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Minireview

Complexities in human herpesvirus-6A and -6B binding to host cells

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Abstract

Human herpesvirus-6A and -6B uses the cellular receptor CD46 for fusion and infection of the host cell. The viral glycoprotein complex gH–gL from HHV-6A binds to the short consensus repeat 2 and 3 in CD46. Although all the major isoforms of CD46 bind the virus, certain isoforms may have higher affinity than others for the virus. Within recent years, elucidation of the viral complex has identified additional HHV-6A and -6B specific glycoproteins. Thus, gH–gL associates with a gQ1–gQ2 dimer to form a heterotetrameric complex. In addition, a novel complex consisting of gH–gL–gO has been described that does not bind CD46. Accumulating evidence suggests that an additional HHV-6A and -6B receptor exists. The previous simple picture of HHV-6A/B-host cell contact therefore includes more layers of complexities on both the viral and the host cell side of the interaction.

Keywords: Human herpesvirus 6A/B; Receptor; CD46; Glycoproteins; Fusion; Viral entry

In its simplest form, viral infection of a host cell is initiated by the interaction of a viral protein with a cellular receptor. For many viruses, this simplistic view is conceptualized in the search of *the* receptor for a given virus. In the case of human herpesvirus-6A and -6B, the conundrum was thought to be solved with the identification of CD46 as a receptor interacting with the viral glycoproteins gH–gL. However, emerging data have taught us that deciphering HHV-6A and -6B interaction with the cellular surface receptor (s) is far more complicated than initially assumed, and once again stressed that HHV-6A and HHV-6B behave as independent viruses.

Isoforms of cellular receptors

HHV-6A and -6B are characterized by a relatively broad tropism for different human cells, whereas the species specificity is narrow with only few susceptible nonhuman primates. The discovery by Lusso and colleagues of CD46 as a HHV-6A/B receptor provided some of the explanations to these findings (Santoro et al., 1999). CD46 is remarkable in that it is a receptor for a large variety of microorganisms including measles virus, all species B adenoviruses except types 3 and 7, *Streptococcus pyogenes*, *Neisseria gonorrhoea*, and *Neisseria meningitides* (Liszewski et al., 2005). The structure of CD46 contains 4 short consensus repeats (SCR), each including 4 cysteines that form two conserved disulfide bonds, a serine–threonine–proline rich domain (ST), a sequence of unknown significance, a transmembrane region (TM) and a cytoplasmic sequence (CYT) (Fig. 1). The CD46 gene comprises 14 exons and gives rise to a number of isoforms caused by alternative splicing of the ST, TM and CYT domains, whereas the SCR domains are constant. CD46 is ubiquitously expressed on all nucleated cells and belongs to a family of regulators of complement activation.

Using deletion mutants of CD46, Mori et al. identified SCR2, SCR3, and SCR4 as binding site for HHV-6A strain U1102 (HHV-$6A_{U1102}$) (Mori et al., 2002). The necessity of SCR4 remains controversial, since Greenstone et al. using a number of truncations and molecular chimeras of CD46 and DAF (CD55) identified SCR2 and SCR3 as the binding domain for HHV-$6A_{GS}$ (Greenstone et al., 2002). Indeed, Mori et al. were able to block fusion of HHV-$6A_{GS}$ by antibodies specific for SCR2 and 3, but not by antibodies against SCR1 (Mori et al., 2002). Despite the presence of SCR in DAF, which also belongs to the family of regulators of complement activation, they do not function as a receptor

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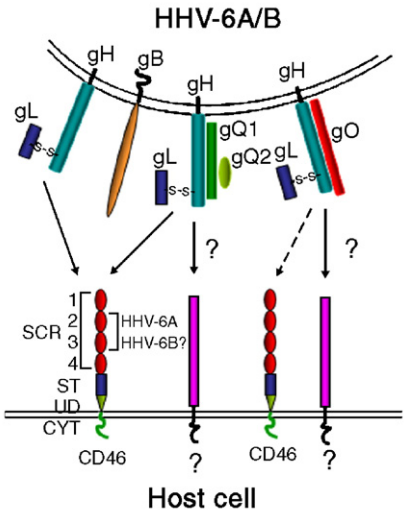

Fig. 1. Complexities in HHV-6A/B glycoprotein interactions with cellular receptors. The tetrameric complex gH–gH–gQ1–gQ2 may interact with CD46 and potentially other cellular receptors. HHV-6A (and probably HHV-6B) binds to the short consensus repeats (SCR) 2 and 3. The complex gH–gL–gO does not bind to or may have low affinity for CD46. In contrast, this complex may interact with a novel HHV-6A/B receptor. It remains to be determined whether HHV-6B is quantitatively or qualitatively different from HHV-6A. See text for further details.

for HHV-$6A_{GS}$. The ST domain is made up of one to three exons denoted A, B, and C. Although all the major isoforms of the ST domain in CD46 bind HHV-$6A_{U1102}$, they do so with different affinities with ST^{C} and ST^{BC} being the most effective as receptors for induction of fusion (Mori et al., 2002). HHV-6B is also dependent on CD46 for fusion, but whether or not it uses the same SCRs as HHV-6A has not been addressed.

