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Stuck in the middle: structural insights into the role of gH/gL heterodimer in herpesvirus entry

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Abstract

Enveloped viruses enter cells by fusing the viral and cellular membranes, and most use a single viral envelope protein that combines receptor-binding and fusogenic functions. In herpesviruses, these functions are distributed among multiple proteins: the conserved fusion protein gB, various non-conserved receptor-binding proteins, and the conserved gH/gL heterodimer that curiously lacks an apparent counterpart in other enveloped viruses. Recent structural studies of gH/gL from HSV-2 and EBV revealed a unique complex with no structural or functional similarity to other viral proteins. Here we analyzed gH/gL structures and highlighted important functional regions. We propose that gH/gL functions as an adaptor that transmits the triggering signals from various non-conserved inputs to the highly conserved fusion protein gB.

Introduction

Enveloped viruses enter host cells by fusing their envelopes with the cellular plasma membrane or the membrane of an endocytic vesicle. This process is initiated by binding of a virus to its cellular receptor and is catalyzed by a viral fusion protein. In most enveloped viruses, a single protein is responsible for the receptor binding and the fusogenic functions. Conformational changes in the receptor-binding module upon receptor interaction are thought to trigger fusogenic conformational changes in the fusion module. In some viruses, such as paramyxoviruses, these two functions are split between two viral proteins.

Herpesviruses are a large family of dsDNA, enveloped viruses. Eight human herpesviruses cause life-long infections and a variety of diseases, including skin lesions, encephalitis, cancers, and disseminated disease in the immunocompromised. Like all enveloped viruses, herpesviruses enter cells by fusing their envelopes with the host membrane. But, unlike most enveloped viruses, all herpesviruses utilize complex multiprotein entry machinery that consists of three conserved proteins: gB, gH, and gL, plus additional non-conserved proteins [1]. This level of complexity is remarkable and raises the question virologists have asked for several decades: why do herpesviruses need several proteins to achieve entry?

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In herpesviruses, the receptor-binding and the fusogenic functions are distributed among multiple proteins. The mechanistic details are best understood for Herpes Simplex viruses Type 1 and 2 (HSV-1 and HSV-2) and Epstein-Barr virus (EBV). The receptor-binding function is typically associated with a non-conserved protein, such as HSV gD [2] and EBV gp42 [3]. Binding of gD or gp42 to their cellular receptors is thought to trigger the conserved membrane fusion machinery of herpesviruses composed of glycoprotein gB and the gH/gL heterodimer. gB is class III viral fusion protein [4], sharing a structural similarity with vesicular stomatitis virus glycoprotein G [5] and baculovirus gp64. The precise function of the gH/gL heterodimer is unclear, although it interacts with gB [6] and is required for gB function. Some herpesviruses such as Kaposi's sarcoma herpesvirus (KSHV) use gH/gL to bind cellular receptors [7], but the requirement of gH/gL for entry in all herpesviruses indicates a more conserved function. Initially postulated to have fusogenic properties, gH/gL does not resemble any known viral fusion proteins nor does it have a known functional counterpart in other enveloped viruses.

This review focuses on how structural biology has shed a new light on this enigmatic component of the conserved fusion machinery of herpesviruses. Because structures are available for HSV-2 [8] and EBV gH/gL complexes [9], the review primarily discusses these proteins. A partial structure of PRV gH [10] is available but is not discussed here in detail. We examine what is known about the gH/gL complex through the prism of the recent structural findings and map functional information onto structures to gain clues regarding the mechanism of gH/gL function. In essence, gH/gL receives a triggering signal from a receptor or receptor-binding protein and transmits it to gB, working as a middleman.

Crystal structures of HSV-2 and EBV gH/gL: similar domains, different arrangements

gH has a large ectodomain and a single C-terminal transmembrane anchor while gL lacks a transmembrane region. The two proteins form a stable 1:1 complex. The crystal structure of gH/gL ectodomain from HSV-2 [8] revealed an unusual boot-shaped complex (Fig. 1a), reminiscent of the 15-20-nm curved glycoprotein spikes observed by cryo-electron tomography [11]. Given the conservation of gH/gL in sequence and function, it was a surprise when the subsequently determined structure of EBV gH/gL [9] showed a cylindrical molecule (Fig. 1a). Yet despite different spatial arrangement, individual domains within HSV-2 gH align well with their EBV counterparts (Fig. 1b). To highlight fundamental similarities between HSV and EBV gH molecules and to reconcile somewhat different domain assignments proposed for the two structures, we divided gH into H1A, H1B, H2A, H2B, and H3 domains, from N to C terminus (Fig. 1a,b). The distinct shapes of two gH/gL complexes, boot *vs* cylinder, arise from differences in relative domain orientations, especially at the H1B/gL and H2A/H2B interfaces, demonstrating a certain structural plasticity.

