been found to be important during entry into monocytes [60]. Notably, integrins α_2, α_6, and β_1 are also reported to play roles during HCMV entry at a post-attachment step [15,16]. The interactions with integrins are thought to involve a "disintegrin-like" gB motif that resembles motifs found in the integrin binding domain of cellular proteins of the "a disintegrin and a metalloproteinase" (ADAM) family. Nonetheless, the disintegrin-like motif is mostly buried in the post-fusion gB structure [12]. A pre-fusion gB structure would help to shed further light on the role of this motif in entry.

Many are called, few are chosen?

Over the years, a great many different cell surface proteins have been reported to function as HCMV entry receptors. Considering the plethora of different cell types that the virus infects, it seems plausible that many if not all of these molecules play *bona fide* roles in early events during infection. That said, it does seem likely that certain cell surface molecules serve as the "primary receptors" that are required for the proximal events that drive physiologically default modes of entry, even though these default modes of entry appear to differ between different types of target cells. PDGFRα can likely be considered a primary receptor for trimer-dependent entry into fibroblasts, since wild-type virus is profoundly defective for entry into fibroblasts under conditions where PDGFRα is absent or unavailable, and pentamer-null virus shows a more severe, virtually absolute entry defect in these settings[33-36]. Based on the evidence, Nrp2, too, should be considered primary receptor for pentamer-dependent entry into epithelial and endothelial cells [37].

Receptors and co-receptors for viral entry often function at steps that are both temporally and spatially distinct from each other [61]. For instance, a given receptor may interact with a viral glycoprotein complex at the cell surface to promote endocytosis of virions, while another cellular

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factor may be required for membrane fusion and escape from the endocytic compartment. Thus, although the evidence for co-receptors is less straightforward, other cellular factors may turn out to absolutely required for downstream events during entry, or may serve in secondary or tertiary roles, in which one of any number of different cellular molecules could substitute. It also seems likely that certain cell surface proteins increase the efficiency of entry but are not required for infection to occur.

Of course, the cell type being infected has important implications for the mechanistic details at play. Trimer-dependent entry into fibroblasts is rapid, does not require clathrin, and is pH-independent, which suggested this mode of entry involved fusion at plasma membrane [62,63] (FIG 1). According to the latest data, however, trimer-dependent into fibroblasts occurs through a rapid macropinocytosis [64]. Pentamer-dependent entry into epithelial and endothelial cells, on the other hand, requires low pH, and presumably involves a more prolonged form of endocytosis [63].

The literature has made clear that pentamer and trimer drive entry into different cell types via distinct cell surface receptors, which strongly suggests that are at least two major modes of HCMV entry: pentamer-dependent and trimer-dependent [33,34,37,65]. Each mode likely involves a unique set of cellular proteins that play roles as receptors, co-receptors, or as accessory factors that enhance infection, and the details for a single mode of entry may differ somewhat between cell-type, for instance pentamer-dependent entry into epithelial cells versus endothelial cells may rely on distinct co-receptors, even if Nrp2 is a primary receptor in both settings.

