Gammaherpesvirus Entry and Fusion -

A Tale how two Human Pathogenic Viruses enter their Host Cells

Britta S. Möhl[1,*], Jia Chen[2], and Richard Longnecker[2]

[1]Institute of Virology, School of Medicine, Technical University of Munich / Helmholtz Zentrum München, Munich, Germany; [2]Department of Microbiology and Immunology, Feinberg School of Medicine, Northwestern University, Chicago, IL, United States;

*Corresponding author: e-mail address: britta.moehl@tum.de

Contents

Keywords: Gammaherpesvirus entry and fusion, Epstein-Barr virus EBV, Kaposi Sarcoma-associated herpesvirus KSHV, virus entry, viral fusion, glycoprotein gB and gH/gL

Abstract

The prototypical human γ-herpesviruses Epstein-Barr virus (EBV) and Kaposi Sarcoma-associated herpesvirus (KSHV) are involved in the development of malignancies. Like all herpesviruses, they share the establishment of latency, the typical architecture, and the conserved fusion machinery to initiate infection. The fusion machinery reflects virus-specific adaptations due to the requirements of the respective herpesvirus. For example, EBV evolved a tropism switch involving either the B- or epithelial cell-tropism complexes to activate fusion driven by gB. Most of the EBV entry proteins and their cellular receptors have been crystallized providing molecular details of the initial steps of infection. For KSHV, a variety of entry and binding receptors has also been reported but the mechanism how receptor binding activates gB-driven fusion is not as well understood as that for EBV. However, the downstream signaling pathways that promote the early steps of KSHV entry are well described. This review summarizes the current knowledge of the key players involved in EBV and KSHV entry and the cell-type specific mechanisms that allow infection of a wide variety of cell types.

1. Introduction

Members of the *Herpesviridae* are large enveloped double-stranded DNA (dsDNA) viruses with a broad host range including almost all vertebrates and even invertebrates (Fields et al., 2013). Herpesviruses are very successful pathogens that have evolved over the last 60-80 million years together with their hosts due to the establishment of life-long latency (Davison et al., 2002). There are nine different human pathogenic herpesviruses that are grouped as α-, β- and γ-herpesviruses. The characteristic for α- and β-herpesviruses is a lytic infection accompanied by tissue destruction and a variety of diseases such as cold sores, corneal blindness, genital herpes, encephalitis, keratitis, chickenpox, shingles, birth defects through congenital infection, and skin lesions (Fields et al., 2013). However, the prototypical γ-herpesviruses Epstein-Barr virus (EBV) and Kaposi Sarcoma-associated

herpesvirus (KSHV) are both associated with quite distinct disease progression, i.e. lymphoproliferative diseases and malignancies.

EBV is the causative agent of infectious mononucleosis in young adults (Longnecker et al., 2013). Infection is associated with epithelial cell malignancies such as gastric carcinoma, nasopharyngeal carcinoma, and B-cell lymphomas such as Burkitt's and Hodgkin's lymphoma. KSHV is involved in endothelial cell malignancies such as Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and Multicentric Castleman's disease (MCD) which are often associated with suppression of the immune system (Damania and Cesarman, 2013). These EBV- and KSHV-associated malignancies are rare even in immunosuppressed patients compared to the ubiquitous seroprevalence in the human population (Damania and Cesarman, 2013, Longnecker et al., 2013). Nevertheless, the genetic and/or environmental triggers and the interplay with compromised immunity is still poorly understood (Ambinder and Cesarman, 2007). KSHV was first identified in Kaposi sarcoma tumor biopsies (Chang et al., 1994), whereas EBV was first found in Burkitt's lymphoma (Epstein et al., 1964). Both viruses are known to infect divergent tissue types in humans that are the origins for the associated malignancies and the establishment of latency. Infection of new hosts requires reactivation of latent virus that results in secretion of newly produced virions into the saliva and primary infections are commonly asymptomatic. Primary infections are controlled by a strong cellular immune response and infected cells are usually efficiently cleared preventing disease. But to evade the immune system, both KSHV and EBV have evolved numerous strategies to modulate host immune pathways facilitating the establishment and maintenance of latency, a hallmark of herpesviruses (Coscoy, 2007, Thorley-Lawson and Allday, 2008). Currently, only seven human oncogenic viruses are known and among them EBV and KSHV are the only herpesviruses (Schäfer et al., 2015).

Despite having different biological characteristics in human infections, EBV and KSHV have very similar mechanisms of infecting target cells in humans. Contrary to EBV, KSHV initially attaches to heparan sulfate proteoglycans (HSPGs). This relatively non-specific attachment step concentrates the virus onto host cell membranes and favors virus-

specific binding to cell type-intrinsic receptors defining the host cell tropism, which then initiate infection. Enveloped viruses such as EBV and KSHV use a fundamental cellular mechanism - membrane fusion -to infect host cells. This process is initiated by receptor binding followed by fusion of the virus envelope with the host cell membrane that requires overcoming the high-energy barrier of fusion of the virion membrane with a host cell membrane. Interestingly, most enveloped viruses require only one protein fulfilling receptor-binding and fusion. In contrast, herpesviruses evolved a fine-tuned and complex entry machinery composed of receptor-binding glycoproteins, the fusion regulatory glycoproteins gH/gL, and the fusogen gB. A specific receptor-binding step enables the interaction of gH/gL with a prefusion form of gB in a metastable conformation on the surface of mature virions. This interaction triggers extensive structural rearrangements within gB resulting in a stable postfusion state that overcomes the high-energy barrier to merge cellular and viral membranes. Full fusion following fusion pore formation ensures the release of the nucleocapsid into the cytoplasm (Connolly et al., 2011, Longnecker et al., 2013). KSHV and EBV have many features in common including the classification as γ-herpesviruses sharing the typical herpesvirus architecture, establishment of latency in lymphocytes and the use of ephrin receptor tyrosine kinase A2 (EphA2) as an entry receptor (Chakraborty et al., 2012a, Chen et al., 2018, Damania and Cesarman, 2013, Fields et al., 2013, Hahn et al., 2012, Longnecker et al., 2013, Zhang et al., 2018). In this chapter, aspects of γ-herpesvirus entry into their host cells will be explored describing EBV and KSHV and their host cell interactions critical for infection and subsequent initiation of the viral life cycle.

