3-1-2018

First Impressions—the Potential of Altering Initial Host-Virus Interactions for Rational Design of Herpesvirus Vaccine Vectors

Paul J.F. Rider
School of Veterinary Medicine

Farhana Musarrat
School of Veterinary Medicine

Rafiq Nabi
School of Veterinary Medicine

Shan Naidu
School of Veterinary Medicine

Konstantin G. Kousoulas
School of Veterinary Medicine

Recommended Citation

Rider, P., Musarrat, F., Nabi, R., Naidu, S., & Kousoulas, K. (2018). First Impressions—the Potential of Altering Initial Host-Virus Interactions for Rational Design of Herpesvirus Vaccine Vectors. *Current Clinical Microbiology Reports, 5* (1), 55-65. https://doi.org/10.1007/s40588-018-0082-1

HHS Public Access

Author manuscript

Curr Clin Microbiol Rep. Author manuscript; available in PMC 2019 March 01.

Published in final edited form as:

Curr Clin Microbiol Rep. 2018 March ; 5(1): 55–65. doi:10.1007/s40588-018-0082-1.

First Impressions-the Potential of Altering Initial Host-Virus Interactions for Rational Design of Herpesvirus Vaccine Vectors

Paul J.F. Rider[1], **Farhana Musarrat**[1], **Rafiq Nabil**[1], **Shan Naidu**[1], and **Konstantin G. Kousoulas**[1,*]

[1]Division of Biotechnology and Molecular Medicine and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA

Abstract

Purpose—The earliest host-virus interactions occur during virus attachment and entry into cells. These initial steps in the virus lifecycle influence the outcome of infection beyond delivery of the viral genome into the cell. Herpesviruses alter host signaling pathways and processes during attachment and entry to facilitate virus infection and modulate innate immune responses. We suggest in this review that understanding these early signaling events may inform the rational design of therapeutic and prevention strategies for herpesvirus infection, as well as the engineering of viral vectors for immunotherapy purposes.

Recent Findings—Recent reports demonstrate that modulation of Herpes Simplex Virus Type-1 (HSV-1) entry results in unexpected enhancement of antiviral immune responses.

Summary—A variety of evidence suggests that herpesviruses promote specific cellular signaling responses that facilitate viral replication after binding to cell surfaces, as well as during virus entry. Of particular interest is the ability of the virus to alter innate immune responses through these cellular signaling events. Uncovering the underlying immune evasion strategies may lead to the design of live-attenuated vaccines that can generate robust and protective anti-viral immune responses against herpesviruses. These adjuvant properties may be extended to a variety of heterologous antigens expressed by herpesviral vectors.

Keywords

HSV; Vaccine; herpesvirus; gK; herpesvirus vector; VC2

*To whom correspondence should be addressed: vtgusk@lsu.edu.

Compliance with Ethics Guidelines

Conflict of Interest

Dr. Kousoulas declares a pending patent for vaccines against genital herpes simplex infections (United States Patent Application 20170266275 pending).

Paul J.F. Rider, Farhana Musarrat, Rafiq Nabil and Shan Naidu declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors

Introduction

Herpes simplex viruses type-1 and type-2 (HSV-1; HSV-2) are responsible primarily for orofacial and genital infections, respectively. Importantly, HSV-1 is the leading viral cause of blindness in developed countries, and both HSV-1 and HSV-2 can cause severe fatal encephalopathies in adults and children (1). HSV-1 and HSV-2 seroprevalence is estimated at 67 percent (0–49 years of age) and at 11 percent (15–49 years of age) respectively, reflecting their ability to replicate and spread throughout the human population very efficiently (2, 3). Currently, despite many efforts over the last 20 years, there is no vaccine for HSV-1 or HSV-2, and drug-based inhibition of viral replication can often lead to the emergence of mutations that render these drugs ineffective (4, 5). As such, an HSV vaccine is desirable.

Approaches to the development of a vaccine(s) against herpes simplex viruses include subunit vaccines, as well as attenuated and replication-defective live viruses (6, 7). Currently, there are several ongoing clinical trials of subunit, DNA and replication-defective approaches to the development of a herpes simplex virus vaccine (7). Challenges include the limited efficacy of subunit vaccines, as well as the establishment of a latent infection by many attenuated and replication-defective vaccine strains. Most approaches to the generation of live virus vaccines have been to delete or mutate genes involved in important aspects of the viral life cycle, such as immune evasion genes (γ34.5), or DNA replication genes (UL5, UL9, UL29). More recently, HSV vaccine candidates harboring deletions or mutations in virion envelope glycoproteins have exhibited exceptional promise and will be highlighted in this review.

The initial interaction of virus with the host cell initiates a cascade of events that determines the outcome of infection at both the cellular and organismal levels. While traditionally these signaling pathways have been viewed from an anti-viral perspective, it is now understood that they represent complex combinations of host anti-viral signaling pathways, as well as pathways that are initiated to benefit viral infection. Recognition of viral proteins by pattern recognition receptors (PRR) such as TLR-2 can result in signaling events that initiate anti-viral pathways resulting in induction of inflammatory and/or interferon responses (8–12). Additionally, fusion of the viral envelope with cellular plasma membranes during virus entry has been shown to lead to the induction of innate immune signaling (13). Importantly, the mechanisms by which herpes simplex viruses modulate cellular signaling processes during virus entry contribute to the establishment of latency in neurons, and the ability of the virus to successfully infect a variety of different cell types (14, 15).

Herpes viruses encode an array of gene products that have been shown to modulate pathways involved in innate and adaptive immunity, differentiation, cell division, apoptosis, autophagy and metabolism (1, 16, 17). For example HSV-1 infection has been shown to induce a biphasic activation of NFκB, where attachment (18) and entry as well the viral UL37 protein induce (19) activation of NFκB pathways initially. In this regard what has been described as the herpesvirus “signalosome” (20) includes both temporal and spatial control of cellular signaling pathways initiated by both host and virus. The outcome of this complex modulation has been shown to have profound impacts on virus entry, tropism,

establishment of either a lytic or latent states, as well as immunogenicity and pathogenesis (1, 20, 21).

Herein, we emphasize that an understanding of the molecular and cell biology of viral entry and associated intracellular signaling may inform the rational design of live-attenuated vaccine candidates. Utilization of these engineered viral vectors for the expression of heterologous antigens specified by other viral, bacterial, or parasitic pathogens may generate significant immune responses against these infections. Similarly, expression of tumor antigens may augment anti-tumor immune responses. Details of the signaling pathways affected during the early stages of viral replication are beyond the scope of this review, but are the subject of excellent recent reviews to which the reader is directed (20, 22). In this review, we examine specific pathways affected by virus infection and provide examples from recent work in the field highlighting underlying cellular signaling mechanisms that lead to significant enhancement of anti-viral immune responses. The review is primarily focused on the prototypic human alphaherpesvirus, herpes simplex virus type 1 (HSV-1) with selected relevant reference to other herpesvirus species where relevant. Overall, it is anticipated that the ideas discussed here may be readily applied to other viruses.

Early events in Herpesvirus infection

Herpesviruses are among the largest and most complex virus families. Herpesvirus entry can occur by fusion and/or endocytosis and is a complex, coordinated process involving multiple virus-specified membrane proteins present in the virus envelope (23, 24). These viruses infect multiple cell types and dependent on cell type, infection can result in lytic, latent or abortive programs. The minimal membrane fusion complex for HSV includes four envelope glycoproteins: gB, gD, gH, and gL, which are necessary and sufficient to promote membrane fusion (25). Further appreciation for the complexity of the HSV fusion process is that the sole fusogeneic glycoprotein gB, unlike other type III fusion proteins, is regulated by complex interactions with other viral glycoproteins and membrane proteins (26–31).