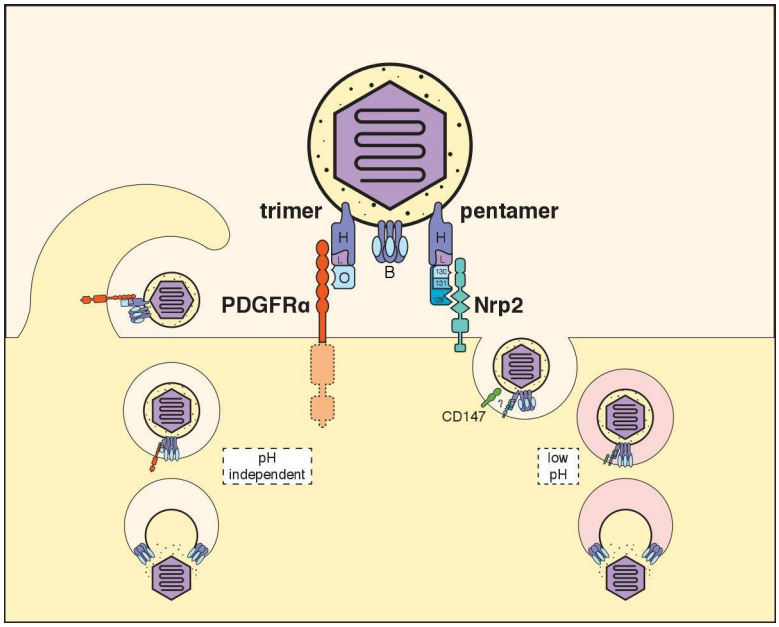

FIGURE 1. Receptors for HCMV gH/gL complexes. The trimeric gH/gL/gO complex interacts with PDGFRα to drive a pH-independent mode of entry that involves macropinocytosis. The pentameric gH/gL/UL128-131 complex interacts with Nrp2 to access a mode of entry that involves endocytosis and a decrease in pH. CD147 has been identified as a co-factor for this mode of entry. Note: gB is depicted as a homotrimer labeled as "B". See text for additional details.

How do gH/gL complexes regulate membrane fusion?

It is assumed that upon recognition of the appropriate cell surface receptor, gH/gL complexes trigger gB to fuse virion and target cell membranes. Precisely how gH/gL complexes regulate the gB fusogen is nevertheless unclear. Data suggesting a physical interaction between gH/gL and gB come mainly from experiments with herpes simplex virus [66-68]. A recent HCMV study, however, reported co-immunoprecipitation (co-IP) results which suggest a stable complex between gB and gH/gL occurs in infected cells and in virions [69]. Future studies leveraging structural and biophysical approaches will be needed to illustrate how gB is regulated in response to gH/gL interactions with cellular receptors. Regardless, since the trimer is observed to be indispensable for

 doi:10.20944/preprints201811.0378.v1

infection all cell types, it has been argued that pentamer may stimulate endocytosis of virions, while the actual membrane fusion may require the trimer to activate gB [32].

Cell-associated versus cell free spread

HCMV is thought to disseminate within the host primarily through cell-to-cell spread rather than via release of extracellular "cell-free" virions that would be susceptible to antibody responses. Most of the infectious virus in the blood of seropositive and acutely infected patients is found in the leukocyte compartment rather than plasma or serum [70-72]. Furthermore, clinical isolates of HCMV spread in a highly cell-associated manner during initial tissue culture passages [73,74], and the progressive loss of this cell-associated phenotype correlates with disruption of several elements within the viral genome [73,75]. We consider the major viral genes known to impact cell-associated vs cell-free spread below.

RL13

RL13, which encodes a virion envelope glycoprotein, is among the viral genes that most rapidly mutate during tissue culture propagation of the virus, often acquiring nonsense or frameshift mutations after one to four passages on fibroblasts, endothelial cells, or epithelial cells [75,76]. Cultured fibroblasts infected with HCMV harboring repaired *RL13* and *UL128-131* loci produce remarkably small amounts of cell-free infectious virus until several weeks post-infection [75,76]. These observations may suggest a function of *RL13* in the dampening of HCMV spread *in vivo*, perhaps to promote long-term persistence, or alternatively, may reflect a mode of selection peculiar to laboratory tissue culture conditions. Unfortunately, little is known about the function of *RL13*. Ectopically expressed RL13 has been shown to traffic to the cell surface and bind the Fc domain of IgG_1 and IgG_2 antibodies, followed by internalization [77]. These findings may be taken to imply an immune-evasive function for RL13, though RL13-dependent internalization of IgG has to date not been demonstrated in the context of the infected cell. RL13 has also been shown to strongly suppress the contribution of gO to cell-free spread in a *UL128*-null Merlin background [78]. Although these findings shed light on the potential interactions of RL13 with cellular and viral factors, they do not readily explain the pronounced instability of *RL13* during tissue culture propagation of HCMV.

gH/gL complexes and cell tropism

The literature suggests that cell-free versus cell-associated modes of spread are governed in large part by the composition of gH/gL complexes expressed in the virion envelope. Repair of the pentamer was observed to increase the cell-associated nature of the virus [46], and a more recent study showed that the pentamer drives a mode of direct cell-to-cell spread that resists neutralization by antibodies [79]. Accordingly, repair of the pentamer in strain AD169 promotes the formation of syncytia during in vitro cultivation of the virus [40]. On the other hand, the trimer is required for the infectivity of cell-free virions [31,38]. Although one might assert that the pentamer promotes cell-associated modes of spread and that the trimer enhances cell-free spread, the pentamer is nonetheless required for cell-free virions to efficiently infect endothelial and epithelial cells, as well as monocytes. Another observation that would confound generalizing the trimer and pentamer into respective roles in "cell-free" versus "cell-to-cell spread" roles is that HCMV strain AD169 deleted for the essential tegument protein pp28 (*UL99*) was found to spread efficiently in cultured fibroblasts [80]. Because strain AD169 harbors a frameshift in *UL131* that renders it unable to express pentamer, the efficient spread observed for the pp28-null virus has led some to argue that the trimer may suffice to drive cell-to-cell spread in fibroblasts [36].