2. History of EBV and KSHV

Even though infectious mononucleosis was reported in the late 19th century and described in 1920 (Sprunt and Evans, 1920), it took nearly half a century to identify EBV as the causative agent (Henle et al., 1968). The discovery of EBV began in 1958 with Burkitt's description of a sarcoma affecting the jaws of African children (Burkitt, 1958) and his talk at

the Middlesex Hospital on this new sarcoma in 1961. This lecture was attended by Anthony Epstein who established a collaboration with Denis Burkitt. In 1964, Epstein first described EBV using electron microscopy detecting virus particles with the typical morphology of *Herpesviridae* in cultured Burkitt lymphoma cells (Epstein et al., 1964). This allowed the development of an immunofluorescent antibody test, which revealed that patients with Burkitt lymphoma and most Americans had antibodies against EBV (Henle and Henle, 1966). Finally in 1978, strong epidemiological evidence indicated a causal relationship between Epstein-Barr virus and Burkitt's lymphoma (de-The et al., 1978). It was the first time that the causal relationship between a lymphoma and a virus had been described. The lymphoproliferative potential has been supported by the finding that EBV transforms and induces proliferation in human B-lymphocytes in cell culture (Nilsson et al., 1971). Besides B-lymphocytes, epithelial cells are the major site for EBV infection in humans and replication in oropharyngeal epithelial cells was firstly described in 1984 (Sixbey et al., 1984). Moreover, the lymphoproliferative potential of EBV has been associated with other malignancies such as nasopharyngeal carcinoma in 1970 (zur Hausen et al., 1970), non-Hodgkin lymphoma in patients with acquired immunodeficiency syndrome (AIDS) in 1982 (Ziegler et al., 1982), T-cell lymphoma in 1988 (Jones et al., 1988), and Hodgkin lymphoma in 1989 (Weiss et al., 1989).

In 1872, the Hungarian physician Moritz Kaposi first reported the idiopathic multiple pigmented sarcoma of the skin, which is now called Kaposi sarcoma or KS (Kaposi, 1872). Classic KS is a rare cancer worldwide and described as a slow-growing, chronic tumor confined to the skin mostly in elderly men of the Mediterranean, Eastern Europe, or the Middle East (Chang and Moore, 2014, Robey and Bower, 2015). In the 1950's, a peak of endemic KS, a more fatal form accompanied by lymph node involvement in adults and children, occurred at extraordinary rates in Central and East Africa as one of the most commonly-reported cancers in certain cancer registries (Cook-Mozaffari et al., 1998). In the 1970's, an increasing incidence of KS was observed among organ transplant recipients involving immunosuppressive therapies and transplantation treatment regiments (Chang and

Moore, 2014, Penn, 1979). An unusual cluster of patients with KS and/or pneumocystis pneumonia in New York City and in California heralded the outbreak of the AIDS epidemic in the early eighties of the last century (Centers for Disease, 1981, Centers for Disease, 1982a). The first report of AIDS described that approximately 37% of the patients had KS (Centers for Disease, 1982b), HIV was only identified shortly afterward (Barre-Sinoussi et al., 1983, Gallo et al., 1984). In 1984 herpes-like structures were found in KS lesions using electron microscopy (Walter et al., 1984), but at that time several other viruses were discussed to be causative. It was another decade and more than a century after the first report of KS that Chang and Moore identified that a herpesvirus was present in tumor cells using representational difference analysis using DNA from a KS lesion from an AIDS patient (Chang et al., 1994). More recently, KSHV has been associated with the B-cell lymphoma designated as primary effusion lymphoma, which is often co-infected with EBV and is also observed in AIDS patients (Damania and Cesarman, 2013).

3. Route of Infection and Host Cell Tropism

More than 90% of the human population are infected with EBV, thus it is one of the most abundant pathogens among human viruses (Auwaerter, 1999, Balfour et al., 2013). Primary infection with EBV is normally asymptomatic and occurs during childhood via droplet and contact infection with saliva. In young adults or adolescents, 30-60% of primary infected patients can develop infectious mononucleosis that is an acute illness manifested by lymphadenopathy, fever and pharyngitis. In fewer cases (10% of patients), patients may have splenomegaly, palatal petechiae, and hepatomegaly (Cohen, 2000). Primary infection with EBV by saliva contact occurs in the oropharynx, where it enters through the squamous oropharyngeal epithelium that lines the nasopharynx (tonsil). It is still controversial whether EBV directly infects either resting naïve B cells or epithelial cells, which in turn infect naïve B cells. Naïve B cells reside below epithelial cells in Waldeyer's ring that includes adenoids and tonsils. It surrounds the nasopharyngeal region and together with this epithelium is part of the

lymphoepithelium. Infected B cells can either produce virus (lytic infection) or express a small subset of viral proteins that facilitate latency. Latently infected B cells become B-cell blasts and mimic a germinal center reaction enabling the virus to persist in the memory B cell compartment (Longnecker et al., 2013).

For spread to naïve individuals, infected memory cells are thought to return to the lymphoepithelium where some of them differentiate into plasma cells and undergo lytic replication (Ambinder and Cesarman, 2007, Laichalk and Thorley-Lawson, 2005, Longnecker et al., 2013). EBV can be amplified in the epithelium for the shedding of virus in saliva or may infect naïve B cells completing the EBV cycle of infection. EBV reactivation is restrained by natural killer (NK) cells and cytotoxic T cells resulting in recognition and destruction of infected cells (Cohen, 2000, Longnecker et al., 2013, Thorley-Lawson and Allday, 2008). This cycle results in a lifelong persistence, which can lead to the development of malignancies originated by B-lymphocytes as in Burkitt and Hodgkin lymphomas or epithelial cells such as nasopharyngeal carcinoma and certain gastric carcinomas (Longnecker et al., 2013). In addition, smooth muscle cells support infection and replication of EBV, which in some cases result in the development of EBV positive smooth muscle tumors, the second most prevalent malignancy of children with AIDS (Dekate and Chetty, 2016, Jenson et al., 1997). EBV can also rarely infect other cell types such as T cells and NK cells in immunosuppressed patients resulting in T/NK-cell lymphoproliferative disorders (Akashi and Mizuno, 2000, Coleman et al., 2015, Isobe et al., 2004, Park and Ko, 2014). Interestingly, the tropism switch, which targets EBV to either epithelial or B cells (Borza and Hutt-Fletcher, 2002) alters its capacity to enter monocytes but blocks monocyte maturation into dendritic cells (Guerreiro-Cacais et al., 2004). EBV infection of monocytes also induces acute maturation into macrophages and infiltration of infected macrophages into cancer tissues associated with EBV are discussed (Shimakage, 2014). The epithelial cell receptor of EBV was identified as EphA2 (Chen et al., 2018, Zhang et al., 2018). EphA2 is known to be involved not only in monocyte differentiation and maturation (Mukai et al., 2017), but also blocks antigen receptor-induced apoptosis by $CD4^+$ T cells (Holen et al., 2008).