Glycoprotein D (gD) is the main receptor binding protein of HSV. Binding of the receptor by gD (discussed in greater detail below) alters its conformation. This gD conformational change is thought to change the conformation of the gH/gL heterodimer, which ultimately triggers a conformational change in gB from a non-fusogenic to a fusogenic state (23, 24). Gaps in current knowledge include available 3-dimensional structure of only the prefusion structure of gB, as well as lack of mechanistic and structural details of the interactions between gD-gH/gL or gH/gL and gB. HSV also enters a variety of cells via endocytosis (32–35). Discussed in greater detail below, the molecular mechanisms facilitating the endocytosis of HSV are beginning to be understood (35–38). While endocytosis has been demonstrated to be critical for the pathogenesis of cytomegalovirus (39–41), it is however not clear what contribution endocytosis makes to the pathogenesis of HSV infection.

Signaling Pathways affected by Herpesvirus Glycoproteins

The most obvious opportunity for induction of intracellular events is via receptor-mediated cellular signaling. Multiple cellular receptors have been identified for HSV entry (23, 42).

HSV-1 gD binds multiple cellular receptors to facilitate entry including: Nectin-1, Nectin-2, HVEM, and 3′-O sulphated heparan sulphate (3-*OS*) (43–46). gB has also been shown to bind to PILRα, myelin associated glycoprotein (MAG) and non-muscle myosin heavy chain IIA (NMHCIIA) to facilitate entry (47–49). While it is important to keep in mind the possible contribution of alternate HSV receptors to the virus-host interaction, for purposes of this review, we will focus on HSV entry via Nectin-1 and HVEM. Nectin-1 and HVEM double knockout mice were not susceptible to infection with HSV-2 indicating these receptors are sufficient for viral infection of mice whereas other receptors may serve secondary functions during virus infection, leastways in mice (50).

The structures of HSV-1 gD bound to nectin-1 and HVEM have been determined (51, 52). Nectin-1 is considered the primary receptor for HSV entry. Intravaginal HSV-2 infection of nectin-1 deficient mice led to decrease in infectivity and spread to neural tissue (50). HSV-2 infection of CaSki cells in which nectin-1 was silenced using siRNA led to greatly reduced calcium release (53). HVEM, a member of the tumor necrosis family, is expressed on many tissues and cellular ligands for HVEM include CD160, BTLA, and LIGHT (54, 55). Dependent on which ligand is bound, HVEM ligation results in either a pro- or anti-inflammatory signaling (56). Binding of soluble gD to HVEM has been shown to activate NFκB pathways and this interaction has been exploited in subunit vaccine design (18, 57–59). Intravaginal infection with HSV-2 in mice deficient for HVEM expression does not alter pathogenesis or establishment of latency (50). However, more recent work has identified HVEM as an important determinant of ocular pathogenesis in experimental infections of mice with HSV-1 (60–62). Specifically, HVEM KO mice were less susceptible to ocular pathogenesis after ocular infection with HSV-1 (61).

While HSV gB has not been shown to bind integrins a number of herpesvirus gBs have been found to bind integrins (63, 64). Two groups have shown that HSV-1 gB binds to TLR-2 with one group demonstrating activation of TLR-2 after binding (11), while a second group did not report activation (65). PILRα, to which gB has been shown to bind, possesses an immunoreceptor tyrosine based inhibitory motif (ITIM) (66). Clustering of PILRα after ligand binding has been shown to negatively regulate neutrophil inflammation by *cis* regulation of β2 integrin activation (67). gH is a membrane protein found as heterodimer with gL and is essential for herpesvirus fusion (1, 23, 24). No membrane fusion activity has been identified for gH/gL and its function in entry is not entirely clear. Recently gH/gL specified by different alphaherpesviruses have been found to bind to integrin receptors, and may function as tropism determinants (35, 68, 69). Several reports have demonstrated the role of calcium ion fluxes and HSV entry (38, 53, 70, 71). HSV-2 gH interaction with αvβ3 is responsible for calcium release from the endoplasmic reticulum (ER) and subsequent phosphorylation of focal adhesion kinase (FAK) during virus entry (69). In support of an important role for the gH-integrin interaction it was shown that siRNA knockdown of αvβ3 prevents HSV entry (69). Consistent with these results, work from the Campadelli-Fiume laboratory has demonstrated an important role for integrins in HSV infection (8, 10, 35, 72–74). They have proposed that these integrins serve as co-receptors. A mechanism includes the finding that αvβ6 and αvβ8 integrins can promote a conformational change in gH that displaces gL to promote entry (72). Further evidence to support a role in HSV entry for these integrins includes data that they facilitate endocytosis of HSV in a variety of cell types (35).

For equine herpesvirus 1(EHV-1), another alphaherpesvirus, it has been shown that binding of gH to α4β1 integrin induces calcium signaling and that abrogation of this pathway redirects entry to the endocytic pathway (38). Multiple groups have shown that gH-integrin complex formation requires the presence of other fusion complex members, such as gD and gB to function (10, 71), implying that either gD receptor binding, or fusion complex formation is important for subsequent gH-integrin complex formation.

A role for gH binding to αvβ3 integrin and activation of innate immune signaling pathways has been demonstrated (8, 10). gH was found to bind TLR-2 directly and simultaneous binding of αvβ3 creates a bridge for TLR-2 activation (73). Binding of gH to TLR-2 and αvβ3 was shown to activate inflammatory (NFκB), as well as type I IFN signaling pathways (8, 73). A second pathway induced by αvβ3 independent of TLR-2 was found to target interferon regulatory factors −3 and −7 (8).

As mentioned earlier, the process of membrane fusion between the HSV-1 viral envelope and cellular plasma membranes during virus entry induces antiviral signaling pathways (13). Induction of a type I interferon response by HSV virus-like particles (VLPs) was shown to depend on phosphatidylinositol-3-OH kinase (PI3K). Inhibition of PI3K, but not calcium release from the ER, blocked the induction of interferon stimulated gene (ISG) expression, placing PI3K activation upstream of calcium release in this process. Interestingly, the induction of ISG by fusion of the viral envelope with cellular plasma membranes was dependent on STING, typically thought of as a sensor of cytoplasmic DNA. Recently, it was also shown that prior to entry, HSV-2 induces an innate antiviral response at the epithelium by an unknown mechanism requiring viral O-linked glycans (75).

In addition to the demonstration that PI3K is important for the induction of the innate response to HSV fusion, several groups have shown that the PI3K pathway is important for HSV entry and cell-to-cell fusion (36, 70, 76, 77); reviewed in (78). It has been reported that PI3K activation is important for actin polymerization critical to virus entry into neuronal cells (79). PI3K inhibitors were shown to block RhoA, a cellular protein involved in cytoskeleton regulation after HSV infection of fibroblast cells (76). There is an emerging role for cytoskeletal dynamics and the sensing of intracellular pathogens (80). Specifically, aberrant RhoA activation has been shown to result in the activation of NOD 1 (81, 82). It is interesting to speculate that manipulation of the cellular cytoskeleton by HSV during entry is not only to facilitate viral infection, but also to alter global cellular host responses.

Tropism

Herpesviruses have the capacity to infect a variety of cell types. It has been shown by a number of groups that herpesviruses encode distinct gene products that facilitate infection of different cell types (83–88). Interestingly, in those herpesviruses for which a tropism determining protein complex has been identified, gH/gL is the common denominator. HCMV, a betaherpesvirus, possesses distinct protein complexes which facilitate entrance into differing cell types. A protein complex of gB, gH/gL and gO mediates fusion in fibroblast cell types where a second protein complex, a pentamer, composed of gB, gH/gL, and UL128, 130 and 131 facilitates entry into epithelial and endothelial cells (89). A second

betaherpesvirus, Human herpesvirus 6 (HHV-6) has been shown to possess a gH-containing tropism determining protein complex that mediates virus-induced membrane fusion in distinct cell types (90). EBV, a gamma herpesvirus, also possesses a gH containing protein complex that mediates entry into epithelial cells or B-cells dependent on the protein complex in which gH is bound (91, 92). Lacking is evidence for a tropism-determining gH/gL complex for any alphaherpesvirus species. Our group has shown that glycoprotein K(gK), a highly conserved alphaherpesvirus glycoprotein, which is present in the virion envelope is an alphaherpesvirus tropism determinant facilitating entry into neuronal cell via axonal termini (87, 93). Supporting its role as a potential alphaherpesvirus tropism determinant, we have also reported that gK interacts with both gB and gH (94). Finally, we have shown that a virus lacking amino acids 31–68 of gK, while indistinguishable from wild type for entry into CHO-nectin1 or CHO-HVEM cells, was unable to enter PILRα expressing CHO cells (95). This suggests that mutations in gK may alter directly or indirectly the ability of gB to mediate membrane fusion during virus entry via either regulation of the viral fusion complex and/or modulation of cellular receptor usage.