Multiple viral protein complexes

In HHV-6A and -6B, as well as in other β-herpesviruses, gH and gL form a complex via intermolecular disulfide bridges, which is important for cell fusion events. However, the HHV-6A glycoprotein complex turned out to include additional proteins. Mori and colleagues defined two major proteins encoded by the HHV-$6A_{GS}$ U100 gene (Akkapaiboon et al., 2004). These proteins of 80 kDa and 37 kDa, denoted gQ1 and gQ2, form dimers within the cell and later interact with the gH–gL complex to form a tetrameric viral complex. Neither gQ1 nor gQ2 are predicted to contain a transmembrane region and they may be transported via association with gH. The stoichiometry of these interactions remains to be determined. Thus, it is not yet clear to what extent other forms of this complex may exist, e.g. gH–gL or gH–gL–gQ1. In analogy, infection of B cells by EBV requires the association of gp42 with the gH–gL complex, which then binds to HLA class II receptor on the host cell. However, during EBV infection of epithelial cells, gp42 is not needed and gH–gL binds a yet unknown receptor. It is therefore an interesting possibility that gH–gL alone or in different combinations may have affinity for different cellular receptors.

Cell-specific differences in glycosylation and post-transcriptional modifications

The discovery of an interaction between gH–gL and gQ proteins opens for additional complexities. The organization of the U100 gene is the most complex in the HHV-6A/B genome with an intron–exon structure that gives rise to multiply spliced mRNA transcripts. We do not know how gQ1–gQ2 form a heterodimer nor do we know how this dimer associates with the gH–gL heterodimer. Both of these gQ proteins have multiple potential N-linked glycosylation sites with prediction of 6 in gQ1 and 4 in gQ2. The broad range of HHV-6A/B tropism may allow for additional variation in cell-specific glycosylation patterns, which ultimately may affect the composition of the viral attachment/entry complex.

Likewise, the CD46 receptor contains N-linked glycosylations in SCR1, 2 and 4, and is heavily O-linked glycosylated in the ST domain. Although the B exon of ST is encoding only 15 amino acids, the majority of O-linked glycosylation sites are within this sequence (Post et al., 1991). Importantly, the distribution of ST^{BC} and ST^{C} is tissue specific with ST^{BC} being the predominant form expressed in the salivary glands and ST^{C} being predominantly expressed in the brain (Johnstone et al., 1993). Indeed, both HHV-6A and -6B may infect the brain, but HHV-6A is thought to be more neurotropic than HHV-6B (De Bolle et al., 2005; Ahlqvist et al., 2005). In contrast, HHV-6B may be more predominantly expressed in the salivary glands. Whether these differences in tropism are influenced by isoforms of the ST domain and thereby by differences in O-linked glycosylation of CD46 remain to be investigated. HHV-6A and HHV-6B also display differences in tropism for T lymphocytes with HHV-6A infecting both CD4+ and CD8+ T cells, whereas HHV-6B appears to be inefficient in infecting CD8+ T cells (Grivel et al., 2003). Peripheral blood lymphocytes may contain both the ST^{BC} and ST^{C} isoform. Thus, the role of CD46 glycosylation during HHV-6A and -6B binding to host cells remains to be established.

Genetic differences between viral strains and variants

Whereas the gH–gL–gQ1–gQ2 complex is a receptor for HHV-$6A_{GS}$, its role as a receptor for the HHV-6B variants is less clear. The U100 gene is located in a region of the HHV-6A/B genome with the greatest variability between HHV-6A and -6B variants. Thus HHV-$6B_{Z29}$ differs up to 17% in certain exons from HHV-$6A_{U1102}$, whereas HHV-$6B_{HST}$ differs by up to 28% at the nucleotide level (Dominguez et al., 1999; Isegawa et al., 1999).

Alternative cellular receptors

The identification of CD46 as a receptor for HHV-6A/B did not entirely explain all the experimental evidence that had

accumulated on HHV-6A and -6B attachment and entry. Lusso and colleagues found that a panel of human CD4+ T-cell lines showed diversity in their ability to be infected by HHV-$6A_{GS}$ and subsequently fuse. This was not due to separate isoforms of CD46, as transfection of CD46 did not make these cells sensitive for fusion or infection. In contrast, these cells did fuse upon infection with measles virus, strongly suggesting the existence of a novel receptor (Santoro et al., 1999). In this respect, it is interesting that Mori et al. have also identified a novel trimer consisting of gH–gL in complex with the U47 gene product, gO (Mori et al., 2004). Importantly, the gH–gL–gO complex has been found on both HHV-$6A_{GS}$ and HHV-$6B_{HST}$ virions, but surprisingly the HHV-$6B_{HST}$ gH–gL–gO or gH–gL–gQ complexes did not bind CD46, and only the complex containing gQ on HHV-$6A_{GS}$ bound CD46. This suggests that gH–gL–gO may be a viral complex for a novel cellular receptor. One should, however, be careful with the interpretation of the lack of CD46 binding of HHV-$6B_{HST}$, since HHV-$6B_{PL-1}$ and HHV-$6B_{Z29}$ clearly use CD46 (Pedersen et al., in press; Santoro et al., 1999). Nevertheless, these findings may either reflect differences in the affinity towards CD46 between the variants and perhaps even between strains within a variant, or the dependency of additional receptors, or both.

What began as a simple one viral protein versus one cellular receptor has broadened substantially in complexity. With a "backbone" of gH–gL as other herpesviruses, HHV-6A and -6B incorporate additional HHV-6A/B-specific glycoproteins to form multichain complexes to adhere, fuse and enter the host cells. There may be further surprises ahead in the composition of this complex, as the recruited glycoproteins originate from highly spliced genes in the virus, and the glycoproteins contain multiple potential glycosylation sites that may lead to different glycosylation or other post-translational modifications in different host cells. In addition, the receptor being recognized is subject to multiple isoforms, and although HHV-$6A_{U1102}$ may bind to all of them, it does so with different affinity. Finally, evidence indicates that at least one yet unknown cellular receptor must exist to explain the differences between the HHV-6A and -6B variants.

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