gL is "sandwiched" between domains H1A and H1B and interacts extensively with both, illustrating why gH and gL are always found as a complex. H1A forms a continuous 5-stranded β-sheet with gL and is probably unstructured in the absence of gL. H1A is the target of several anti-HSV neutralizing (or inhibiting spread) antibodies [12, 13] and some mutations that disrupt HSV fusion [14] or affect VZV tropism [15]. No functional role has yet been assigned to the much smaller H1A of EBV gH/gL. The relatively unstructured N terminus of domain H1B has many contacts with gL as well as a long lasso-like loop that interacts with H2A and H2B. The C terminus of domain H1B forms a 5-stranded β-sheet, a "picket-fence" [9] that wraps around domain H2A. Both EBV and HSV gH/gL contain non-conserved integrin-binding motifs within domain H1B.

Sequence conservation in gH increases from N to C terminus, so domains H2A, H2B, and H3 share a higher degree of structural similarity. The presence of a three-helix bundle in H2A evokes a structural similarity with other helical bundles, e.g., syntaxin, while the helical arrangement in the crescent-shaped H2B is reminiscent of HEAT repeats [10].

The C-terminal domain H3 has a β-sandwich fold. It is the most highly conserved domain and likely critical for fusion. H3 in HSV, EBV, and VZV gH is very sensitive to point mutations and insertions as most abolish fusion [15-19] or alter tropism in EBV [18-20]. A long extended polypeptide, termed a “flap” [10], wraps around one side of the β-sandwich (Fig. 2a,b). The hydrophobic surface patch underneath the flap was postulated to interact with membranes, were the flap to relocate [10]. In support of this idea, replacement of some residues with more hydrophobic ones enhances fusion or rescues fusion reduced by other nearby mutations [15, 19] (Fig. 2a,b). The relocation of the flap and membrane interaction remains to be demonstrated.

Herpesvirus gL: a chemokine connection?

gL is required for correct folding, surface expression, and function of gH in HSV [13, 21, 22] and EBV [23]. gL is thus a scaffolding protein for gH [8], perhaps, with additional functions. Despite the lack of obvious sequence similarity, the HSV-2 and EBV gL structures can be aligned (Fig. 1c). Both structures feature a chemokine fold - a three-stranded β-sheet and a helix (Fig. 1c) - recognized earlier through bioinformatics analysis [24]. A CC chemokine motif consisting of two adjacent conserved disulfide bonds is found in EBV gL, while in HSV gL, a single disulfide bond stabilizes the chemokine fold (Fig. 1c). These gL structures align well to both CC and CXC chemokines. Many chemokines dimerize by forming an intermolecular β-sheet. Interestingly, a structurally analogous β-sheet is formed by residues from domain H1A and gL (Fig. 1d). These similarities raise the interesting possibility that the gL gene may have originated from a chemokine. Surprisingly, the HCMV gL sequence cannot be aligned with either HSV or EBV gL and probably does not share a chemokine fold. Instead, CMV UL128, a component of CMV gH/gL/UL128/UL130/UL131 complex, shares sequence similarity with CC chemokines [25]. Moreover, its rat homolog functions as a CC chemokine [26]. HSV gL is secreted from mammalian cells in the absence of gH [27, 28], and soluble gL could, in principle, be produced during infection. It is unknown, however, whether gL can function as a chemokine, and this possibility awaits future investigation.

Activation of gH/gL by receptor-binding proteins and receptors

The ability of herpesviruses to enter multiple cell types is reflected in the variety of viral glycoproteins used to engage cellular receptors [1]. HSV-1 and HSV-2 use gD to bind nectin-1 on epithelial cells and neurons and herpesvirus entry mediator (HVEM) on lymphocytes [2, 29, 30]. In EBV, gp42 binds HLA Class II molecules during B cell entry [3, 31] while gH/gL binds αvβ5, αvβ6, and αvβ8 integrins on epithelial cells [32, 33]. Entry of CMV into fibroblasts requires a gH/gL heterodimer [34] but entry into epithelial and endothelial cells requires a pentameric complex gH/gL/UL128/UL130/UL131 [35, 36].

Binding of these viral proteins to their cellular receptors triggers membrane fusion, likely by first activating gH/gL. In HSV, receptor-bound gD presumably interacts with gH/gL [6, 37], but the gD-gH/gL complex has not yet been captured directly and the gD-binding site on gH/gL has not been identified. More is known about the site in EBV gH/gL that receives a triggering signal from the receptor. During entry into epithelial cells, gH/gL receives a triggering signal directly from integrin receptors [32, 33]. During B cell entry, gH/gL receives a triggering signal from HLA class II through gp42, which it binds [38]. The binding sites for integrins and gp42 in gH/gL likely overlap because mutation of the integrin

motif KGD reduces not only binding to epithelial cells and epithelial cell fusion but also binding to gp42 and fusion with B cells [39]. Thus, gp42 acts as a tropism switch by binding the integrin-binding site and inhibiting the gH/integrin interaction while simultaneously promoting entry into B cells. Importantly, regardless of where the triggering signal originates, EBV gH/gL receives it in the same location (Fig. 2b). The integrin-binding motif RGD in HSV gH [40] is neither conserved nor required for gH/gL function [17].