Primary infection with KSHV occurs during childhood by salivary transmission (Ambinder and Cesarman, 2007, Henke-Gendo and Schulz, 2004, Pica and Volpi, 2007). In the oropharynx, KSHV infects the tonsillar epithelium that functions as the entrance for the virus to infect underlying B-lymphocytes, which are the site of latency (Chagas et al., 2006, Duus et al., 2004, Pauk et al., 2000, Pica and Volpi, 2007, Vieira et al., 1997, Webster-Cyriaque et al., 2006). Primary infection during childhood is usually asymptomatic but can be associated with febrile illness and maculopapular rash without compromised immunity. But in organ transplant recipients, primary infection can result in fever, lymphoid hyperplasia, splenomegaly and pancytopenia (Ambinder and Cesarman, 2007). In immune-competent individuals, KSHV establishes asymptomatic life-long latency in a subset of B cells, the lambda expressing B cells (CD19+ B cells) that do not circulate in large numbers in peripheral blood (Ambroziak et al., 1995, Hassman et al., 2011, Rappocciolo et al., 2008). The healthy, competent immune system of infected individuals controls lytic reactivation of KSHV and compels latency, thus the immune status is one of the key factors controlling reactivation (Lukac and Yuan, 2007). However, reactivation of KSHV from latency is the critical pathogenic step during progression to KS and the development of other KSHV-associated malignancies such as PEL and MCD. In addition, another key factor in KS progression is the occurrence of co-pathogenic infections that can perturb this regulatory mechanism and thereby influence the pathologic outcome of KSHV infection (Ambinder and Cesarman, 2007, Lukac and Yuan, 2007). Sexual transmission in adults within high-risk groups can result in lymphadenopathy associated with seroconversion (Ambinder and Cesarman, 2007, Henke-Gendo and Schulz, 2004, Lukac and Yuan, 2007, Martin et al., 1998, Pica and Volpi, 2007).

Primary infection with KS in AIDS-KS patients occurs at least ten years prior to clinically apparent signs of KS lesions (Lukac and Yuan, 2007, Martin et al., 1998). AIDS-KS progression can be followed first by seroconversion and then by appearance of measurable virus DNA in peripheral blood. The viremia of the peripheral blood is accompanied by infection of endothelial cells of the skin resulting in the typical KS lesions. The severity of KS

outcome correlates with the viral load in peripheral blood and the risk of developing KS is ten-fold higher in patients with viremia of the peripheral blood mononuclear cells (PBMCs) than in those without this viremia (Lukac and Yuan, 2007). The cell tropism of KSHV is also reflected by the origin of associated malignancies such as KS, which is a slow growing, highly vascularized tumor of endothelial cell origin influenced by lytic replication, immune modulation and production of multiple cytokines as well as immune cells. KS is differentiated into: (i) the classic rarely aggressive KS in elderly men of Mediterranean and Middle Eastern ancestry, (ii) severe African-endemic KS with significant morbidity and mortality, (iii) iatrogenic KS of pharmacological immunosuppressed patients, (iv) the most aggressive clinical manifestation, endemic AIDS-KS predominantly in Sub-Saharan countries (Thakker and Verma, 2016). In addition, KSHV is associated with two other relatively rare lymphoproliferative disorders including PEL and KSHV-associated MCD. This rare but aggressive immunoblastic or anaplastic large-cell PEL lymphomas are of B cell origin and are characterized by KSHV-infection commonly accompanied by co-infection with EBV. This late manifestation during AIDS progression is described as malignant effusion involving body cavities and occasionally extra-nodal sites (Ambinder and Cesarman, 2007). KSHV is also associated with MCD - an atypical, heterogeneous group of lymphoproliferative disorders that share common histopathological features defined as angiofollicular lymph node hyperplasia. KSHV shows a distinct tropism for both B-cells (CD19+) and T-lymphocytes in KSHV-associated MCD patients (Ambroziak et al., 1995, Hassman et al., 2011, Kikuta et al., 1997, Rappocciolo et al., 2008). Castleman´s Disease (CD) is classified as unicentric localized or multicentric CD that involves many lymph node groups and spleen (multicentric angiofollicular hyperplasia) and is further subclassified due to presence/absence of KSHV infection (KSHV-associated MCD) (Ambinder and Cesarman, 2007). Based on these findings in human infection, KSHV has a broad host cell tropism *in vivo* including epithelial cells, endothelial cells, keratinocytes, fibroblasts, B- and T-lymphocytes, monocytes and macrophages as well as dendritic cells (Chandran and Hutt-Fletcher, 2007, Damania and Cesarman, 2013, Kikuta et al., 1997, Pica and Volpi, 2007). Contrary to EBV, KSHV has a

very broad tropism regarding susceptible host cell lines. Surprisingly, it can also infect cells across species-barriers such as owl monkey kidney cells, baby hamster kidney fibroblast cells, Chinese hamster ovary cells, and mouse fibroblasts (Chandran and Hutt-Fletcher, 2007, Damania and Cesarman, 2013).

4. The Herpesvirus Fusion Machinery

Herpesviruses require three to six viral envelope proteins forming the fine-tuned complex fusion machinery involving binding to a specific host cell receptor, activation of the fusogen and the virus-driven fusion process. Most enveloped viruses only use one or two proteins driving entry into their host cells. In contrast, the core fusion machinery conserved among *Herpesviridae* consists of gH/gL, which functions as activator, and gB, which is the driver of membrane fusion (Connolly et al., 2011, Heldwein, 2016, Möhl et al., 2016). The heterodimeric complex of gH/gL is the key to the divergent virus-specific tropism among *Herpesviridae* by recruiting additional non-conserved viral proteins engaged in binding cell-specific host receptors (Chandran and Hutt-Fletcher, 2007, Connolly et al., 2011, Longnecker et al., 2013). For example, the α-herpesviruses herpes simplex virus (HSV) and Pseudorabies virus (PrV) only require gD for determining cell tropism (Connolly et al., 2011). The β-herpesviruses such as human cytomegalovirus (HCMV) utilize gO and UL128/130/131 complexes (Adler, 2015, Revello and Gerna, 2010, Zhou et al., 2015), and human herpesvirus 6 (HHV-6) requires gO and gQ1/Q2 (Jasirwan et al., 2014, Mori, 2009, Tang et al., 2014) that complex with gH/gL. EBV utilizes gp42 that also binds gH/gL to facilitate its cell-specific tropism for B cells, but for epithelial cell entry only gH/gL is required for receptor binding (Borza and Hutt-Fletcher, 2002, Chen et al., 2018, Kirschner et al., 2009, Sathiyamoorthy et al., 2016, Sathiyamoorthy et al., 2014, Wang et al., 1998, Zhang et al., 2018). These tropism-determinant complexes form membrane bridging intermediates engaging and activating gB to mediate membrane fusion (Sathiyamoorthy et al., 2017).