Novel approaches to vaccine design have incorporated knowledge of protein complexes mediating tropism (96–98). Not surprisingly given the wealth of molecular data regarding cytomegalovirus (CMV) tropism, most progress has been made in the development of CMV vaccines. The Barry and Diamond groups have tested modified vaccinia Ankara virus (MVA) which express CMV pentamer complexes (99, 100). In the macaque system administration of MVA expressing the rhesus pentamer complex generated neutralizing antibodies and reduced plasma viral loads (99). They demonstrated similar results using MVA expressing the human pentamer complex (100). Hansen et al. tested an attenuated RhCMV viral vector that lacks members of a “pentamer” complex orthologous to UL128, UL130 and UL131 (97). This vector contained SIV inserts and was successful in generating anti-SIV responses (97). Interestingly, mutations in the pentamer complex resulted in T-cell responses to unconventional epitopes and the authors suggested that possibly a change in the tropism due to lack of the pentamer complex results in differences in T-cell priming (97). Whether this is the case remains to be seen, however, this provides fascinating insight into the potential to alter this virus to affect differential host responses to vaccination.

An interesting recent approach to the alteration of HSV tropism for therapeutic purposes is work on retargeting (101–109). HSV is retargeted by inserting a single chain antibody into a fusion complex member, typically gD, but more recently insertion into gB and gH (106, 109). Most recently, retargeted virus entry was engineered by inserting a single chain antibody against human epidermal growth factor receptor 2 (HER2) into gB (109). Apparently, this retargeted virus no longer required receptor binding by either gD or gH/gL for entry (109). While the authors of the above mentioned work suggested that retargeting through gB may affect the signalosome, an attempt wasn’t made to address this experimentally. It is conceivable that altered intracellular signaling may enhance the efficacy of these viruses for oncolytic immunotherapy.

Current HSV vaccine strains or HSV-derived vectors containing mutations expected to affect signaling pathways and tropism

Glycoprotein D

The Herold group has pursued an HSV vaccine that is deleted for gD (HSV-2 ΔgD$^{-/+gD-1}$) (110–112). This vaccine protects rodents from lethal infection with both laboratory and clinical strains of HSV-1 or -2 (110–112). Produced in complementing cells that express HSV-1 gD, the vaccine is competent only for an initial entry event after which only virus particles which lack gD, and hence are non-infectious, will be produced. The HSV-2 ΔgD $^{-/+gD-1}$ vaccine elicited antibody dependent cellular cytotoxicity (ADCC) as its major mechanism of action (112). How the absence of gD elicits a humoral response characterized by cytotoxicity and little neutralizing activity is an interesting question. The authors speculate that perhaps the gD-HVEM interaction is important in producing a humoral response dominated by neutralizing antibody and that when gD is absent after one round of replication, the ADCC response predominates. Alternatively, they suggest the presence of gD could mask viral antigens and prevent the development of protective antibody responses. Interestingly, similar to the HSV-2 ΔgD$^{-/+gD-1}$ virus Royer et al. found that FcRn was important for vaccine candidate HSV-2 0ΔNLS efficacy as siRNA depletion of FcRn in mouse eyes abrogated protection upon corneal challenge (113). This group also identified a role for the complement system in mediating protection from ocular challenge. Specifically, vaccinated C3 deficient mice cleared virus more slowly after ocular challenge than vaccinated wild type mice (113).

The Cohen laboratory has generated an HSV-2 vaccine candidate, HSV2-gD27, which contains mutations that abrogate interaction between gD and Nectin-1, the primary receptor for entry into neurons (96, 114). As such, this virus should be unable to infect neurons and establish latent infection boosting its safety profile. The mutations in gD were found to be remarkably stable and HSV2-gD27 was attenuated in ocular, intracranial and intravaginal routes of infection (114). Importantly, HSV2-gD27 exhibited superior protection of mice after vaginal challenge as compared to mice vaccinated with a gD subunit vaccine (114). The authors describe, similar to HSV-2 ΔgD$^{-/+gD-1}$, that while more protective than the gD subunit vaccine, HSV2 gD-27 elicited low titers of neutralizing antibodies compared to mice vaccinated with gD subunit (114). While this is not entirely surprising given that HSV2 gD-27 may not be expected to select for many gD-nectin-1 neutralizing antibodies, it would be interesting to test the ADCC activity or determine if there is a similar dependency on FcRn or FCγRIII for HSV2 gD-27 efficacy as was shown for HSV-2 ΔgD$^{-/+gD-1}$. Finally, although infection of neurons via nectin-1 is considered the primary route of infection HSV2-gD27, as well as challenge viral DNA could be found in DRGs (114). Thus this virus would be expected to establish, maintain, and although not examined in this study perhaps reactivate from latency.

Glycoprotein K(gK)

gK is a four transmembrane domain virion envelope protein with the amino and carboxyl termini located within the extracellular and intracellular spaces. gK functions in close association with the virally encoded UL20 membrane protein, which assumes a similar

membrane topography with the exception that the amino and carboxy termini are located intracellularly and extracellulary, respectively. Our working hypothesis supported by our published results suggests that the gK/UL20 heterodimer may regulate gB's fusogenicity through direct interactions with gB, as well as regulating additional functions carried out by gH/gL and gM. In this regard, it is important to note that syncytial mutations that cause extensive virus-induced cell fusion, a phenomenon thought to be highly similar to fusion between the viral envelope and cellular plasma membranes during virus entry, are predominantly located in the carboxyl terminus of gB and amino termini of gK and UL20 (115–125). We have shown that the amino terminus of gK binds to the amino terminus of gB, while the amino terminus of UL20 binds to the carboxyl terminus of gB. In addition, the gK/UL20 complex binds gH and gM glycoproteins. (94, 126–129).

Both gK and UL20 are highly conserved among all alphaherpesvirus species (87, 130). For other herpesvirus subfamilies (beta and gamma) multiple complexes mediating entry have been identified. HCMV, EBV, and HHV-6 have all been shown to use multiple distinct protein complexes to facilitate entry. As mentioned above, these complexes facilitate entry into differing cell types and mediate distinct signaling events. For HSV-1 this has not been shown. We have hypothesized that gK is component of a distinct alphaherpesvirus entry complex that facilitates virus entry and downstream cellular signaling. Primary evidence supporting a role for gK as a tropism determinant is our finding that deletion of 38 amino acids from the amino terminus of gK renders the virus incapable of entry into neurons via axonal termini (87).

VC2 is a recombinant HSV-1 (F)-strain in which the amino terminal 38 amino acids of gK have been deleted in combination with the 18 amino acids in the N-terminus of UL20 (131). We have shown that intramuscular immunization with VC2 protected mice from lethal intravaginal infection with either HSV-1 or HSV-2 (131). Additionally, VC2 is immunogenic in rhesus macaques and protects guinea pigs from a lethal infection with HSV-2 (submitted and (132)). The gK mutation renders the virus unable to enter into neurons via the axonal termini in vitro and no virus is present in trigeminal ganglia of vaccinated mice (131). This mutation also leads to the hyper-induction of proinflammatory pathways upon entry into fibroblasts or epithelial cells *in vitro*. In human corneal epithelial cells infected with wild type or virus with a deletion of amino acids 31–68 in gK, we found IkBα to be more rapidly degraded in cells infected with mutant virus compared to cells infected with parental virus (*Rider and Kousoulas*). Furthermore, we found that inflammatory mRNAs associated with induction of NFκB activity were more highly induced after infection with mutant virus compared to cells infected with parental virus (*Rider and Kousoulas*). The way in which this adjuvant-like activity of VC2 affects the development of a protective response to subsequent challenge with HSV is the subject of active investigation in our laboratory.