gB-gH/gL binding and activation of gB by gH/gL

Activated gH/gL is thought to help gB achieve its fusogenic potential [8, 41]. In HSV, gB and gH/gL interact, presumably through their ectodomains, only after gH/gL has been exposed to receptor-bound gD [6, 42]. This suggests that gH/gL activates gB in response to a gD/receptor interaction rather than represses gB in its absence. The gB-gH/gL interaction precedes membrane fusion, is inhibited by neutralizing antibodies, and is required for fusion [8, 41, 42]. Although the gB-binding site on gH/gL has not yet been identified, anti-HSV-1 gH/gL neutralizing antibody LP11 blocks interaction. The LP11 epitope is defined by monoclonal antibody neutralization resistance (mar) mutations [43] and several insertion mutants that block LP11 binding [17]. Because LP11 competes with gB for binding to gH/gL, the gB binding site on gH/gL likely overlaps the putative LP11 epitope and has been proposed to be located in a nearby groove [8] (Fig. 2a).

Little is known about how gB-gH/gL binding triggers membrane fusion. Interestingly, anti-HSV-1 gH/gL neutralizing antibody 52S blocks fusion without blocking the gB-gH/gL interaction [8]. The 52S epitope in HSV-1 gH is defined by mar mutations [43] and maps to the opposite side of gH/gL from the LP11 epitope (Fig. 2c). This explains why LP11-resistant viruses are sensitive to 52S and *vice versa* [43]. Less clear is how 52S blocks fusion. While it allows gB-gH/gL interaction, it may block the next step: gB activation. We propose that 52S prevents a conformational change that gH/gL must undergo to activate bound gB. In our model, gB binds to its putative site near the LP11 epitope (Fig. 2a,c). This binding impinges upon the well-aligned series of alpha helices in domains H2A and H2B, displacing them along with the helix containing the 52S epitope (Fig. 2c). We propose that gH/gL requires this or a similar kind of a conformational change to activate gB, and that 52S binding blocks this conformational change and thus gB activation. Such long-range conformational change would require a certain structural flexibility within gH/gL, which is consistent with the fact that both H2A and H2B tolerate insertions, a few in buried regions [17]. A double point mutation in VZV gH, designed to destabilize helix-helix interactions, is fusion-null [15] and pinpoints a region that could be critical in transmitting the gB-activating intramolecular signal within gH/gL (Fig. 2c).

No known functional sites have yet been reported within a structurally analogous region of EBV gH/gL. Interestingly, in EBV gH/gL, a nearby site may be involved in binding gB or controlling its activation through a long-range effect (Fig. 2b). gL from a closely-related rhesus lymphocryptovirus (Rh-LCV) binds EBV gH, but does not substitute for EBV gL in virus-free cell fusion assays. Yet, replacing residues 54 and 94 in Rh-LCV gL with their EBV gL counterparts restores full function [44], suggesting that these residues somehow specify the selection of gB. Further experiments are needed to clarify where gH/gL binds gB and how this interaction activates gB for fusion. Considering conservation of gH/gL and gB, we expect that the gB-binding site is likely conserved in all herpesviruses. But, given the structural plasticity of gH/gL domain arrangement, the gB-activating conformational changes within gH/gL could vary.

Conclusions

Why do all herpesviruses require gH/gL, an additional protein shuttling signals from the receptor-binding protein to the fusion protein? Why not cut out this middleman? It is notable that fusion protein gB is highly conserved across all herpesviruses while cellular entry receptors and viral receptor-binding proteins vary greatly (Fig. 3). The evolutionary pressure on gB to maintain function as a fusogen may be too high to allow it to evolve to accommodate many different inputs. The high sequence and structure conservation of gB supports this idea. Perhaps, the solution was to use an “adaptor” that could relate different triggering inputs to a conserved fusion protein. We propose that the main function of gH/gL is to adapt and transmit activating signals to gB, thus allowing gB to be triggered by varied receptors in different herpesviruses and even within the same virus. While conserved, gH/gL sequences and structures are more divergent than those of gB. Structural plasticity in gH/gL may be uniquely suited for intermolecular signal transmission from the receptor or a receptor-binding protein to fusion protein gB. This plasticity may have permitted the divergent evolution of gH/gL and customization for different triggering partners. The versatility provided by gH/gL allows activation signals from a multitude of sources to be transmitted to gB to accommodate entry of herpesviruses into different cell types. Herpesviruses are among the most ubiquitous in nature, targeting nearly all mammals and even non-mammals. The presence of gH/gL as an evolutionarily malleable adaptor could have allowed herpesviruses to acquire new cell targets without compromising the function in fusion protein gB.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

1*. Heldwein EE, Krummenacher C. Entry of herpesviruses into mammalian cells. Cell Mol Life Sci. 2008; 65(11):1653–68. [PubMed: 18351291] This paper provides a comprehensive review of multiple entry receptors used by various human herpesviruses and their viral ligands.