Viral genes and polymorphisms that impact HCMV cell tropism

HCMV strains show large differences in the relative levels of pentamer and trimer incorporated into virions, and these differences correlate with cell tropism differences between strains [32,81]. A number of HCMV genes have the capacity to influence viral cell tropism at the stage of entry, and

 doi:10.20944/preprints201811.0378.v1

most of these presumably act via effects on the composition of gH/gL complexes that are incorporated in virions. Certain HCMV strains that maintain intact or largely-intact U$_L$*b'* regions express the pentamer at low levels. Examples include viruses derived from BAC-clones of strains TR [82], TB40/E [83] and VR1814 /FIX [84]. These strains, at least when reconstituted on fibroblasts, express high levels of gH/gL/gO (trimer) and low levels of gH/gL/UL128-131 (pentamer). Although a mutation in an intron of *UL128* has been identified to limit pentamer expression in strain TB40/E [85], why FIX and TR express similarly low levels of pentamer is unknown. Given their low levels of pentamer expression, it is perhaps unsurprising that these HCMV strains replicate inefficiently on epithelial cells [40]. On the other hand, the highly passaged strain AD169, which carries a UL*b'* region that has undergone rearrangements and deletions leading to loss of ~12 kbp of coding content, replicates robustly on epithelial cells when pentamer expression is restored [40].

Intriguingly, ablation of *UL148*, a gene within the U$_L$*b'*, enhances the ability of FIX, TR, and TB40/E to replicate to high levels on ARPE-19 epithelial cells, and leads to a striking reduction in overall levels of gH/gL and of trimer in virions [[86,87] and Siddiquey, M. and Kamil J.P. unpublished results] (FIG. 2). The *UL148*-null phenotype in these strains is accompanied by markedly reduced expression of gO, which likely explains why the *UL148*-null mutants express low levels of trimer. Nonetheless, the increase in epithelial cell tropism does not appear to involve enhanced levels of pentamer expression.

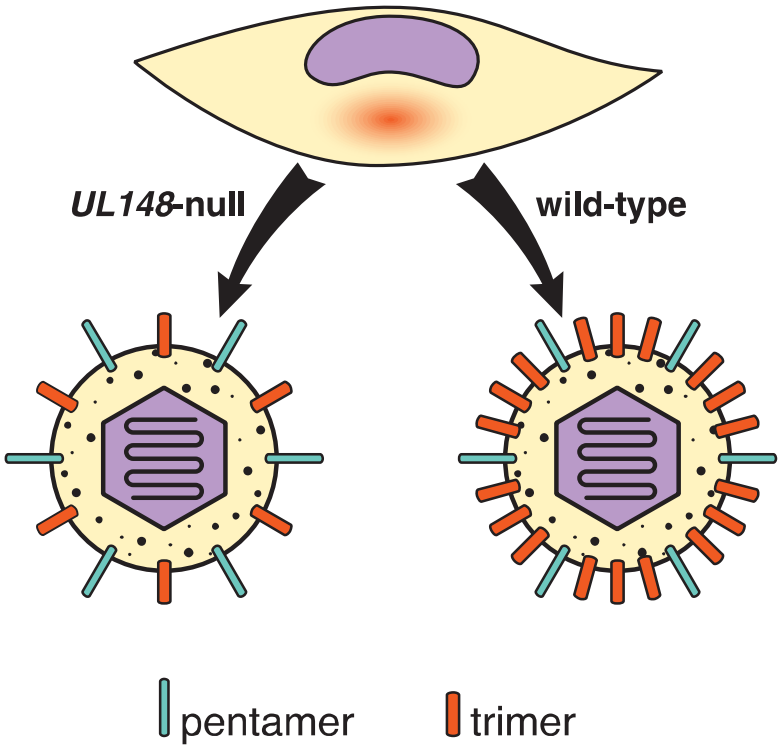

FIGURE 2. Regulation of alternative gH/gL complexes by UL148. UL148, a viral endoplasmic reticulum (ER)-resident glycoprotein, promotes high level expression of the trimer during infection (UL148$^+$) by stabilizing gO within the endoplasmic reticulum, resulting in the production of trimer-rich progeny virions. In *UL148*-null infections (UL148$^-$), virions with lower levels of trimer are produced, which are observed to more efficiently infect and replicate in epithelial cells. See text for additional details.