We have recently found that deletion of the first 38 amino acids of gK abolishes the ability of the virus to enter into cells via fusion of the viral envelope with cellular plasma membranes, while allowing the virus to enter via chlathrin-mediated endocytosis (*Musarrat et al. manuscript submitted*). It is increasingly appreciated that distinct signaling pathways are activated by identical receptors via endosomes versus at the plasma membrane (133). These endosomal signaling events are important mediators of a variety of important

biological processes including innate and adaptive immunity (133–135). We have hypothesized that VC2 enhanced immunogenicity and elication of protective immune responses may be associated with the inability of the virus to enter via a fusion mechanism, and conversely, that forced entry through endocytosis may eliminate certain immune evasion strategies allowing the production of robust immune responses. A differential host response to vaccination by VC2 versus parental virus is reflected in the finding that ocular challenge with highly pathogenic strains of HSV-1 results in no ocular pathogenesis in VC2-vaccinated mice compared to mice vaccinated with parental virus (*Naidu et al., manuscript in preparation*). The mechanisms are currently under investigation in our laboratory.

Conclusions and Future Directions

There has been a great deal of progress in the herpes simplex virus vaccine field (7). With recent FDA approval for the first oncolytic virotherapy (TVEC, Amgen, Inc), an HSV-1 derived virus, safety has been demonstrated for HSV-1 derived vectors. Despite significant progress in the understanding of protective immune responses against HSV infections, there are no available vaccines. A comprehensive analysis of the ways in which HSV vaccine candidates affect the herpesvirus “signalasome” as part of their efficacy is lacking. It is to be expected that mechanisms of protection such as ADCC and complement activation seen with HSV-2 $\Delta gD^{-/+gD\text{-}1}$ and HSV2-gD27 are influenced by early stages of virus-host cell interaction of a potential live-attenuated vaccine strain, as well as with the subsequent challenge with wild-type virus. Overall, the ways in which affected signaling pathways translate to protective innate or adaptive immune responses need to be elucidated. A greater understanding of the role that diverse receptor usage plays during infection and pathogenesis and how they affect downstream cell signaling and innate immune responses will lead to greater opportunities to engineer effective live-attenuated vaccines.

Precluding a complete analysis of HSV-1 signaling activity at attachment and entry is the dearth of information regarding infection of neurons. This information may be most clearly relevant to the rational design of vaccine strategies to abrogate infection of neurons and thus block the establishment or maintenance of latent infection. The difficulty of experimental approaches to the analysis of biologically relevant signaling events during entry into axons needs to be overcome before a complete picture can emerge. Significant progress regarding neuronal culture and single cell transcriptomics will help here. The predilection of HSV to enter into neurons and establish latency suggests that intracellular signaling events in neurons are of specific interest and may be more complicated than those elicited in other cell types. Ultimately, understanding of neuronal signaling in viral infection may shed light of how the virus prepares neurons for the establishment of latency and reactivation from latency, and the role of the immune system in these phenomena. Finally, initial encounter of virus in the host is by polarized epithelial cells which are expected to differ greatly from widely used tissue culture models of infection. A greater emphasis on established and using polarized epithelial models of infection for signaling and tissue culture studies may yield additional biologically meaningful data from which to rationalize future vaccine design.

Acknowledgments

We regret that the important works of many of our colleagues was outside the scope of this review and were thus unable to be discussed. We would like to thank Rhonda Cardin for comments and critical reading of the manuscript. The work was supported by the LSU Division of Biotechnology & Molecular Medicine, by a Governor's Biotechnology Initiative Grant (KGK) and Cores of the Center for Experimental Infectious Disease Research (CEIDR) supported by the NIH:NIGMS Grant P30GM110670.

References

Recently published papers of particular interest have been highlighted as:

• Of importance

•• Of major importance

1. Roizman, B, Knipe, D, Whitley, RJ. Herpes simplex viruses. In: Knipe, DMHP, editorFields Virology. 6. Lippincott Williams & Wilkins; 2013. 1823–97.
2. Looker KJ, Magaret AS, May MT, Turner KM, Vickerman P, Gottlieb SL, et al. Global and Regional Estimates of Prevalent and Incident Herpes Simplex Virus Type 1 Infections in 2012. PloS one. 2015; 10(10):e0140765. [PubMed: 26510007]
3. Looker KJ, Magaret AS, Turner KM, Vickerman P, Gottlieb SL, Newman LM. Global estimates of prevalent and incident herpes simplex virus type 2 infections in 2012. PloS one. 2015; 10(1):e114989. [PubMed: 25608026]
4. Pottage JC Jr, Kessler HA. Herpes simplex virus resistance to acyclovir: clinical relevance. Infectious agents and disease. 1995; 4(3):115–24. [PubMed: 8548189]
5. Castelo-Soccio L, Bernardin R, Stern J, Goldstein SA, Kovarik C. Successful treatment of acyclovir-resistant herpes simplex virus with intralesional cidofovir. Archives of dermatology. 2010; 146(2): 124–6. [PubMed: 20157021]
6. Kemble, G, Spaete, R. Herpes simplex vaccines. In: Arvin, A, Campadelli-Fiume, G, Mocarski, E, Moore, PS, Roizman, B, Whitley, R. , et al., editorsHuman Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge: 2007.
7. Stanfield B, Kousoulas KG. Herpes Simplex Vaccines: Prospects of Live-attenuated HSV Vaccines to Combat Genital and Ocular infections. Current clinical microbiology reports. 2015; 2(3):125–36. [PubMed: 27114893]
8. Gianni T, Leoni V, Chesnokova LS, Hutt-Fletcher LM, Campadelli-Fiume G. alphavbeta3-integrin is a major sensor and activator of innate immunity to herpes simplex virus-1. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109(48):19792–7. [PubMed: 23150579]
9. Paludan SR, Bowie AG, Horan KA, Fitzgerald KA. Recognition of herpesviruses by the innate immune system. Nature reviews Immunology. 2011; 11(2):143–54.
10. Casiraghi C, Gianni T, Campadelli-Fiume G. alphavbeta3 Integrin Boosts the Innate Immune Response Elicited in Epithelial Cells through Plasma Membrane and Endosomal Toll-Like Receptors. Journal of virology. 2016; 90(8):4243–8. [PubMed: 26842473]
11. Cai M, Li M, Wang K, Wang S, Lu Q, Yan J, et al. The herpes simplex virus 1-encoded envelope glycoprotein B activates NF-kappaB through the Toll-like receptor 2 and MyD88/TRAF6-dependent signaling pathway. PloS one. 2013; 8(1):e54586. [PubMed: 23382920]
12. Kim M, Osborne NR, Zeng W, Donaghy H, McKinnon K, Jackson DC, et al. Herpes simplex virus antigens directly activate NK cells via TLR2, thus facilitating their presentation to CD4 T lymphocytes. Journal of immunology. 2012; 188(9):4158–70.
13. Holm CK, Jensen SB, Jakobsen MR, Cheshenko N, Horan KA, Moeller HB, et al. Virus-cell fusion as a trigger of innate immunity dependent on the adaptor STING. Nature immunology. 2012; 13(8):737–43. [PubMed: 22706339]