2. Spear PG. Herpes simplex virus: receptors and ligands for cell entry. Cell Microbiol. 2004; 6(5): 401–10. [PubMed: 15056211]

3. Li Q, et al. Epstein-Barr virus uses HLA class II as a cofactor for infection of B lymphocytes. J Virol. 1997; 71(6):4657–62. [PubMed: 9151859]

4. Heldwein EE, et al. Crystal structure of glycoprotein B from herpes simplex virus 1. Science. 2006; 313(5784):217–220. [PubMed: 16840698]

5. Roche S, et al. Crystal structure of the low-pH form of the vesicular stomatitis virus glycoprotein G. Science. 2006; 313(5784):187–191. [PubMed: 16840692]

6. Atanasiu D, et al. Bimolecular complementation reveals that glycoproteins gB and gH/gL of herpes simplex virus interact with each other during cell fusion. Proc Natl Acad Sci U S A. 2007; 104(47): 18718–23. [PubMed: 18003913]

7. Hahn AS, et al. The ephrin receptor tyrosine kinase A2 is a cellular receptor for Kaposi’s sarcoma-associated herpesvirus. Nature medicine. 2012; 18(6):961–6.

8*. Chowdary TK, et al. Crystal structure of the conserved herpesvirus fusion regulator complex gH-gL. Nat Struct Mol Biol. 2010; 17(7):882–8. [PubMed: 20601960] This paper reports the first

gH/gL structure, reveals that gH/gL does not resemble a viral fusion protein, and provides evidence that gH/gL may activate gB for fusion.

9*. Matsuura H, et al. Crystal structure of the Epstein-Barr virus (EBV) glycoprotein H/glycoprotein L (gH/gL) complex. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107(52):22641–6. [PubMed: 21149717] This paper reports the second gH/gL structure showing a different overall shape despite similarity within individual domains.

10. Backovic M, et al. Structure of a core fragment of glycoprotein H from pseudorabies virus in complex with antibody. Proc Natl Acad Sci U S A. 2010; 107(52):22635–40. [PubMed: 21149698]

11. Grunewald K, et al. Three-dimensional structure of herpes simplex virus from cryo-electron tomography. Science. 2003; 302(5649):1396–8. [PubMed: 14631040]

12. Peng T, et al. Structural and antigenic analysis of a truncated form of the herpes simplex virus glycoprotein gH-gL complex. J Virol. 1998; 72(7):6092–6103. [PubMed: 9621073]

13. Cairns TM, et al. Epitope mapping of herpes simplex virus type 2 gH/gL defines distinct antigenic sites, including some associated with biological function. J Virol. 2006; 80(6):2596–2608. [PubMed: 16501070]

14. Jackson JO, et al. Insertion mutations in herpes simplex virus 1 glycoprotein H reduce cell surface expression, slow the rate of cell fusion, or abrogate functions in cell fusion and viral entry. J Virol. 2010; 84(4):2038–46. [PubMed: 20007280]

15. Vleck SE, et al. Structure-function analysis of varicella-zoster virus glycoprotein H identifies domain-specific roles for fusion and skin tropism. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(45):18412–7. [PubMed: 22025718]

16. Cairns TM, et al. Contribution of cysteine residues to the structure and function of herpes simplex virus gH/gL. Virology. 2005; 332(2):550–562. [PubMed: 15680420]

17. Galdiero M, et al. Site-Directed and linker Insertion Mutangenesis of Herpes Simplex Virus Type 1 Glycoprotein H. J. Virol. 1997; 71(3):2163–2170. [PubMed: 9032350]

18. Wu L, Borza CM, Hutt-Fletcher LM. Mutations of Epstein-Barr virus gH that are differentially able to support fusion with B cells or epithelial cells. Journal of virology. 2005; 79(17):10923–30. [PubMed: 16103144]

19. Wu L, Hutt-Fletcher LM. Point mutations in EBV gH that abrogate or differentially affect B cell and epithelial cell fusion. Virology. 2007; 363(1):148–55. [PubMed: 17307213]

20. Molesworth SJ, et al. Epstein-Barr virus gH is essential for penetration of B cells but also plays a role in attachment of virus to epithelial cells. J Virol. 2000; 74(14):6324–6332. [PubMed: 10864642]

21. Hutchinson L, et al. A novel herpes simplex virus glycoprotein, gL, forms a complex with glycoprotein H (gH) and affects normal folding and surface expression of gH. J Virol. 1992; 66(4): 2240–50. [PubMed: 1312629]