We recently reported that gO, but not other viral glycoproteins, is intrinsically unstable within the ER and is constitutively targeted for ER-associated degradation (ERAD) during infection [87].

UL148 appears to reduce the rate at which gO is degraded, possibly by interacting with SEL1L, a core component of the ERAD machinery. The observation that gO behaves as a constitutive ERAD substrate suggests that modulation of ERAD could provide a platform for viral regulation of cell tropism in HCMV and perhaps other betaherpesviruses. Nonetheless, whether UL148 is somehow regulated to stabilize gO in a cell-type specific manner remains to be seen.

Interestingly, UL148 was also recently identified to prevent surface presentation of CD58 (LFA-3), a co-stimulatory ligand for natural killer cells and T-cells [88], and to strongly contribute to the induction of the unfolded protein response (UPR) during infection [89]. It is not yet clear how roles for UL148 in CD58 retention or stabilization of gO relate to the mechanism by which it activates the UPR. However, pharmacologic and short interfering RNA treatments that inhibit or deplete the ERAD machinery stabilize gO expression [87]. Since blockade of ERAD would also be expected to activate the UPR, it seems reasonable to hypothesize that UL148 functions in part by inhibiting ERAD.

US16 is another viral factor that was recently identified to impact the composition of gH/gL complexes in HCMV virions [90]. *US16*-null mutant viruses fail to incorporate pentamer into progeny virions, and accordingly, are unable to efficiently infect epithelial cells or endothelial cells. Unlike UL148, which resides in the ER, US16 localizes to the cytoplasmic viral assembly compartment (cVAC), where virions acquire their infectious envelope. How US16 promotes pentamer incorporation is unclear, but co-immunoprecipitation results suggest that US16 interacts with the pentamer subunit UL130. The observation that US16 localizes to – and presumably functions at the cVAC may suggest that the trans-Golgi network-derived vesicles that provide virion envelopes are heterogeneous. Alternatively, US16 may regulate processes by which lysosomes that localize to the cVAC during infection degrade pentamer complexes, or otherwise make them unavailable for incorporation into virions.

It is intriguing to speculate that gene products like US16 and UL148 might play tissue specific roles in modulating the composition of viral gH/gL complexes in vivo. Since production of pentamer-rich pools of gH/gL might favor cell-associated modes of spread that evade humoral immunity, while trimer-rich particles could provide a fitness advantage during horizontal shedding, it would be crucial to identify how viral modulators of these complexes might be regulated. It would be helpful to know, for instance, whether virions with increased levels of trimer are produced in saliva or breast milk during natural infection.

In the example of EBV, a viral tropism switch that drives alternating cycles of viral replication in B-cells and epithelial cells depends on the cell type producing virus to regulate the levels in progeny virions of gH/gL and gH/gL/gp42, the latter being required for infection of B-cells [91]. Although there is at least one report that gH/gL composition and that the degree of heterogeneity in the cell tropism of progeny virions depends on the cell type producing virus [1], additional research is this area is certainly warranted. Regardless, if one or more bona fide tropism switches exists in HCMV, the mechanisms are likely to differ from those found in EBV.

Conclusion and Outlook.

Although the processes by which HCMV enters human cells and navigates through the human body remain to be fully understood, crucial new information on the cellular receptors and viral entry machinery has shed new light on HCMV entry and cell tropism. It is hoped that the coming decade will see investigators leverage these advances to develop new therapies to limit disease, as well as to reveal how HCMV spreads through the host to establish infection at sites of latency, long-term persistence, and horizontal shedding.

ACKNOWLEDGEMENTS: Research in the Kamil Laboratory is supported by grants R01-AI116851 and P30-GM110703 from the National Institutes of Health. This content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. C.C.N. is supported by a Malcolm Feist Fellowship in the Cardiovascular Sciences.

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