14. Stevenson EV, Collins-McMillen D, Kim JH, Cieply SJ, Bentz GL, Yurochko AD. HCMV reprogramming of infected monocyte survival and differentiation: a Goldilocks phenomenon. Viruses. 2014; 6(2):782–807. [PubMed: 24531335]
15. Yurochko AD. Human cytomegalovirus modulation of signal transduction. Current topics in microbiology and immunology. 2008; 325:205–20. [PubMed: 18637508]
16. Kurt-Jones EA, Orzalli MH, Knipe DM. Innate Immune Mechanisms and Herpes Simplex Virus Infection and Disease. Advances in anatomy, embryology, and cell biology. 2017; 223:49–75.
17. McCormick, AL, Mocarski, ES, Jr. Viral modulation of the host response to infection. In: Arvin, A, Campadelli-Fiume, G, Mocarski, E, Moore, PS, Roizman, B, Whitley, R. , et al., editorsHuman Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge: 2007.
18. Medici MA, Sciortino MT, Perri D, Amici C, Avitabile E, Ciotti M, et al. Protection by herpes simplex virus glycoprotein D against Fas-mediated apoptosis: role of nuclear factor kappaB. The Journal of biological chemistry. 2003; 278(38):36059–67. [PubMed: 12844494]
19. Liu X, Fitzgerald K, Kurt-Jones E, Finberg R, Knipe DM. Herpesvirus tegument protein activates NF-kappaB signaling through the TRAF6 adaptor protein. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(32):11335–9. [PubMed: 18682563]
20. Campadelli-Fiume G, Collins-McMillen D, Gianni T, Yurochko AD. Integrins as Herpesvirus Receptors and Mediators of the Host Signalosome. Annual review of virology. 2016; 3(1):215–36.
21. Chan G, Nogalski MT, Stevenson EV, Yurochko AD. Human cytomegalovirus induction of a unique signalsome during viral entry into monocytes mediates distinct functional changes: a strategy for viral dissemination. Journal of leukocyte biology. 2012; 92(4):743–52. [PubMed: 22715139]
22. Azab W, Osterrieder K. Initial Contact: The First Steps in Herpesvirus Entry. Advances in anatomy, embryology, and cell biology. 2017; 223:1–27.
23. Eisenberg RJ, Atanasiu D, Cairns TM, Gallagher JR, Krummenacher C, Cohen GH. Herpes virus fusion and entry: a story with many characters. Viruses. 2012; 4(5):800–32. [PubMed: 22754650]
24. Heldwein EE, Krummenacher C. Entry of herpesviruses into mammalian cells. Cellular and molecular life sciences: CMLS. 2008; 65(11):1653–68. [PubMed: 18351291]
25. Turner A, Bruun B, Minson T, Browne H. Glycoproteins gB, gD, and gHgL of herpes simplex virus type 1 are necessary and sufficient to mediate membrane fusion in a Cos cell transfection system. Journal of virology. 1998; 72(1):873–5. [PubMed: 9420303]
26. Ruyechan WT, Morse LS, Knipe DM, Roizman B. Molecular genetics of herpes simplex virus. II. Mapping of the major viral glycoproteins and of the genetic loci specifying the social behavior of infected cells. Journal of virology. 1979; 29(2):677–97. [PubMed: 219254]
27. Dolter KE, Ramaswamy R, Holland TC. Syncytial mutations in the herpes simplex virus type 1 gK (UL53) gene occur in two distinct domains. Journal of virology. 1994; 68(12):8277–81. [PubMed: 7966620]
28. Bond VC, Person S, Warner SC. The isolation and characterization of mutants of herpes simplex virus type 1 that induce cell fusion. The Journal of general virology. 1982; 61(Pt 2):245–54. [PubMed: 6288856]
29. Read GS, Person S, Keller PM. Genetic studies of cell fusion induced by herpes simplex virus type 1. Journal of virology. 1980; 35(1):105–13. [PubMed: 6251259]
30. Pogue-Geile KL, Spear PG. The single base pair substitution responsible for the Syn phenotype of herpes simplex virus type 1, strain MP. Virology. 1987; 157(1):67–74. [PubMed: 3029967]
31. Avitabile E, Lombardi G, Gianni T, Capri M, Campadelli-Fiume G. Coexpression of UL20p and gK inhibits cell-cell fusion mediated by herpes simplex virus glycoproteins gD, gH-gL, and wild-type gB or an endocytosis-defective gB mutant and downmodulates their cell surface expression. Journal of virology. 2004; 78(15):8015–25. [PubMed: 15254173]
32. Agelidis AM, Shukla D. Cell entry mechanisms of HSV: what we have learned in recent years. Future virology. 2015; 10(10):1145–54. [PubMed: 27066105]
33. Nicola AV, Hou J, Major EO, Straus SE. Herpes simplex virus type 1 enters human epidermal keratinocytes, but not neurons, via a pH-dependent endocytic pathway. Journal of virology. 2005; 79(12):7609–16. [PubMed: 15919913]

34. Clement C, Tiwari V, Scanlan PM, Valyi-Nagy T, Yue BY, Shukla D. A novel role for phagocytosis-like uptake in herpes simplex virus entry. The Journal of cell biology. 2006; 174(7): 1009–21. [PubMed: 17000878]
35. Gianni T, Salvioli S, Chesnokova LS, Hutt-Fletcher LM, Campadelli-Fiume G. alphavbeta6- and alphavbeta8-integrins serve as interchangeable receptors for HSV gH/gL to promote endocytosis and activation of membrane fusion. PLoS pathogens. 2013; 9(12):e1003806. [PubMed: 24367260]
36. Nicola AV, Straus SE. Cellular and viral requirements for rapid endocytic entry of herpes simplex virus. Journal of virology. 2004; 78(14):7508–17. [PubMed: 15220424]
37. Nicola AV. Herpesvirus Entry into Host Cells Mediated by Endosomal Low pH. Traffic. 2016; 17(9):965–75. [PubMed: 27126894]
38. Azab W, Gramatica A, Herrmann A, Osterrieder N. Binding of alphaherpesvirus glycoprotein H to surface alpha4beta1-integrins activates calcium-signaling pathways and induces phosphatidylserine exposure on the plasma membrane. mBio. 2015; 6(5):e01552–15. [PubMed: 26489864]
39. Ryckman BJ, Jarvis MA, Drummond DD, Nelson JA, Johnson DC. Human cytomegalovirus entry into epithelial and endothelial cells depends on genes UL128 to UL150 and occurs by endocytosis and low-pH fusion. Journal of virology. 2006; 80(2):710–22. [PubMed: 16378974]
40. Archer MA, Brechtel TM, Davis LE, Parmar RC, Hasan MH, Tandon R. Inhibition of endocytic pathways impacts cytomegalovirus maturation. Scientific reports. 2017; 7:46069. [PubMed: 28406138]
41. Oxford KL, Strelow L, Yue Y, Chang WL, Schmidt KA, Diamond DJ, et al. Open reading frames carried on UL/b′ are implicated in shedding and horizontal transmission of rhesus cytomegalovirus in rhesus monkeys. Journal of virology. 2011; 85(10):5105–14. [PubMed: 21389128]
42. Spear PG. Herpes simplex virus: receptors and ligands for cell entry. Cellular microbiology. 2004; 6(5):401–10. [PubMed: 15056211]
43. Shukla D, Liu J, Blaiklock P, Shworak NW, Bai X, Esko JD, et al. A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry. Cell. 1999; 99(1):13–22. [PubMed: 10520990]
44. Montgomery RI, Warner MS, Lum BJ, Spear PG. Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. Cell. 1996; 87(3):427–36. [PubMed: 8898196]
45. Cocchi F, Menotti L, Mirandola P, Lopez M, Campadelli-Fiume G. The ectodomain of a novel member of the immunoglobulin subfamily related to the poliovirus receptor has the attributes of a bona fide receptor for herpes simplex virus types 1 and 2 in human cells. Journal of virology. 1998; 72(12):9992–10002. [PubMed: 9811737]
46. Geraghty RJ, Krummenacher C, Cohen GH, Eisenberg RJ, Spear PG. Entry of alphaherpesviruses mediated by poliovirus receptor-related protein 1 and poliovirus receptor. Science. 1998; 280(5369):1618–20. [PubMed: 9616127]
47. Satoh T, Arii J, Suenaga T, Wang J, Kogure A, Uehori J, et al. PILRalpha is a herpes simplex virus-1 entry coreceptor that associates with glycoprotein B. Cell. 2008; 132(6):935–44. [PubMed: 18358807]
48. Arii J, Goto H, Suenaga T, Oyama M, Kozuka-Hata H, Imai T, et al. Non-muscle myosin IIA is a functional entry receptor for herpes simplex virus-1. Nature. 2010; 467(7317):859–62. [PubMed: 20944748]
49. Suenaga T, Satoh T, Somboonthum P, Kawaguchi Y, Mori Y, Arase H. Myelin-associated glycoprotein mediates membrane fusion and entry of neurotropic herpesviruses. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107(2):866–71. [PubMed: 20080767]
50. Taylor JM, Lin E, Susmarski N, Yoon M, Zago A, Ware CF, et al. Alternative entry receptors for herpes simplex virus and their roles in disease. Cell host & microbe. 2007; 2(1):19–28. [PubMed: 18005714]
51. Di Giovine P, Settembre EC, Bhargava AK, Luftig MA, Lou H, Cohen GH, et al. Structure of herpes simplex virus glycoprotein D bound to the human receptor nectin-1. PLoS pathogens. 2011; 7(9):e1002277. [PubMed: 21980294]