22. Roop C, Hutchinson L, Johnson DC. A mutant herpes simplex virus type 1 unable to express glycoprotein L cannot enter cells, and its particles lack glycoprotein H. J Virol. 1993; 67(4):2285–97. [PubMed: 8383241]

23. Yaswen LR, et al. Epstein-Barr virus glycoprotein gp85 associates with the BKRF2 gene product and is incompletely processed as a recombinant protein. Virology. 1993; 195(2):387–396. [PubMed: 8393232]

24. Wyrwicz LS, Rychlewski L. Herpes glycoprotein gL is distantly related to chemokine receptor ligands. Antiviral research. 2007; 75(1):83–6. [PubMed: 17215049]

25. Akter P, et al. Two novel spliced genes in human cytomegalovirus. The Journal of general virology. 2003; 84(Pt 5):1117–22. [PubMed: 12692276]

26. Vomaske J, et al. Cytomegalovirus CC-Chemokine Promotes Immune Cell Migration. Journal of virology. 2012

27. Dubin G, Jiang H. Expression of herpes simplex virus type 1 glycoprotein L (gL) in transfected mammalian cells: evidence that gL is not independently anchored to cell membranes. J. Virol. 1995; 69(7):4564–4568. [PubMed: 7769724]

28. Peng T, et al. The gH-gL complex of herpes simplex virus (HSV) stimulates neutralizing antibody and protects mice against HSV type 1 challenge. J. Virol. 1998; 72:65–72. [PubMed: 9420201]

29. Carfi A, et al. Herpes simplex virus glycoprotein D bound to the human receptor HveA. Mol Cell. 2001; 8(1):169–179. [PubMed: 11511370]

30. Di Giovine P, et al. Structure of herpes simplex virus glycoprotein D bound to the human receptor nectin-1. PLoS pathogens. 2011; 7(9):e1002277. [PubMed: 21980294]

31. Mullen MM, et al. Structure of the Epstein-Barr virus gp42 protein bound to the MHC class II receptor HLA-DR1. Mol Cell. 2002; 9(2):375–385. [PubMed: 11864610]

32. Chesnokova LS, Hutt-Fletcher LM. Fusion of Epstein-Barr virus with epithelial cells can be triggered by alphavbeta5 in addition to alphavbeta6 and alphavbeta8, and integrin binding triggers a conformational change in glycoproteins gHgL. Journal of virology. 2011; 85(24):13214–23. [PubMed: 21957301]

33. Chesnokova LS, Nishimura SL, Hutt-Fletcher LM. Fusion of epithelial cells by Epstein-Barr virus proteins is triggered by binding of viral glycoproteins gHgL to integrins alphavbeta6 or alphavbeta8. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106(48):20464–9. [PubMed: 19920174]

34. Wille PT, et al. A human cytomegalovirus gO-null mutant fails to incorporate gH/gL into the virion envelope and is unable to enter fibroblasts and epithelial and endothelial cells. Journal of virology. 2010; 84(5):2585–96. [PubMed: 20032184]

35*. Ryckman BJ, et al. Characterization of the human cytomegalovirus gH/gL/UL128-131 complex that mediates entry into epithelial and endothelial cells. J Virol. 2008; 82(1):60–70. [PubMed: 17942555] This paper describes the unusual pentameric complex containing gH/gL allowing CMV to enter epithelial cells.

36. Ryckman BJ, Chase MC, Johnson DC. HCMV gH/gL/UL128-131 interferes with virus entry into epithelial cells: evidence for cell type-specific receptors. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(37):14118–23. [PubMed: 18768787]

37. Avitabile E, Forghieri C, Campadelli-Fiume G. Complexes between herpes simplex virus glycoproteins gD, gB, and gH detected in cells by complementation of split enhanced green fluorescent protein. J Virol. 2007; 81(20):11532–11537. [PubMed: 17670828]

38. Kirschner AN, et al. Soluble Epstein-Barr virus glycoproteins gH, gL, and gp42 form a 1:1:1 stable complex that acts like soluble gp42 in B-cell fusion but not in epithelial cell fusion. J Virol. 2006; 80(19):9444–9454. [PubMed: 16973550]

39*. Chen J, et al. The KGD motif of Epstein-Barr virus gH/gL is bifunctional, orchestrating infection of B cells and epithelial cells. MBio. 2012; 3(1) This paper shows that the KGD motif in EBV gH/gL is critical for binding both gp42 and integrins and clarifies the mechanism by which EBV switches tropism (B cells vs epithelial cells).

40. Parry C, et al. Herpes simplex virus type 1 glycoprotein H binds to alphavbeta3 integrins. J Gen Virol. 2005; 86(Pt 1):7–10. [PubMed: 15604426]

41*. Atanasiu D, et al. Cascade of events governing cell-cell fusion induced by herpes simplex virus glycoproteins gD, gH/gL, and gB. Journal of virology. 2010; 84(23):12292–9. [PubMed: 20861251] This paper provides the first strong evidence to support the theory of triggering signal transmitted from gD/receptor to gH/gL and then gB.