The crystal structure of EBV gB revealed similarities to the postfusion form of vesicular stomatitis virus glycoprotein G (VSV G), thus being classified as class III viral fusion protein (Backovic et al., 2009). Crystal structure studies of gB across *Herpesviridae* revealed that the herpesvirus postfusion form exists as spike-like homotrimer formed by five individual domains that adopt an elongated conformation. The EBV gB structure is very similar to the postfusion structures of HSV-1, PrV, and HCMV gB but the domain orientations vary suggesting species-specific functional adaptations (Backovic et al., 2009, Burke and Heldwein, 2015, Chandramouli et al., 2015, Heldwein, 2016, Vallbracht et al., 2017). Besides the presumed role of gH/gL in initiating gB-driven fusion, it is also an important determinant for cell tropism of certain herpesviruses such as EBV, HCMV, and HHV6 (Adler, 2015, Connolly et al., 2011, Möhl et al., 2016, Mori, 2009). The crystal structures of gH/gL across *Herpesviridae* revealed no similarity to known fusogens. However, despite low amino acid conservation, they share a striking structure adopting an elongated rod- or boot-like shape (Backovic et al., 2010, Chowdary et al., 2010, Matsuura et al., 2010, Xing et al., 2015). The overall structure is formed by four structural domains with divergent domain interfaces, whose flexibility is important for gH/gL functioning as a trigger for fusion (Böhm et al., 2015, Chen et al., 2013, Möhl et al., 2015, Wu et al., 2005). Although the crystal structures of KSHV entry proteins have not been solved, the sequence homology between EBV and KSHV glycoproteins, 42% for gB, 26.8% for gH and 27.1% for gL (Gish and States, 1993, Papadopoulos and Agarwala, 2007), allowed homology modeling of KSHV gB, gH and gL. The homology models of KSHV gB, gH and gL (Fig. 1) were generated using the SWISS-MODEL program (Waterhouse et al., 2018) based on the crystal structures of EBV gB (PDB ID: 3FVC), gH (6C5V) and gL (5T1D) (Backovic et al., 2009, Sathiyamoorthy et al., 2016, Snijder et al., 2018).

The structural sequence alignment of EBV and KSHV gB based on the crystal structure of EBV gB (3FVC) (Backovic et al., 2009) was performed using T-COFFEE Expresso (Notredame et al., 2000). The alignment revealed that the overall domain (D) distribution is similar but that DIV (the “head” of the gB postfusion structure) is enlarged in

KSHV (Fig. 1A, B). The hypothetical structure of KSHV gB generated by SWISS-MODEL shows an overall similar elongated rod-like structure as EBV gB, reminiscent of a slouchy and sad stick figure (Fig. 1B). The feet are the fusion loops, the plekstrin-homology modules of DI and DII are the legs. One long α-helix represents the spine that kinks down on the tip giving the impression that the figure is bent over. This impression is supported by the following disordered DIV looking like the slouchy stick figure with head down. The elongated helical DV resembles the loose arms stretching towards its feet. In the homotrimer of gB, this loose extended α-helical arm (DV) reaches into the cavity, formed by the other two monomers together with the α-helical spine of DIII, which wraps around the helices of the other two monomers and stably ties together the homotrimer (Backovic et al., 2009). DIV of EBV gB is more disordered than HSV-1 gB which might result in more flexibility (Backovic et al., 2009). The potentially dynamic structure of DIV could be a common feature of class III viral fusion proteins as suggested by the disordered analogous DIV of baculovirus gp64 (Backovic et al., 2009). DI, with the plekstrin-homology module and the exposed loops ("feet") connecting the β-sheets at the extreme bottom of the KSHV gB structure (Fig. 1B), may resemble the fusions loops similar to all gB homologues crystallized to date. A similar plekstrin-homology module is found in DII that is a common feature for a membrane-binding domain but also for ligand-binding epitopes (Backovic et al., 2009). These modules could be involved in gH/gL-binding or interaction with cellular membranes as discussed for HSV-1 gB (Atanasiu et al., 2010a, Atanasiu et al., 2010b, Chandramouli et al., 2015). DII of KSHV gB is not only composed of this module but contains also an exposed helix, and, contrary to EBV gB, carries several predicted N-glycosylation sites (Fig. 1A, B circled). Like HCMV gB, KSHV gB is characterized by several predicted N-glycosylation sites in the interface of DI/DII and in DII around the predominant α-helix structure. HSV-1 gB has only 5 N-glycan's, whereas EBV gB has 10 predicted N-glycosylation sites and 3 (N163, N290, N629) of them showed electron density in the crystal structure (Backovic et al., 2009, Heldwein et al., 2006). Two of the 15 predicted N-glycosylation sites of KSHV gB correspond to N163 and N629 of EBV gB (Fig. 1B). Similar to KSHV gB, HCMV gB carries 18 predicted N-glycosylation sites and 10

could be proven by the crystal structure, whereas the other six are located in unstructured regions and two outside of the crystallized ectodomain (Chandramouli et al., 2015). As discussed for HCMV gB, the heavy glycosylation around the plekstrin-homology modules might protect these important binding regions from a host antibody response (Chandramouli et al., 2015). It would be interesting to understand why only certain herpesviruses evolved this feature and whether it co-evolved with certain tropism requirements.

The structural sequence alignments of KSHV and EBV gH and gL based on the crystal structures of EBV gH (6C5V) and gL (5T1D) (Chandramouli et al., 2015, Sathiyamoorthy et al., 2016, Snijder et al., 2018) were performed using T-COFFEE Expresso (Notredame et al., 2000). The alignments revealed an overall similar domain distribution (Fig. 1A) but with extended N-terminal domains (DI) for gH and gL that are known to facilitate the intimate complexing between both (Fig. 1C) suggesting a virus-specific adaptation of KSHV. The hypothetical KSHV gH configuration reveals a similar structure of the complex composed of four domains including four conserved disulfide bonds within DII, DIII and DIV and the two predicted, conserved N-glycosylation sites within DIV (Fig. 1C). EBV gL includes two disulfide bonds and three confirmed N-glycans (Matsuura et al., 2010), whereas the hypothetical model of KSHV gL indicates structurally homologous disulfide bonds but distinct N-glycosylation sites (Fig. 1C). KSHV has 15 predicted N-glycosylation sites that differ from EBV gH with its five N-glycans. Most of these predicted N-glycans are localized in a region that corresponds to an epitope in EBV gH responsible for gp42-binding (Fig. 1C). Thus, it will be important to understand if this region in KSHV gH also plays a role for binding to another viral entry protein or direct to a specific host cell receptor and if KSHV for this purpose evolved the predicted N-glycans masking the epitope from the host antibody response. In detail, gL and DI of gH are closely linked and connected to the rest of gH by one hinge assembled by the DI/DII-linker helix. DII starts with a striking antiparallel β-sheet structure that shields the rest of gH against DI, the so-called fence. Direct underneath the fence, a bundle of three α-helices resembles a syntaxin-like bundle (SLB) structure. In case of EBV, two disulfide bonds flank this SLB, one is conserved in KSHV gH and a triad of cysteine

residues is located around the region corresponding to disulfide bond 1 of EBV gH (Fig. 1C). DIII is α-helical with a stabilizing disulfide bond conserved across gH homologues and followed by DIV with its antiparallel β-sandwich structure and two disulfide bonds that act as hinges connected by a long loop. This loop hides an extended hydrophobic patch that might be exposed during the cascaded fusion process enabling interaction with membranes. The loop and the β-sandwich structure carry the conserved N-glycan's of EBV and KSHV gH (Fig. 1C).