52. Carfi A, Willis SH, Whitbeck JC, Krummenacher C, Cohen GH, Eisenberg RJ, et al. Herpes simplex virus glycoprotein D bound to the human receptor HveA. Molecular cell. 2001; 8(1):169–79. [PubMed: 11511370]

53. Cheshenko N, Liu W, Satlin LM, Herold BC. Multiple receptor interactions trigger release of membrane and intracellular calcium stores critical for herpes simplex virus entry. Molecular biology of the cell. 2007; 18(8):3119–30. [PubMed: 17553929]

54. Shui JW, Kronenberg M. HVEM: An unusual TNF receptor family member important for mucosal innate immune responses to microbes. Gut microbes. 2013; 4(2):146–51. [PubMed: 23333859]

55. Steinberg MW, Cheung TC, Ware CF. The signaling networks of the herpesvirus entry mediator (TNFRSF14) in immune regulation. Immunological reviews. 2011; 244(1):169–87. [PubMed: 22017438]

56. Murphy TL, Murphy KM. Slow down and survive: Enigmatic immunoregulation by BTLA and HVEM. Annual review of immunology. 2010; 28:389–411.

57. Cheung TC, Steinberg MW, Oborne LM, Macauley MG, Fukuyama S, Sanjo H, et al. Unconventional ligand activation of herpesvirus entry mediator signals cell survival. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106(15):6244–9. [PubMed: 19332782]

58. Lasaro MO, Tatsis N, Hensley SE, Whitbeck JC, Lin SW, Rux JJ, et al. Targeting of antigen to the herpesvirus entry mediator augments primary adaptive immune responses. Nature medicine. 2008; 14(2):205–12.

59. Sciortino MT, Medici MA, Marino-Merlo F, Zaccaria D, Giuffre-Cuculletto M, Venuti A, et al. Involvement of gD/HVEM interaction in NF-kB-dependent inhibition of apoptosis by HSV-1 gD. Biochemical pharmacology. 2008; 76(11):1522–32. [PubMed: 18723002]

60. Edwards RG, Longnecker R. Herpesvirus Entry Mediator and Ocular Herpesvirus Infection: More than Meets the Eye. Journal of virology. 2017; 91(13)

61. Karaba AH, Kopp SJ, Longnecker R. Herpesvirus entry mediator is a serotype specific determinant of pathogenesis in ocular herpes. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109(50):20649–54. [PubMed: 23184983]

62. Karaba AH, Kopp SJ, Longnecker R. Herpesvirus entry mediator and nectin-1 mediate herpes simplex virus 1 infection of the murine cornea. Journal of virology. 2011; 85(19):10041–7. [PubMed: 21795335]

63. Feire AL, Roy RM, Manley K, Compton T. The glycoprotein B disintegrin-like domain binds beta 1 integrin to mediate cytomegalovirus entry. Journal of virology. 2010; 84(19):10026–37. [PubMed: 20660204]

64. Garrigues HJ, Rubinchikova YE, Dipersio CM, Rose TM. Integrin alphaVbeta3 Binds to the RGD motif of glycoprotein B of Kaposi's sarcoma-associated herpesvirus and functions as an RGD-dependent entry receptor. Journal of virology. 2008; 82(3):1570–80. [PubMed: 18045938]

65. Leoni V, Gianni T, Salvioli S, Campadelli-Fiume G. Herpes simplex virus glycoproteins gH/gL and gB bind Toll-like receptor 2, and soluble gH/gL is sufficient to activate NF-kappaB. Journal of virology. 2012; 86(12):6555–62. [PubMed: 22496225]

66. Fournier N, Chalus L, Durand I, Garcia E, Pin JJ, Churakova T, et al. FDF03, a novel inhibitory receptor of the immunoglobulin superfamily, is expressed by human dendritic and myeloid cells. Journal of immunology. 2000; 165(3):1197–209.

67. Wang J, Shiratori I, Uehori J, Ikawa M, Arase H. Neutrophil infiltration during inflammation is regulated by PILRalpha via modulation of integrin activation. Nature immunology. 2013; 14(1): 34–40. [PubMed: 23142774]

68. Parry C, Bell S, Minson T, Browne H. Herpes simplex virus type 1 glycoprotein H binds to alphavbeta3 integrins. The Journal of general virology. 2005; 86(Pt 1):7–10. [PubMed: 15604426]

69. Cheshenko N, Trepanier JB, Gonzalez PA, Eugenin EA, Jacobs WR Jr, Herold BC. Herpes simplex virus type 2 glycoprotein H interacts with integrin alphavbeta3 to facilitate viral entry and calcium signaling in human genital tract epithelial cells. Journal of virology. 2014; 88(17):10026–38. [PubMed: 24942591]

70. Cheshenko N, Trepanier JB, Stefanidou M, Buckley N, Gonzalez P, Jacobs W, et al. HSV activates Akt to trigger calcium release and promote viral entry: novel candidate target for treatment and

suppression. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2013; 27(7):2584–99. [PubMed: 23507869]

