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Viral Regulation of Cell Tropism in Human Cytomegalovirus

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The viral glycoproteins that decorate enveloped viruses play crucial roles in cell entry and in large part dictate the spectrum of cell types that a virus can infect. The identification in human cytomegalovirus (HCMV) of a viral endoplasmic reticulum (ER)-resident glycoprotein that regulates the composition of alternative viral envelope glycoprotein complexes raises the intriguing possibility that certain viruses might actively regulate the tropism of progeny virions to improve their fitness or to navigate through the host.

Despite their infamy as opportunistic pathogens, viruses can turn out to be picky eaters. An age-old challenge in virology has been to figure out why a given virus will replicate prodigiously in one cell type while failing to infect another. In certain examples, most famously from bacteriophages and retroviruses, efforts to crack such riddles have led to the discovery of restriction factors that act after entry to block infection. Many tropism enigmas, however, reflect an incomplete understanding of how the viral entry machinery is regulated. In the herpesviruses, a complex of viral envelope glycoproteins H and L (gH/gL) has proven itself a source of sharp insights and stubborn riddles concerning cell tropism and entry.

Together with glycoprotein B (gB), the gH/gL dimer comprises the "core membrane fusion machinery" conserved among all herpesviruses (reviewed in reference 1). The prevailing view is that gH/gL complexes regulate the fusogenic activity of gB. Although alphaherpesviruses express only one form of gH/gL, a number of beta- and gammaherpesviruses express gH/gL in two alternative forms. The Epstein-Barr virus (EBV), a gammaherpesvirus, provides the most well understood example of how a herpesvirus can deploy alternative gH/gL complexes in a fascinating phenomenon called tropism switching (reviewed in reference 2). EBV alternates between infection of B cells and epithelial cells by adjusting its gH/gL complement in a manner that depends on which cell type is producing virions. Epithelial cells produce EBV virions that are enriched for a trimeric gH/gL complex that includes gp42, a viral glycoprotein necessary for engaging HLA II, which serves as a coreceptor for entry into B cells. Hence, virions shed from epithelial cells preferentially infect B cells. During replication in B cells, however, some gp42-containing complexes bind to immature HLA class II molecules in the endoplasmic reticulum (ER) and traffic to the peptide-loading compartment where they are degraded. EBV virions produced from B cells therefore contain more gH/gL complexes that lack gp42 and preferentially infect epithelial cells.

Human cytomegalovirus (HCMV), a betaherpesvirus, likewise expresses two alternative forms of gH/gL on its virions and infects a broad range of cell types during natural infection (3), but how—or even whether—the virus adjusts its gH/gL complexes is not at all clear. The first of the two HCMV gH/gL complexes to be described, gH/gL/gO (trimer), is a heterotrimeric complex between gH/gL and a heavily glycosylated protein encoded by the *UL74* gene (4, 5). The UL74 glycoprotein was named glycoprotein O (gO), much to the chagrin of those who had sought to reserve alphabet letter designations only for virion glycoproteins that were conserved across all herpesvirus subfamilies (gH, gL, and gB are conserved among all herpesviruses, but there is no gO homolog in any of the alpha- or gammaherpesviruses). The second HCMV gH/gL complex, gH/gL/UL128-131 (pentamer), was discovered more recently. It comprises a pentameric complex between gH/gL and three small glycoproteins encoded by *UL128*, *UL130*, and *UL131* (6–9). It is now established that the pentameric complex is required for infection of epithelial cells and endothelial cells. During adaptation to culture in fibroblasts, natural isolates of HCMV quickly accumulate mutations in *UL128*, *UL130*, or *UL131* that either prevent maturation of the pentamer or substantially diminish its expression on virions (10, 11). Therefore, most laboratory-adapted HCMV strains express only gH/gL/gO (trimer). HCMV virions that lack the pentamer are fully capable of infecting fibroblasts, but they fail to infect epithelial and endothelial cells (6, 8).

Two of the best-studied HCMV strains, Towne and AD169, not only lack expression of the pentamer, but they also contain extensive deletions in a region of the viral genome termed UL*b*′, leading to the loss of ~15 kb of coding content and at least 15 genes (12). It was within this region that we recently identified an open reading frame, *UL148*, which encodes an ER-resident glycoprotein that influences virion incorporation of the trimer, and depending on the genetic background of the virus, may also affect levels of the pentamer (13).

Identification of *UL148* as an HCMV tropism determinant. What led us to identify a role for *UL148* in HCMV cell tropism? We had been working with HCMV strain TB40/E, which harbors a virtually intact (full-length) UL*b*′ region and preserves expression of the pentamer (14). We were puzzled to learn that this strain fails to produce appreciable levels of cell-free virus when grown in ARPE-19 human epithelial cells, since derivatives of the laborato-