42. Atanasiu D, et al. Bimolecular complementation defines functional regions of Herpes simplex virus gB that are involved with gH/gL as a necessary step leading to cell fusion. J Virol. 2010; 84(8): 3825–34. [PubMed: 20130048]

43. Gompels UA, et al. Characterization and sequence analyses of antibody-selected antigenic variants of herpes simplex virus show a conformationally complex epitope on glycoprotein H. J Virol. 1991; 65(5):2393–401. [PubMed: 1707982]

44. Plate AE, et al. Functional analysis of glycoprotein L (gL) from rhesus lymphocryptovirus in Epstein-Barr virus-mediated cell fusion indicates a direct role of gL in gB-induced membrane fusion. J Virol. 2009; 83(15):7678–89. [PubMed: 19457993]

45. Ren M, et al. Polymerization of MIP-1 chemokine (CCL3 and CCL4) and clearance of MIP-1 by insulin-degrading enzyme. The EMBO journal. 2010; 29(23):3952–66. [PubMed: 20959807]

46. Holm L, Rosenstrom P. Dali server: conservation mapping in 3D. Nucleic acids research. 2010; 38(Web Server issue):W545–9. [PubMed: 20457744]

47. Emsley P, et al. Features and development of Coot. Acta crystallographica. Section D, Biological crystallography. 2010; 66(Pt 4):486–501.

48. Xiong JP, et al. Crystal structure of the complete integrin alphaVbeta3 ectodomain plus an alpha/beta transmembrane fragment. The Journal of cell biology. 2009; 186(4):589–600. [PubMed: 19704023]

49. Backovic M, Longnecker R, Jardetzky TS. Structure of a trimeric variant of the Epstein-Barr virus glycoprotein B. Proc Natl Acad Sci U S A. 2009; 106(8):2880–5. [PubMed: 19196955]

Highlights

- Herpesviruses require multiple viral proteins to achieve cell entry.
- Receptor-binding and fusogenic functions are distributed among several proteins.
- Conserved gH/gL lacks apparent functional analogs in other viruses.
- HSV-2 and EBV gH/gL structures reveal structural plasticity and important functional regions.
- gH/gL is an adaptor transmitting different signals to conserved fusion protein gB.