71. Cheshenko N, Del Rosario B, Woda C, Marcellino D, Satlin LM, Herold BC. Herpes simplex virus triggers activation of calcium-signaling pathways. The Journal of cell biology. 2003; 163(2):283–93. [PubMed: 14568989]
72. Gianni T, Massaro R, Campadelli-Fiume G. Dissociation of HSV gL from gH by alphavbeta6- or alphavbeta8-integrin promotes gH activation and virus entry. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112(29):E3901–10. [PubMed: 26157134]
73. Gianni T, Campadelli-Fiume G. The epithelial alphavbeta3-integrin boosts the MYD88-dependent TLR2 signaling in response to viral and bacterial components. PLoS pathogens. 2014; 10(11):e1004477. [PubMed: 25375272]
74. Gianni T, Campadelli-Fiume G. alphaVbeta3-integrin relocalizes nectin1 and routes herpes simplex virus to lipid rafts. Journal of virology. 2012; 86(5):2850–5. [PubMed: 22171266]
75. Iversen MB, Reinert LS, Thomsen MK, Bagdonaite I, Nandakumar R, Cheshenko N, et al. An innate antiviral pathway acting before interferons at epithelial surfaces. Nature immunology. 2016; 17(2):150–8. [PubMed: 26595890]
76. Tiwari V, Shukla D. Phosphoinositide 3 kinase signalling may affect multiple steps during herpes simplex virus type-1 entry. The Journal of general virology. 2010; 91(Pt 12):3002–9. [PubMed: 20810749]
77. MacLeod IJ, Minson T. Binding of herpes simplex virus type-1 virions leads to the induction of intracellular signalling in the absence of virus entry. PloS one. 2010; 5(3):e9560. [PubMed: 20221426]
78. Liu X, Cohen JI. The role of PI3K/Akt in human herpesvirus infection: From the bench to the bedside. Virology. 2015; 479–480:568–77.
79. Zheng K, Xiang Y, Wang X, Wang Q, Zhong M, Wang S, et al. Epidermal growth factor receptor-PI3K signaling controls cofilin activity to facilitate herpes simplex virus 1 entry into neuronal cells. mBio. 2014; 5(1):e00958–13. [PubMed: 24425731]
80. Mostowy S, Shenoy AR. The cytoskeleton in cell-autonomous immunity: structural determinants of host defence. Nature reviews Immunology. 2015; 15(9):559–73.
81. Fukazawa A, Alonso C, Kurachi K, Gupta S, Lesser CF, McCormick BA, et al. GEF-H1 mediated control of NOD1 dependent NF-kappaB activation by Shigella effectors. PLoS pathogens. 2008; 4(11):e1000228. [PubMed: 19043560]
82. Keestra AM, Winter MG, Auburger JJ, Frassle SP, Xavier MN, Winter SE, et al. Manipulation of small Rho GTPases is a pathogen-induced process detected by NOD1. Nature. 2013; 496(7444): 233–7. [PubMed: 23542589]
83. Dunn W, Chou C, Li H, Hai R, Patterson D, Stolc V, et al. Functional profiling of a human cytomegalovirus genome. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100(24):14223–8. [PubMed: 14623981]
84. Wang D, Shenk T. Human cytomegalovirus virion protein complex required for epithelial and endothelial cell tropism. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102(50):18153–8. [PubMed: 16319222]
85. Hai R, Chu A, Li H, Umamoto S, Rider P, Liu F. Infection of human cytomegalovirus in cultured human gingival tissue. Virology journal. 2006; 3:84. [PubMed: 17022821]
86. Borza CM, Hutt-Fletcher LM. Alternate replication in B cells and epithelial cells switches tropism of Epstein-Barr virus. Nature medicine. 2002; 8(6):594–9.
87. Jambunathan N, Charles AS, Subramanian R, Saied AA, Naderi M, Rider P, et al. Deletion of a Predicted beta-Sheet Domain within the Amino Terminus of Herpes Simplex Virus Glycoprotein K Conserved among Alphaherpesviruses Prevents Virus Entry into Neuronal Axons. Journal of virology. 2015; 90(5):2230–9. [PubMed: 26656706]
88. Li G, Nguyen CC, Ryckman BJ, Britt WJ, Kamil JP. A viral regulator of glycoprotein complexes contributes to human cytomegalovirus cell tropism. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112(14):4471–6. [PubMed: 25831500]

89. Li G, Kamil JP. Viral Regulation of Cell Tropism in Human Cytomegalovirus. Journal of virology. 2015; 90(2):626–9. [PubMed: 26559829]

90. Mori Y. Recent topics related to human herpesvirus 6 cell tropism. Cellular microbiology. 2009; 11(7):1001–6. [PubMed: 19290911]

91. Hutt-Fletcher LM, Lake CM. Two Epstein-Barr virus glycoprotein complexes. Current topics in microbiology and immunology. 2001; 258:51–64. [PubMed: 11443867]

92. Sathiyamoorthy K, Hu YX, Mohl BS, Chen J, Longnecker R, Jardetzky TS. Structural basis for Epstein-Barr virus host cell tropism mediated by gp42 and gHgL entry glycoproteins. Nature communications. 2016; 7:13557.

93. Saied AA, Chouljenko VN, Subramanian R, Kousoulas KG. A replication competent HSV-1(McKrae) with a mutation in the amino-terminus of glycoprotein K (gK) is unable to infect mouse trigeminal ganglia after cornea infection. Current eye research. 2014; 39(6):596–603. [PubMed: 24401006]

94. Chouljenko VN, Iyer AV, Chowdhury S, Kim J, Kousoulas KG. The herpes simplex virus type 1 UL20 protein and the amino terminus of glycoprotein K (gK) physically interact with gB. Journal of virology. 2010; 84(17):8596–606. [PubMed: 20573833]

95. Chowdhury S, Chouljenko VN, Naderi M, Kousoulas KG. The amino terminus of herpes simplex virus 1 glycoprotein K is required for virion entry via the paired immunoglobulin-like type-2 receptor alpha. Journal of virology. 2013; 87(6):3305–13. [PubMed: 23302878]

96. Wang K, Kappel JD, Canders C, Davila WF, Sayre D, Chavez M, et al. A herpes simplex virus 2 glycoprotein D mutant generated by bacterial artificial chromosome mutagenesis is severely impaired for infecting neuronal cells and infects only Vero cells expressing exogenous HVEM. Journal of virology. 2012; 86(23):12891–902. [PubMed: 22993162]

97. Hansen SG, Sacha JB, Hughes CM, Ford JC, Burwitz BJ, Scholz I, et al. Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. Science. 2013; 340(6135):1237874. [PubMed: 23704576]

98. Barouch DH, Picker LJ. Novel vaccine vectors for HIV-1. Nature reviews Microbiology. 2014; 12(11):765–71. [PubMed: 25296195]

99. Wussow F, Yue Y, Martinez J, Deere JD, Longmate J, Herrmann A, et al. A vaccine based on the rhesus cytomegalovirus UL128 complex induces broadly neutralizing antibodies in rhesus macaques. Journal of virology. 2013; 87(3):1322–32. [PubMed: 23152525]

100. Wussow F, Chiuppesi F, Martinez J, Campo J, Johnson E, Flechsig C, et al. Human cytomegalovirus vaccine based on the envelope gH/gL pentamer complex. PLoS pathogens. 2014; 10(11):e1004524. [PubMed: 25412505]

101. Zhou G, Roizman B. Construction and properties of a herpes simplex virus 1 designed to enter cells solely via the IL-13alpha2 receptor. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103(14):5508–13. [PubMed: 16554374]

102. Zhou G, Ye GJ, Debinski W, Roizman B. Engineered herpes simplex virus 1 is dependent on IL13Ralpha 2 receptor for cell entry and independent of glycoprotein D receptor interaction. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99(23): 15124–9. [PubMed: 12417744]

103. Campadelli-Fiume G, Petrovic B, Leoni V, Gianni T, Avitabile E, Casiraghi C, et al. Retargeting Strategies for Oncolytic Herpes Simplex Viruses. Viruses. 2016; 8(3):63. [PubMed: 26927159]

104. Gambini E, Reisoli E, Appolloni I, Gatta V, Campadelli-Fiume G, Menotti L, et al. Replication-competent herpes simplex virus retargeted to HER2 as therapy for high-grade glioma. Molecular therapy: the journal of the American Society of Gene Therapy. 2012; 20(5):994–1001. [PubMed: 22354378]

105. Nanni P, Gatta V, Menotti L, De Giovanni C, Ianzano M, Palladini A, et al. Preclinical therapy of disseminated HER-2(+) ovarian and breast carcinomas with a HER-2-retargeted oncolytic herpesvirus. PLoS pathogens. 2013; 9(1):e1003155. [PubMed: 23382683]

106. Gatta V, Petrovic B, Campadelli-Fiume G. The Engineering of a Novel Ligand in gH Confers to HSV an Expanded Tropism Independent of gD Activation by Its Receptors. PLoS pathogens. 2015; 11(5):e1004907. [PubMed: 25996983]

107. Leoni V, Gatta V, Palladini A, Nicoletti G, Ranieri D, Dall'Ora M, et al. Systemic delivery of HER2-retargeted oncolytic-HSV by mesenchymal stromal cells protects from lung and brain metastases. Oncotarget. 2015; 6(33):34774–87. [PubMed: 26430966]