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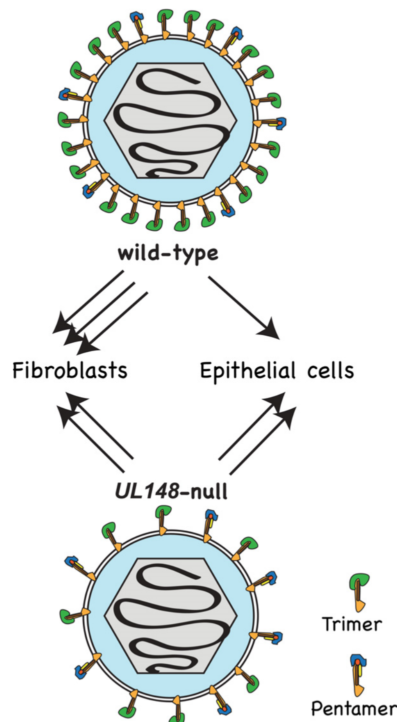

FIG 1 Influence of *UL148* on HCMV virion gH/gL complexes. Schematic of gH/gL composition and cell tropism of HCMV virions produced from fibroblasts infected with either wild-type or *UL148* null mutant viruses (based on data from strain TB40/E).

ry-adapted strain AD169 repaired for expression of the pentamer replicate robustly in ARPE-19 cells, producing very high titers of cell-free virus (8). Because the most prominent genetic difference between the AD169 and TB40/E strains is that the AD169 strain lacks most of the UL*b*′ region, we reasoned that a gene within this region might account for the difference in tropism for epithelial cells. We decided to screen a set of mutants that we had on hand which were deleted for different portions of the UL*b*′ region. We found that a mutant deleted for *UL142-UL148* replicated to titers approximately 100-fold higher on ARPE-19 cells compared to the parental TB40/E virus (G. Li and J. P. Kamil, unpublished results). Ultimately, disruption of *UL148* proved sufficient to improve tropism for epithelial cells, and we observed that this phenotype was associated with defects in maturation of the trimer (13) (Fig. 1). Although the pentamer is crucial for entry into epithelial cells, the enhanced epithelial cell tropism in *UL148* null TB40/E occurred without any appreciable increase in virion pentamer levels. This finding suggests that the absolute level of the pentamer does not *per se* predict the tropism of HCMV virions for epithelial cells, because the ratio of trimer to pentamer is also important.

A viral balancing act: alternative partners competing for gH/gL. HCMV may have evolved in *UL148* a mechanism to balance the expression of its two alternative gH/gL complexes, as UL128, UL130, and UL131 in theory have a 3:1 advantage over gO in competing for gH/gL. Indeed, it has been reported that each of the three pentamer-specific components can interfere with maturation of the trimer (7) (Fig. 2). It therefore seems plausible that in the absence of UL148, a portion of gH/gL that might otherwise become trimer is trapped in the ER, because UL128, UL130, and

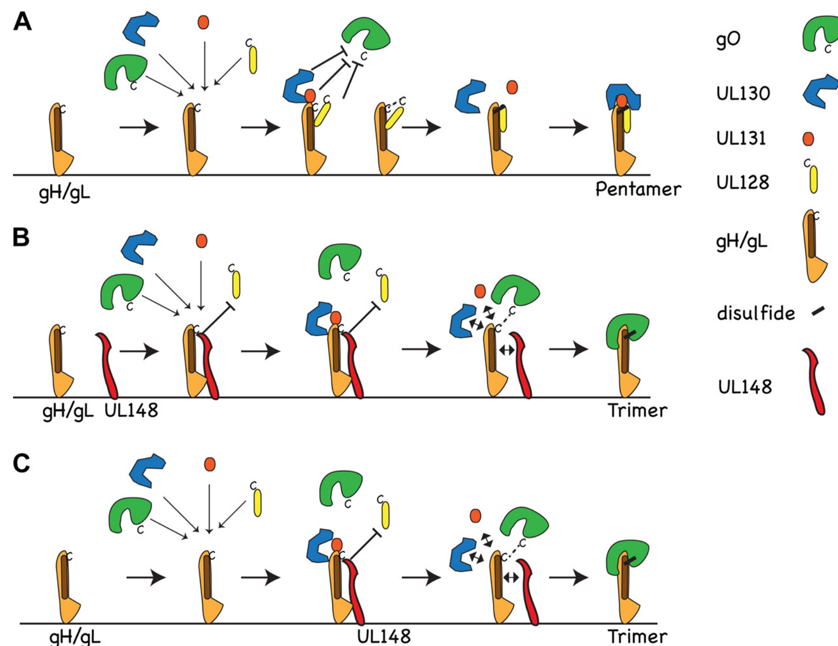

FIG 2 Models for the role of UL148 in modulation of alternative gH/gL complexes. (A) Maturation in the absence of UL148. gO, UL128, UL130, and UL131 complete within the ER for assembly onto the gH/gL dimer. UL128, UL130, and UL131 can each interfere with the loading of gO (7), and UL128 and gO compete to form a disulfide bond with gL Cys144 (15). Under these circumstances, UL128 forms its disulfide bond with gH/gL more efficiently than gO does, committing most gH/gL dimers to mature into pentamer. (B and C) Maturation in the presence of UL148. (B) UL148 binds gH/gL dimers prior to loading of accessory proteins. UL148 binds to gH/gL in a manner that prevents UL128, but not gO, from loading and forming its disulfide bond with gL. UL148-bound gH/gL complexes containing UL130 and UL130 are retained in the ER until the pentamer-specific components disassociate, which allows gO to load and form its disulfide bond with gL. (C) UL148 binds specifically to partially assembled gH/gL complexes containing UL130 and/or UL131 and prevents UL128 from loading and forming its disulfide bond with gL. The incompletely assembled gH/gL complexes are retained in the ER until UL130 and UL131 dissociate to allow loading of gO.