Herpesviruses can engage divergent entry pathways for different target cells (Connolly et al., 2011) despite conserved fusion machinery. An example of this is the observation that EBV infects B-lymphocytes, the site of latency, by endocytosis, but infects epithelial cells by direct fusion with the host cell plasma membrane (Miller and Hutt-Fletcher, 1992, Nemerow and Cooper, 1984). EBV has a distinct tropism for both cell types due to their role in facilitating primary infection, and virions carry two diverse gH/gL complexes, the epithelial cell-tropism complex or the B cell-tropism complex. For epithelial cell entry, only the core fusion machinery of gB and gH/gL and the epithelial cell entry-specific receptor EphA2 are required. Thus, the gH/gL complex is designated as the epithelial cell-tropism complex (Fig. 2A) (Chen et al., 2018; Longnecker, Kieff and Cohen, 2013; Zhang et al., 2018). For B cell entry, EBV requires an additional glycoprotein (gp)42 that is unique for EBV and other closely related lymphocryptoviruses. EBV gp42 binds to the B cell receptor human leukocyte antigen (HLA) class II (Longnecker, Kieff and Cohen, 2013) for B cell entry and the gH/gL-gp42 complex acts as the B cell-tropism entry complex (Fig. 2B). Structural analysis of the gH/gL-gp42 complex revealed that the elongated N-terminus of gp42 wraps around gH involving three of its four external surfaces (Fig. 2B) (Sathiyamoorthy et al., 2016). The receptor binding causes a widening of the hydrophobic pocket within the C-terminus of gp42 and it is proposed that this feature is involved in the cascaded fusion process (Kirschner et al., 2009). The crystal structure of gH/gL-gp42 indicates an intimate interaction of gp42 with gH suggesting that this change is translated to gH/gL activating gB for cell fusion.

5. EBV-driven entry and fusion

B cells are the site of latency for EBV and therefore the major target cells for infection. Infection of B cells is initiated by the interaction of the abundant viral attachment protein gp350/220 to complement receptor 2 (CD21 or CR2) or CD35 (Fig. 3) (Ogembo et al., 2013, Tanner et al., 1987). This binding results in capping of CD21 followed by endocytosis of the virion into the host cell (Nemerow and Cooper, 1984, Tanner et al., 1987). This attachment step tethers EBV to its host cell and thereby increases the efficiency of infection, although this step is not essential for B cell entry (Janz et al., 2000). Interestingly, contrary to B-lymphocytes, B lymphoblastoid cells are infected by direct fusion with the viral envelope (Nemerow and Cooper, 1984). Fusion is mediated by the binding of gp42 in the gH/gL-gp42 complex to the B cell-specific HLA class II receptor (Fig. 3). This binding is accompanied by a widening of the hydrophobic pocket within gp42. This conformational change may expose additional binding sites for engaging other components of the entry machinery or a yet unidentified cellular interaction partner (Kirschner et al., 2009). Structural data indicate that the conformational change within gp42 is involved in triggering the overall fusion process (Kirschner et al., 2009). After receptor binding, the hydrophobic pocket of the C-terminus, including the HLA-binding region, is engaged closer to the complex enabling interaction with the DII/III-interface of gH (Sathiyamoorthy et al., 2016). The stability of the gH/gL-gp42 complex with HLA class II provided the opportunity to discern the overall structure of the B cell-triggering complex required for infection by EBV, a first for herpesviruses (Sathiyamoorthy et al., 2016, Sathiyamoorthy et al., 2014). In these electron micrographs, an “open” and “closed” state were observed. The “open” state was characterized by a highly variable orientation of HLA and gH/gL to each other resulting in larger angles, whereas the “closed” state, likely the structural view of the gH/gL-gp42 complex, revealed a more rigid orientation with the C-terminus of gp42 closely engaged to the interface of DII/III of gH (Sathiyamoorthy et al., 2016, Sathiyamoorthy et al., 2014). Mutational analysis of the hydrophobic pocket of gp42 indicated that the closed state, which draws the transmembrane domains of gH and HLA closer to each other, is important for activating the cascaded fusion

process driven by gB (Sathiyamoorthy et al., 2016). Following HLA-binding and conformational change within gp42, the gH/gL-gp42 complex is then thought to bind to the metastable prefusion gB (Fig. 3). The binding to gB triggers a conformational change of gB from the hypothetical prefusion state to the extended pre-hairpin intermediate enabling the insertion of the fusion loops into the host cell membrane, thereby acting as additional bridge between the two bilayers (Connolly et al., 2011, Heldwein, 2016, Longnecker et al., 2013, Möhl et al., 2016, Sathiyamoorthy et al., 2017). Based on previous studies, a molecular model of herpesvirus gB postulates that the tip of the second fusion loop can further penetrate into the hydrophobic core of the cellular lipid bilayer enabling the side chains forming a rim structure. Thereby, the fusion loops form a stable interaction with the host cell membrane ensuring that the two membranes will fuse (Vallbracht et al., 2017). The collapse and refolding of this extended pre-hairpin intermediate to a trimer of hairpins catalyzes enough free energy to overcome the high kinetic barrier for merging membranes (Connolly et al., 2011, Harrison, 2015, Sathiyamoorthy et al., 2017). The structural analysis of the postfusion form of EBV gB highlighted a long C-terminal arm arranged antiparallel to the coiled-coil core of the trimeric hairpin (Backovic et al., 2009, Connolly and Longnecker, 2012). The formation of this coil-arm complex may facilitate the reorganization of the gB structure into the postfusion form of gB similar to class I fusogens (Connolly and Longnecker, 2012). Thus, the activation of gB triggers the highly orchestrated fusion process of both membranes releasing the nucleocapsid into the cytoplasm of the target cells. B cell-derived virions are gp42-deficient due to g42 being sequestrated by HLA class II during maturation, and thereby acts as tropism switch rendering virions without gp42 capable of infecting epithelial cells. In contrast, virions derived from epithelial cells are enriched in the B cell-tropism complex gH/gL-gp42, which can facilitate entry into B cells (Borza and Hutt-Fletcher, 2002, Wang et al., 1998, Longnecker et al., 2013).