108. Leoni V, Gatta V, Casiraghi C, Nicosia A, Petrovic B, Campadelli-Fiume G. A Strategy for Cultivation of Retargeted Oncolytic Herpes Simplex Viruses in Non-cancer Cells. Journal of virology. 2017; 91(10)

109. Petrovic B, Gianni T, Gatta V, Campadelli-Fiume G. Insertion of a ligand to HER2 in gB retargets HSV tropism and obviates the need for activation of the other entry glycoproteins. PLoS pathogens. 2017; 13(4):e1006352. [PubMed: 28423057]

110. Petro CD, Weinrick B, Khajoueinejad N, Burn C, Sellers R, Jacobs WR Jr, et al. HSV-2 DeltagD elicits FcgammaR-effector antibodies that protect against clinical isolates. JCI insight. 2016; 1(12)

111. Boukhvalova M, McKay J, Mbaye A, Sanford-Crane H, Blanco JC, Huber A, et al. Efficacy of the Herpes Simplex Virus 2 (HSV-2) Glycoprotein D/AS04 Vaccine against Genital HSV-2 and HSV-1 Infection and Disease in the Cotton Rat Sigmodon hispidus Model. Journal of virology. 2015; 89(19):9825–40. [PubMed: 26178984]

112•. Petro C, Gonzalez PA, Cheshenko N, Jandl T, Khajoueinejad N, Benard A, et al. Herpes simplex type 2 virus deleted in glycoprotein D protects against vaginal, skin and neural disease. eLife. 2015:4.

113. Royer DJ, Carr MM, Gurung HR, Halford WP, Carr DJJ. The Neonatal Fc Receptor and Complement Fixation Facilitate Prophylactic Vaccine-Mediated Humoral Protection against Viral Infection in the Ocular Mucosa. Journal of immunology. 2017; 199(5):1898–911.

114•. Wang K, Goodman KN, Li DY, Raffeld M, Chavez M, Cohen JI. A Herpes Simplex Virus 2 (HSV-2) gD Mutant Impaired for Neural Tropism Is Superior to an HSV-2 gD Subunit Vaccine To Protect Animals from Challenge with HSV-2. Journal of virology. 2015; 90(1):562–74. [PubMed: 26559846]

115. MacLean CA, Efstathiou S, Elliott ML, Jamieson FE, McGeoch DJ. Investigation of herpes simplex virus type 1 genes encoding multiply inserted membrane proteins. Journal of General Virology. 1991; 72(Pt 4):897–906. [PubMed: 1849972]

116. Melancon JM, Foster TP, Kousoulas KG. Genetic analysis of the herpes simplex virus type 1 (HSV-1) UL20 protein domains involved in cytoplasmic virion envelopment and virus-induced cell fusion. J Virol. 2004; 78(14):7329–43. [PubMed: 15220406]

117. Baines JD, Ward PL, Campadelli-Fiume G, Roizman B. The UL20 gene of herpes simplex virus 1 encodes a function necessary for viral egress. Journal of Virology. 1991; 65(12):6414–24. [PubMed: 1719228]

118. Bzik DJ, Fox BA, DeLuca NA, Person S. Nucleotide sequence of a region of the herpes simplex virus type 1 gB glycoprotein gene: mutations affecting rate of virus entry and cell fusion. Virology. 1984; 137(1):185–90. [PubMed: 6089415]

119. Pellett PE, Kousoulas KG, Pereira L, Roizman B. Anatomy of the herpes simplex virus 1 strain F glycoprotein B gene: primary sequence and predicted protein structure of the wild type and of monoclonal antibody-resistant mutants. Journal of Virology. 1985; 53(1):243–53. [PubMed: 2981343]

120. Bond VC, Person S. Fine structure physical map locations of alterations that affect cell fusion in herpes simplex virus type 1. Virology. 1984; 132(2):368–76. [PubMed: 6322419]

121. Debroy C, Pederson N, Person S. Nucleotide sequence of a herpes simplex virus type 1 gene that causes cell fusion. Virology. 1985; 145(1):36–48. [PubMed: 2990101]

122. Hutchinson L, Goldsmith K, Snoddy D, Ghosh H, Graham FL, Johnson DC. Identification and characterization of a novel herpes simplex virus glycoprotein, gK, involved in cell fusion. J Virol. 1992; 66(9):5603–9. [PubMed: 1323714]

123. Pogue-Geile KL, Lee GT, Shapira SK, Spear PG. Fine mapping of mutations in the fusion-inducing MP strain of herpes simplex virus type 1. Virology. 1984; 136(1):100–9. [PubMed: 6330988]

124. Pogue-Geile KL, Spear PG. The single base pair substitution responsible for the syn phenotype of herpes simplex virus type 1, strain MP. Virology. 1987; 157:67–74. [PubMed: 3029967]

125. Ruyechan WT, Morse LS, Knipe DM, Roizman B. Molecular genetics of herpes simplex virus. II. Mapping of the major viral glycoproteins and of the genetic loci specifying the social behavior of infected cells. J Virol. 1979; 29:677–97. [PubMed: 219254]

126. Kim IJ, Chouljenko VN, Walker JD, Kousoulas KG. Herpes simplex virus 1 glycoprotein M and the membrane-associated protein UL11 are required for virus-induced cell fusion and efficient virus entry. Journal of virology. 2013; 87(14):8029–37. [PubMed: 23678175]

127. Foster TP, Chouljenko VN, Kousoulas KG. Functional and physical interactions of the herpes simplex virus type 1 UL20 membrane protein with glycoprotein K. Journal of virology. 2008; 82(13):6310–23. [PubMed: 18434401]

128. Foster TP, Melancon JM, Baines JD, Kousoulas KG. The herpes simplex virus type 1 UL20 protein modulates membrane fusion events during cytoplasmic virion morphogenesis and virus-induced cell fusion. Journal of virology. 2004; 78(10):5347–57. [PubMed: 15113914]

129. Melancon JM, Luna RE, Foster TP, Kousoulas KG. Herpes simplex virus type 1 gK is required for gB-mediated virus-induced cell fusion, while neither gB and gK nor gB and UL20p function redundantly in virion de-envelopment. Journal of virology. 2005; 79(1):299–313. [PubMed: 15596825]

130. Rider PJF, Naderi M, Bergeron S, Chouljenko VN, Brylinski M, Kousoulas KG. Cysteines and N-glycosylation Sites Conserved Among all Alphaherpesviruses Regulate Membrane Fusion in Herpes Simplex Virus Type 1 Infection. Journal of virology. 2017

131•. Stanfield BA, Stahl J, Chouljenko VN, Subramanian R, Charles AS, Saied AA, et al. A single intramuscular vaccination of mice with the HSV-1 VC2 virus with mutations in the glycoprotein K and the membrane protein UL20 confers full protection against lethal intravaginal challenge with virulent HSV-1 and HSV-2 strains. PloS one. 2014; 9(10):e109890. [PubMed: 25350288]

132. Stanfield BA, Pahar B, Chouljenko VN, Veazey R, Kousoulas KG. Vaccination of rhesus macaques with the live-attenuated HSV-1 vaccine VC2 stimulates the proliferation of mucosal T cells and germinal center responses resulting in sustained production of highly neutralizing antibodies. Vaccine. 2017; 35(4):536–43. [PubMed: 28017425]

133. Murphy JE, Padilla BE, Hasdemir B, Cottrell GS, Bunnett NW. Endosomes: a legitimate platform for the signaling train. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106(42):17615–22. [PubMed: 19822761]

134. Gleeson PA. The role of endosomes in innate and adaptive immunity. Seminars in cell & developmental biology. 2014; 31:64–72. [PubMed: 24631355]

135. Brubaker SW, Bonham KS, Zanoni I, Kagan JC. Innate immune pattern recognition: a cell biological perspective. Annual review of immunology. 2015; 33:257–90.