UL131 interfere with its assembly. Further, while UL130 and UL131 attach to gH/gL via noncovalent interactions, UL128 and gO each form a disulfide bond to a cysteine residue at position 144 of gL, suggesting that these accessory proteins compete in a mutually exclusive manner for formation of the trimer versus the pentamer (9, 15).

The mechanistic underpinnings that explain how UL148 influences the maturation of gH/gL complexes remain unknown. Our results suggested that UL148 might participate in protein-protein interactions with gH/gL complexes during their assembly in the ER, and indeed, we found UL148 to coimmunoprecipitate gH, gL, UL130, and UL131, but not gO or UL128 (13). Because we observed coimmunoprecipitation of all of the pentamer-specific components except for the one that forms a disulfide bond with gH/gL (UL128), we proposed that UL148 might function, at least in part, by retaining in the ER immature gH/gL complexes that contain either UL130 and/or UL131 while preventing UL128 from forming its disulfide bond with gL (13) (Fig. 2B and C). Moreover, because UL130 and UL131 do not form disulfide bonds with gH/gL, these pentamer-specific components might dissociate from the ER-retained complexes to allow gO to load. Even though we did not find evidence for any association between UL148 and complexes containing gO, we cannot exclude the possibility that UL148 binds gH/gL in a manner that still allows or even promotes gO assembly. If so, gO loading would likely have to displace UL148. At this time, we also do not know whether UL148 binds specifically to immature gH/gL complexes that contain UL130 and or UL131 or whether it also binds to gH/gL dimers in the absence of the pentamer-specific components (Fig. 2).

A recent commentary suggested that our model could not explain why the *UL148* null TB40/E mutant showed a profound decrease in the total level of gH/gL, not just in the level of trimer (16). We suggest, however, that because trimer accounts for most of the gH/gL on TB40/E virions (17), a decrease in overall virion levels of gH/gL might be expected when a factor important for its efficient maturation is absent. In addition, TB40/E contains a mutation in intron 1 of *UL128* that causes inefficient splicing of UL128 transcripts and hence, inefficient expression of the pentamer (11). Therefore, a strain-specific bottleneck in UL128 expression may have prevented an increase in pentamer levels from occurring when *UL148* was disrupted. In contrast, when we restored *UL148* to laboratory strain AD169 repaired for pentamer, we observed the expected increase in trimer together with a decrease in pentamer (13). Further work is needed to clarify the effects of UL148 in a wider array of viral genetic backgrounds.

HCMV strain Merlin (18) should provide an especially informative context for investigation of the function of UL148. While most HCMV strains that preserve the complete UL*b′* region express much higher levels of trimer than pentamer on their particles (at least when cultivated on fibroblasts), Merlin is an exception, as it shows much higher levels of pentamer than trimer (17). Merlin is also notable for the fact that it is the only available HCMV strain in which the cloned viral genome was restored to match the sequence found in the original clinical sample (prior to laboratory passage in cultured cells) (18). Whether Merlin is representative of all wild-type HCMVs in nature is not yet clear, but nonetheless, it should be illuminating to find out how UL148 affects the gH/gL composition of its virions.

A role for UL148 in HCMV tropism switching? HCMV infects many different types of cells during natural infection (3). Epithelial cells might be the first cell type to become infected and would likely transfer virus to monocytes which are thought to be particularly important for disseminating HCMV throughout the body. Along the way to establishing latency in $CD34^+$ hematopoietic progenitor cells and en route to the epithelial cells in the salivary gland and kidney, which shed the virus for horizontal transmission, the virus can infect endothelial cells, fibroblasts, hepatocytes, and sometimes smooth muscle cells and neural cells. Cell type-specific regulation of UL148 would offer a conceptually attractive mechanism by which the virus could adjust its gH/gL complement and optimize its infectivity to a particular tissue milieu during its journey through the host.

The evidence for tropism switching in HCMV remains limited. It has been reported that HCMV particles produced from fibroblasts display a broader cell tropism than those shed from endothelial cells and that this difference involves alternative gH/gL complexes (19). An earlier report, albeit using a strain that lacks *UL148*, found that the cell entry route used by the virus to infect epithelial cells is influenced by whether virus is produced from epithelial cells or fibroblasts (20). Further studies are clearly needed to carefully define the differences in the gH/gL composition and cell tropism of virions produced from various biologically relevant cell types and to determine whether *UL148* mutants show any notable differences. Systems for expressing recombinant gH/gL complexes, either by ectopic coexpression in uninfected cells or by *in vitro* translation in the presence of microsomal membranes, together with a rigorous characterization of the protein-protein interactions that occur, should likewise prove helpful for deciphering the mechanisms underlying the influence of UL148.

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