The attachment step tethering EBV to epithelial cells is facilitated by gp350/220 binding to CD21 expressed on tonsillar epithelial cells and BMRF2 interaction with integrins on polarized epithelial cells (Fig. 4) (Jiang et al., 2008, Tugizov et al., 2003, Xiao et al.,

2007). The epithelial cell-tropism complex gH/gL binds to the ligand-binding domain of EphA2 similar to KSHV (Chen et al., 2018, Zhang et al., 2018). Interestingly, EBV enters epithelial cells by direct fusion with the plasma membrane, whereas KSHV enters its host cells via an endocytosis pathway involving gH/gL-binding to EphA2, A4 or A5 (Chen et al., 2018, Miller and Hutt-Fletcher, 1992, Zhang et al., 2018, Chakraborty et al., 2012a, Hahn et al., 2012, Chandran, 2010, Chen et al., 2019, TerBush et al., 2018). EBV gH/gL acts as tropism switch by either interacting with gp42 that facilitates B cell receptor binding or directly binding to the epithelial cell receptor EphA2 (Borza and Hutt-Fletcher, 2002, Chen et al., 2018, Matsuura et al., 2010, Möhl et al., 2016, Sathiyamoorthy et al., 2016, Sathiyamoorthy et al., 2014, Zhang et al., 2018). Receptor binding by gH/gL enables the transient and dynamic interaction with gB initiating the fusion process (Fig. 4). Similar to the B cell entry-triggering complex, it is likely that the epithelial cell entry-mediating complex requires conformational changes within gH/gL that enable interaction with gB (Longnecker et al., 2013). Compatible with this hypothesis, the domain interfaces of the four domains and their flexibility are required for gH/gL-activated gB-driven fusion process (Böhm et al., 2015, Chen et al., 2013, Möhl et al., 2015, Wu et al., 2005). In addition, the disulfide bond of DII tightens the syntaxin-like bundles and thereby induces a local rigidity that is required for epithelial cell fusion. In contrast, a larger perturbation of this region next to the DII/III interface that binds to gp42 disturbs the formation of the B cell entry-triggering complex (Möhl et al., 2014, Sathiyamoorthy et al., 2014). Despite the striking structural similarity of the postfusion forms of gB conserved across *Herpesviridae*, the differences in domain orientations indicate virus-specific functional adaptations regarding its receptor-binding partners (Backovic et al., 2009, Burke and Heldwein, 2015, Chandramouli et al., 2015, Connolly et al., 2011, Heldwein et al., 2006, Stampfer and Heldwein, 2012, Vallbracht et al., 2017).

6. KSHV-driven entry and fusion

KSHV predominantly infects endothelial cells that are the origin for development of KS and activated B cells, which are the origin for a rare type of B cell lymphoma or PEL. Besides these two target cell types, KSHV can enter multiple other cell types including PBMCs, fibroblasts, keratinocytes, and epithelial cells (Chandran, 2010, Chandran and Hutt-Fletcher, 2007). The main entry route are diverse pH-dependent endocytosis pathways such as dynamin-dependent, clathrin-mediated endocytosis for fibroblasts and clathrin/caveolin-mediated endocytosis for monocytes (Akula et al., 2003, Chandran, 2010, Damania and Cesarman, 2013, Kerur et al., 2010). In contrast, early stages of KSHV infection targeting endothelial cells involve actin reorganization attended by actin-dependent macropinocytosis and c-Cbl-dependent membrane blebbing (Chandran, 2010, Damania and Cesarman, 2013, Raghu et al., 2009). Activated primary human B cells are infected by endocytosis involving DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing non-integrin - CD209) binding (Chandran, 2010, Damania and Cesarman, 2013, Rappocciolo et al., 2008). The broad KSHV cell tropism is supported by the usage of ubiquitous cell surface HSPGs as attachment receptor bound by gH, gB, or the KSHV specific glycoproteins open reading frame 4 (ORF4) and gpK8.1A (Fig. 5) (Akula et al., 2001a, Akula et al., 2001b, Chakraborty et al., 2012b, Hahn et al., 2009, Mark et al., 2006, Wang et al., 2001). Binding to HSPG tethers KSHV to its host cells increasing the efficiency of infection (Chandran, 2010, Damania and Cesarman, 2013). In addition, KSHV uses DC-SIGN as cell-specific receptor on human myeloid dendritic cells, macrophages, and activated B cells, which are the site of latency. A potential interaction partner of DC-SIGN is the highly mannosylated fusogen gB (Fig. 5) (Chandran, 2010, Damania and Cesarman, 2013, Rappocciolo et al., 2008). In addition, other entry receptors have been described including the cysteine transporter xCT and the multifunctional protein CD98 as well as integrins that promote entry into fibroblasts, endothelial and epithelial cells (Fig. 5). Interestingly, KSHV gB is the only gB-homolog with an integrin-binding motif (RGD-sequence). Previous studies demonstrated that several integrins such as $\alpha V\beta 3$, $\alpha V\beta 5$ and $\alpha 3\beta 1$ are important for infection of human epithelial and

endothelial cells as well as esophageal fibroblasts (Akula et al., 2002, Chandran, 2010, Damania and Cesarman, 2013, Wang et al., 2003). More recent studies suggest that KSHV infection of epithelial cells is independent of αν- and β1-family integrin expression (TerBush et al., 2018), but requires HSPGs and either EphA2, EphA4, or EphA5 (Chakraborty et al., 2012a, Chandran, 2010, Chen et al., 2019, Damania and Cesarman, 2013, Hahn et al., 2012, TerBush et al., 2018). Interestingly, EphA7 has recently been found to be a receptor required for KSHV entry into B cells (Grosskopf et al., 2019). KSHV attachment to its host cells via HSPG and DC-SIGN is predominately mediated by gB and gpK8.1A (Fig. 5). This binding may promote interaction of viral glycoproteins to the receptors integrin and xCT/CD98 inducing conformational changes within the receptor-binding glycoproteins rendering them accessible to interact with specific entry receptors (Schäfer et al., 2015, Veettil et al., 2014). Interestingly, integrin binding is followed by the interaction of gH/gL with an EphA family member initiating phosphorylation of EphA2 and thereby triggering coordinated integrin-associated downstream signaling that mediates clathrin-mediated endocytosis into fibroblasts (Chakraborty et al., 2012a, Dutta et al., 2013). Activation of cell receptor-associated signaling pathways creates a friendly environment for early events during infection (Kumar et al., 2018). KSHV infection is also known to rely on cell signaling pathways triggered by activation of FAK (focal adhesion kinase), and phosphorylated Src as well as PI3K (phosphoinositide 3-kinase) (Krishnan et al., 2006, Naranatt et al., 2003, Veettil et al., 2006). Moreover, KSHV uses the core fusion machinery of gB and gH/gL as well as KSHV-specific receptor-binding proteins gpK8.1A and ORF4 in divergent settings facilitating entry into a variety of human host cells (Schäfer et al., 2015, Veettil et al., 2014). KSHV entry into endothelial cells is initiated by tethering KSHV to the target cells by HSPG-binding via gB, gH, gpK8.1A, and ORF4 (Kumar et al., 2018). Tethering may enable the interaction with the specific entry receptors such as integrins, xCT and EphA family members (Fig. 5). Binding of specific entry receptors is thought to induce the activation of a variety of cell signaling pathways involved in actin-rearrangement, acetylation of microtubules, and endocytosis thereby mediating a preferable environment for entry stages (Kumar et al.,

2018). For example, KSHV entry into endothelial cells also involves actin-driven formation of blebs facilitating macropinocytosis (Kumar et al., 2018).