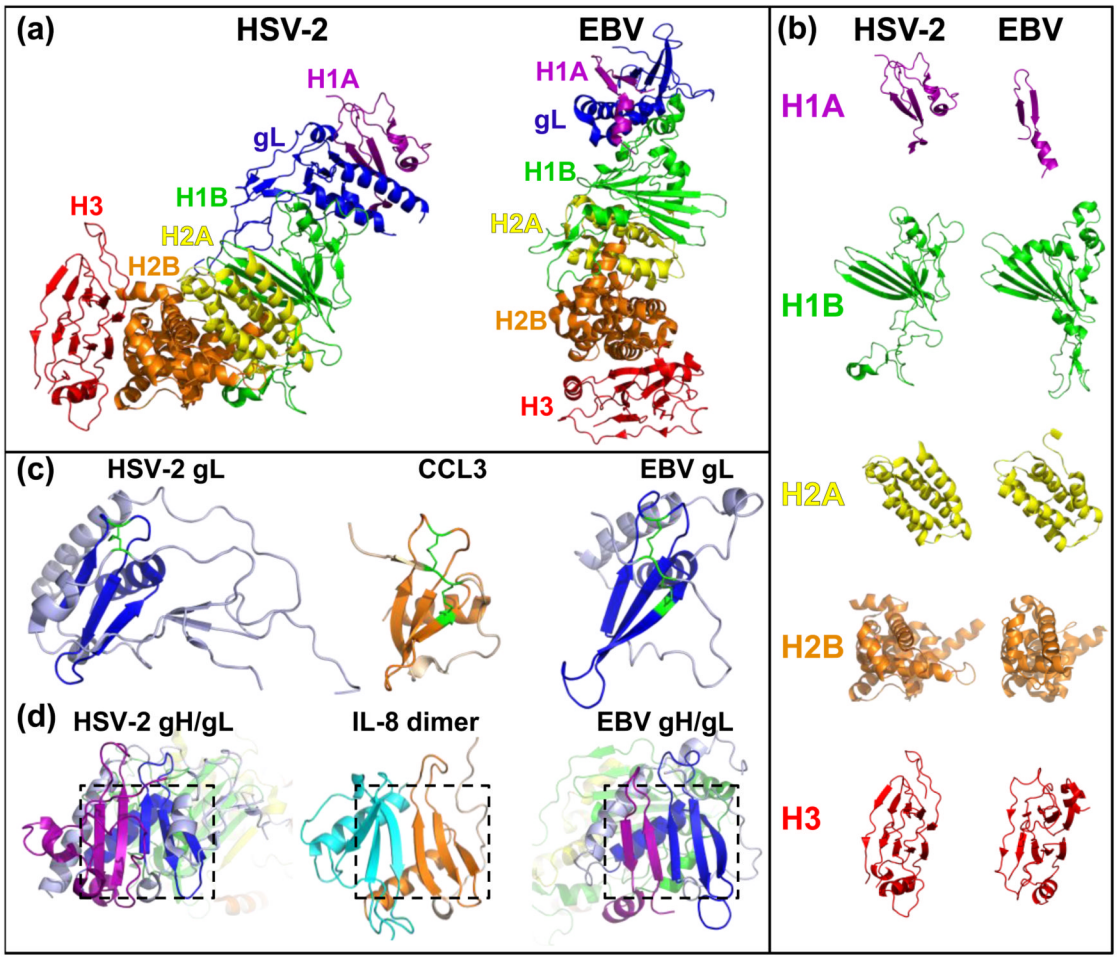

Fig. 1.

Structural similarities between HSV and EBV gH/gL. (a) Structures of HSV-2 gH/gL (PDB: 3M1C) [8] and EBV gH/gL (PDB: 3PHF) [9] are colored by domain, and all domains are labeled. (b) Aligned individual domains are shown side by side and labeled. Color scheme matches that in (a). HSV-2 gH domains are shown in the same orientation as in (a). EBV gH domains are aligned onto HSV domains and are shown in the same orientation. HSV Domains: H1A: 19-120, H1B: 121-331, H2A: 332-441, H2B: 442-645, H3: 646-797. EBV Domains: H1A: 19-48, H1B: 49-238, H2A: 239-344, H2B: 345-527, H3: 528-677. (c-d) HSV-2 and EBV gL share structural similarities with chemokines. (c) Chemokine folds of HSV-2 and EBV gL (blue) align well with the CC chemokine CCL3 (orange, PDB: 2X6G-P) [45] (orange) and other chemokines (not shown) using DALI [46]. Disulfides are shown in green. Non-chemokine-fold regions in all three molecules are shown using lighter shades. (d) A continuous intermolecular β-sheet in IL8 homodimer is mimicked in gH/gL heterodimer. IL8 monomers are shown in orange and cyan, HSV-2 gH/gL and EBV gH/gL are shown using the same colors as in (a) and (b). Intermolecular sheets are outlined. All structures were made using Pymol (http://www.pymol.org). Alignments were carried out using Coot [47].

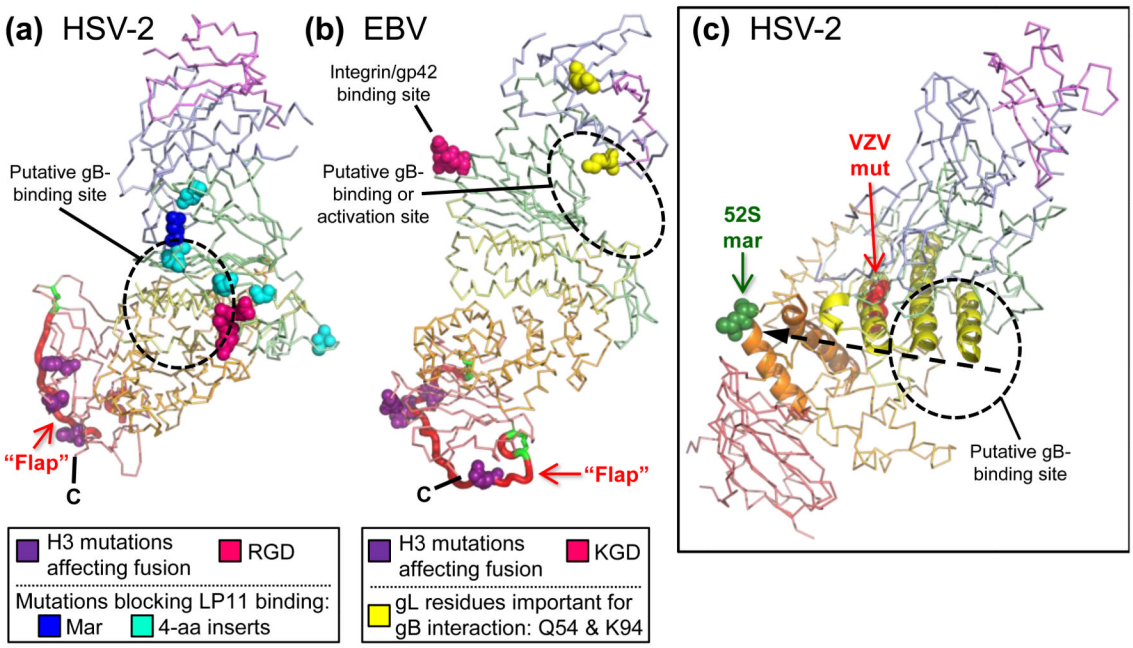

Fig. 2.