The receptor-binding step may activate gH/gL to bind to the metastable prefusion gB (Fig. 5) as indicated for EBV and other herpesviruses such as HSV and PrV. It was previously shown that only gH/gL and gB can mediate cell-cell fusion in a virus-free assay (Pertel, 2002). The conserved structures of the fusogen gB among *Herpesviridae* suggest a fundamental mechanism of gB-driven fusion initiated by the binding of gH/gL to gB. This binding is thought to trigger a conformational change of gB into an extended pre-hairpin intermediate enabling the insertion of the gB fusion loops into the host cell membrane. This bridges both bilayers and subsequent gB refolding brings the membranes together to allow fusion to occur (Connolly et al., 2011, Harrison, 2015, Heldwein, 2016, Longnecker et al., 2013, Sathiyamoorthy et al., 2017).

7. Conclusion

In summary, EBV and KSHV have many features in common including the induction of lymphoproliferation and the expression of conserved key proteins such as gB and gH/gL, which are required for virus-cell fusion (Chakraborty et al., 2012a, Chandran and Hutt-Fletcher, 2007, Chen et al., 2018, Hahn et al., 2012, Zhang et al., 2018). Nevertheless, the molecular details of entry and fusion vary dependent on the diverse cells types that these two herpesviruses infect (Tab. 1). EBV infection of its major host cells is fine-tuned by two different receptor-binding complexes, which have been characterized by crystal structures of all key players involved in entry except for the prefusion form of gB (Möhl et al., 2016). In contrast, there are no crystal structures for the proteins essential for KSHV entry, but the functions of the KSHV proteins can readily be discerned based on the amino acid sequence homology to EBV. In contrast, the importance of downstream signaling pathways supporting entry and later steps during replication are well described for KSHV (Chandran, 2010, Kumar et al., 2018). Future research using new techniques such as cryo-electron microscopy will

allow a full understanding of the complex entry pathway of these important human pathogens. The IARC-classification of KSHV and EBV as human carcinogens makes it important to understand the molecular details of the diverse entry and fusion pathway adaptions due to cell-type specific requirements to better understand their roles in the development of cancer associated with infections by these two pathogens.

Acknowledgments

The authors thank current and former members of the Longnecker and Jardetzky laboratories for their contributions to the work described. The research was supported by the National Institute of Allergy and Infectious Diseases (grant AI076183 to R.L.) and by the National Cancer Institute (grant CA117794 to R.L.). The DZIF Maternity Leave Stipend (TI 07.005) has been awarded to B.S.M by the German Center for Infection Research (Deutsches Zentrum für Infektionsforschung - DZIF). J.C. acknowledges funding by the National Institute of Allergy and Infectious Diseases, IRG-15-173-21 American Cancer Society and the Third Coast Center for AIDS Research pilot to J.C. B.S.M. wants to thank especially her little son, who tirelessly tried to contribute to text and figures.

References

ADLER, B. 2015. A Viral Pilot for HCMV Navigation? *Viruses,* 7**,** 3857-62.

AKASHI, K. & MIZUNO, S. 2000. Epstein-Barr virus-infected natural killer cell leukemia. *Leuk Lymphoma,* 40**,** 57-66.

AKULA, S. M., NARANATT, P. P., WALIA, N. S., WANG, F. Z., FEGLEY, B. & CHANDRAN, B. 2003. Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) infection of human fibroblast cells occurs through endocytosis. *J Virol,* 77**,** 7978-90.

AKULA, S. M., PRAMOD, N. P., WANG, F. Z. & CHANDRAN, B. 2001a. Human herpesvirus 8 envelope-associated glycoprotein B interacts with heparan sulfate-like moieties. *Virology,* 284**,** 235-49.

AKULA, S. M., PRAMOD, N. P., WANG, F. Z. & CHANDRAN, B. 2002. Integrin alpha3beta1 (CD 49c/29) is a cellular receptor for Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) entry into the target cells. *Cell,* 108**,** 407-19.

AKULA, S. M., WANG, F. Z., VIEIRA, J. & CHANDRAN, B. 2001b. Human herpesvirus 8 interaction with target cells involves heparan sulfate. *Virology,* 282**,** 245-55.

AMBINDER, R. F. & CESARMAN, E. 2007. Clinical and pathological aspects of EBV and KSHV infection. *In:* ARVIN, A., CAMPADELLI-FIUME, G., MOCARSKI, E., MOORE, P. S., ROIZMAN, B., WHITLEY,

R. & YAMANISHI, K. (eds.) *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis.* Cambridge University Press, Cambridge.

AMBROZIAK, J. A., BLACKBOURN, D. J., HERNDIER, B. G., GLOGAU, R. G., GULLETT, J. H., MCDONALD, A. R., LENNETTE, E. T. & LEVY, J. A. 1995. Herpes-like sequences in HIV-infected and uninfected Kaposi's sarcoma patients. *Science,* 268**,** 582-3.

ATANASIU, D., SAW, W. T., COHEN, G. H. & EISENBERG, R. J. 2010a. Cascade of events governing cell-cell fusion induced by herpes simplex virus glycoproteins gD, gH/gL, and gB. *J Virol,* 84**,** 12292-9.

ATANASIU, D., WHITBECK, J. C., DE LEON, M. P., LOU, H., HANNAH, B. P., COHEN, G. H. & EISENBERG, R. J. 2010b. Bimolecular complementation defines functional regions of Herpes simplex virus gB that are involved with gH/gL as a necessary step leading to cell fusion. *Journal of virology,* 84**,** 3825-34.

AUWAERTER, P. G. 1999. Infectious mononucleosis in middle age. *JAMA,* 281**,** 454-9.

BACKOVIC, M., DUBOIS, R. M., COCKBURN, J. J., SHARFF, A. J., VANEY, M. C., GRANZOW, H., KLUPP, B. G., BRICOGNE, G., METTENLEITER, T. C. & REY, F. A. 2010. Structure of a core fragment of glycoprotein H from pseudorabies virus in complex with antibody. *Proc Natl Acad Sci U S A,* 107**,** 22635-40.

BACKOVIC, M., LONGNECKER, R. & JARDETZKY, T. S. 2009. Structure of a trimeric variant of the Epstein-Barr virus glycoprotein B. *Proceedings of the National Academy of Sciences of the United States of America,* 106**,** 2880-5.

BALFOUR, H. H., JR., SIFAKIS, F., SLIMAN, J. A., KNIGHT, J. A., SCHMELING, D. O. & THOMAS, W. 2013. Age-specific prevalence of Epstein-Barr virus infection among individuals aged 6-19 years in the United States and factors affecting its acquisition. *J Infect Dis,* 208**,** 1286-93.

BARRE-SINOUSSI, F., CHERMANN, J. C., REY, F., NUGEYRE, M. T., CHAMARET, S., GRUEST, J., DAUGUET, C., AXLER-BLIN, C., VEZINET-BRUN, F., ROUZIOUX, C., ROZENBAUM, W. & MONTAGNIER, L. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science,* 220**,** 868-71.