Functional sites in HSV-2 and EBV gH/gL. HSV-1 gH/gL (a) and EBV gH/gL (b) are shown using wireframe representation and paler colors than in Fig. 1. An extended polypeptide in H3, the "flap", is shown as a thick tube. Disulfide bonds that anchor it are shown in green, and key functional residues, in purple. (a) Functional sites in HSV gH/gL. Residues that define the LP11 epitope are shown in blue (mar mutations [43]) and insertions that block LP11 binding [17] are shown in cyan. The putative gB-binding site [8] is outlined. A RGD motif is shown in magenta. (b) Functional sites in EBV gH/gL. A KGD motif (magenta) is the putative binding site for integrins and gp42 as both are affected by mutations in the KGD motif [39]. gL residues Q54 and K94 within the putative gB activation site [44] (outlined) are shown in blue. (c) Proposed mechanism of fusion inhibition by anti-HSV-1-gH/gL mAb 52S. Binding of gB in a conserved groove (outlined) displaces a stack of α helices (shown as ribbons) and impinges on the 52S epitope. Arrow indicates the hypothetical direction of the displacement. Concomitant conformational changes may allow gH/gL to bend around gB, delivering an activation signal to gB. Binding of 52S antibody may prevent such dislocation and thus transmission of the activation signal. Residues mutated in 52S mar mutant [43] are shown in green; the putative gB-binding site in gH/gL is outlined. The fusion-null mutation in VZV gH/gL (red) marks the region that may be involved in transmitting the gB-activating intramolecular signal within gH/gL.

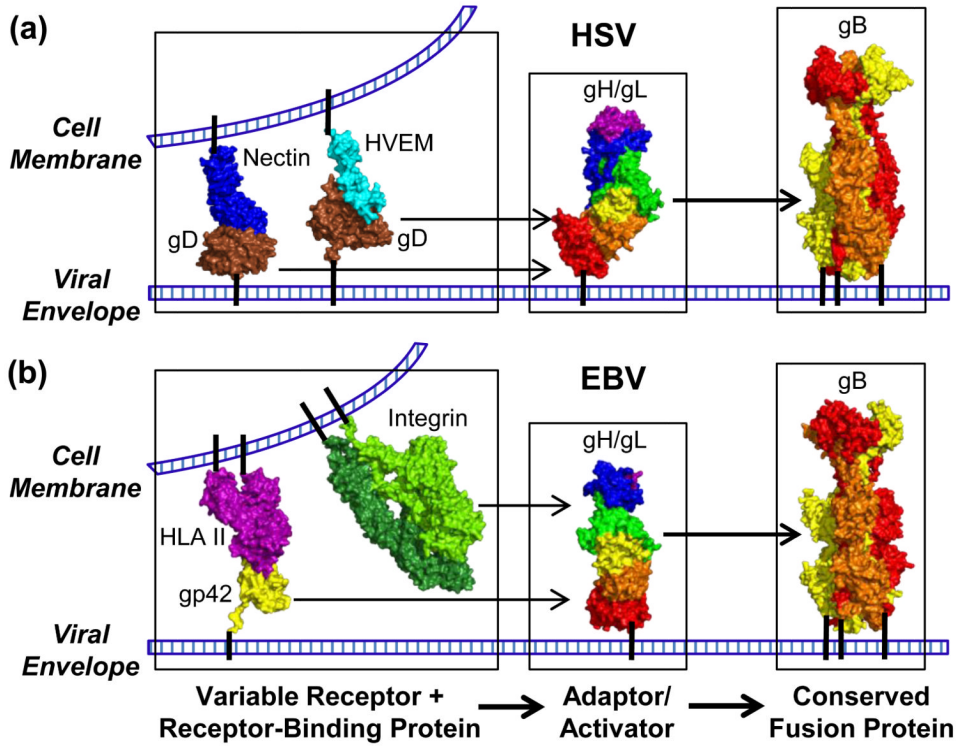

Fig. 3.

A variety of receptor inputs are transmitted through gH/gL to the conserved fusion protein gB. Cell entry machinery of HSV-2 (top) and EBV (bottom). All crystal structures are shown in surface representation. Top: HSV-1 gD/HVEM (PDB: 1JMA) [29], HSV-1 gD/nectin (PDB: 3SKU) [30], HSV-2 gH/gL (PDB: 3M1C) [8], and HSV-1 gB (PDB: 2GUM) [4]. Bottom: EBV gp42/HLA-DR1 (PDB: 1KG0) [31], αVβ3 integrin (PDB: 3IJE) [48], (used in place of αVβ5, αVβ6, and αVβ8, for which no structures are available), EBV gH/gL (PDB: 3PHF) [9], and EBV gB (PDB: 3FVC) [49].