BÖHM, S. W., ECKROTH, E., BACKOVIC, M., KLUPP, B. G., REY, F. A., METTENLEITER, T. C. & FUCHS, W. 2015. Structure-based functional analyses of domains II and III of pseudorabies virus glycoprotein H. *J Virol,* 89**,** 1364-76.

BORZA, C. M. & HUTT-FLETCHER, L. M. 2002. Alternate replication in B cells and epithelial cells switches tropism of Epstein-Barr virus. *Nature medicine,* 8**,** 594-9.

BURKE, H. G. & HELDWEIN, E. E. 2015. Crystal Structure of the Human Cytomegalovirus Glycoprotein B. *PLoS Pathog,* 11**,** e1005227.

BURKITT, D. 1958. A sarcoma involving the jaws in African children. *Br J Surg,* 46**,** 218-23.

CENTERS FOR DISEASE, C. 1981. Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men--New York City and California. *MMWR Morb Mortal Wkly Rep,* 30**,** 305-8.

CENTERS FOR DISEASE, C. 1982a. A cluster of Kaposi's sarcoma and Pneumocystis carinii pneumonia among homosexual male residents of Los Angeles and Orange Counties, California. *MMWR Morb Mortal Wkly Rep,* 31**,** 305-7.

CENTERS FOR DISEASE, C. 1982b. Update on acquired immune deficiency syndrome (AIDS)--United States. *MMWR Morb Mortal Wkly Rep,* 31**,** 507-8, 513-4.

CHAGAS, C. A., ENDO, L. H., SAKANO, E., PINTO, G. A., BROUSSET, P. & VASSALLO, J. 2006. Detection of herpesvirus type 8 (HHV8) in children's tonsils and adenoids by immunohistochemistry and in situ hybridization. *Int J Pediatr Otorhinolaryngol,* 70**,** 65-72.

CHAKRABORTY, S., VEETTIL, M. V., BOTTERO, V. & CHANDRAN, B. 2012a. Kaposi's sarcoma-associated herpesvirus interacts with EphrinA2 receptor to amplify signaling essential for productive infection. *Proc Natl Acad Sci U S A,* 109**,** E1163-72.

CHAKRABORTY, S., VEETTIL, M. V. & CHANDRAN, B. 2012b. Kaposi's Sarcoma Associated Herpesvirus Entry into Target Cells. *Front Microbiol,* 3**,** 6.

CHANDRAMOULI, S., CIFERRI, C., NIKITIN, P. A., CALO, S., GERREIN, R., BALABANIS, K., MONROE, J., HEBNER, C., LILJA, A. E., SETTEMBRE, E. C. & CARFI, A. 2015. Structure of HCMV glycoprotein

B in the postfusion conformation bound to a neutralizing human antibody. *Nat Commun,* 6**,** 8176.

CHANDRAN, B. 2010. Early events in Kaposi's sarcoma-associated herpesvirus infection of target cells. *J Virol,* 84**,** 2188-99.

CHANDRAN, B. & HUTT-FLETCHER, L. 2007. Gammaherpesviruses entry and early events during infection. *In:* ARVIN, A., CAMPADELLI-FIUME, G., MOCARSKI, E., MOORE, P. S., ROIZMAN, B., WHITLEY, R. & YAMANISHI, K. (eds.) *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis.* Cambridge.

CHANG, Y., CESARMAN, E., PESSIN, M. S., LEE, F., CULPEPPER, J., KNOWLES, D. M. & MOORE, P. S. 1994. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science,* 266**,** 1865-9.

CHANG, Y. & MOORE, P. 2014. Twenty years of KSHV. *Viruses,* 6**,** 4258-64.

CHEN, J., JARDETZKY, T. S. & LONGNECKER, R. 2013. The large groove found in the gH/gL structure is an important functional domain for Epstein-Barr virus fusion. *Journal of virology,* 87**,** 3620-7.

CHEN, J., SATHIYAMOORTHY, K., ZHANG, X., SCHALLER, S., PEREZ WHITE, B. E., JARDETZKY, T. S. & LONGNECKER, R. 2018. Ephrin receptor A2 is a functional entry receptor for Epstein-Barr virus. *Nat Microbiol,* 3**,** 172-180.

CHEN, J., ZHANG, X., SCHALLER, S., JARDETZKY, T. S. & LONGNECKER, R. 2019. Ephrin Receptor A4 is a New Kaposi's Sarcoma-Associated Herpesvirus Virus Entry Receptor. *MBio,* 10, https://doi.org/10.1128/mBio.02892-18.

CHOWDARY, T. K., CAIRNS, T. M., ATANASIU, D., COHEN, G. H., EISENBERG, R. J. & HELDWEIN, E. E. 2010. Crystal structure of the conserved herpesvirus fusion regulator complex gH-gL. *Nature structural & molecular biology,* 17**,** 882-8.

COHEN, J. I. 2000. Epstein-Barr virus infection. *N Engl J Med,* 343**,** 481-92.

COLEMAN, C. B., WOHLFORD, E. M., SMITH, N. A., KING, C. A., RITCHIE, J. A., BARESEL, P. C., KIMURA, H. & ROCHFORD, R. 2015. Epstein-Barr virus type 2 latently infects T cells, inducing an atypical activation characterized by expression of lymphotactic cytokines. *J Virol,* 89**,** 2301-12.

CONNOLLY, S. A., JACKSON, J. O., JARDETZKY, T. S. & LONGNECKER, R. 2011. Fusing structure and function: a structural view of the herpesvirus entry machinery. *Nature reviews. Microbiology,* 9**,** 369-81.

CONNOLLY, S. A. & LONGNECKER, R. 2012. Residues within the C-Terminal Arm of the Herpes Simplex Virus 1 Glycoprotein B Ectodomain Contribute to Its Refolding during the Fusion Step of Virus Entry. *Journal of virology,* 86**,** 6386-93.

COOK-MOZAFFARI, P., NEWTON, R., BERAL, V. & BURKITT, D. P. 1998. The geographical distribution of Kaposi's sarcoma and of lymphomas in Africa before the AIDS epidemic. *Br J Cancer,* 78**,** 1521-8.

COSCOY, L. 2007. Immune evasion by Kaposi's sarcoma-associated herpesvirus. *Nat Rev Immunol,* 7**,** 391-401.

DAMANIA, B. & CESARMAN, E. 2013. *Kaposi's A. Sarcoma-Associated Herpesvirus,* United States, Philadelphia: Wolters Kluwer Health/Lippincott, Williams & Wilkins, c2013.

DAVISON, A. J., DARGAN, D. J. & STOW, N. D. 2002. Fundamental and accessory systems in herpesviruses. *Antiviral Res,* 56**,** 1-11.

DE-THE, G., GESER, A., DAY, N. E., TUKEI, P. M., WILLIAMS, E. H., BERI, D. P., SMITH, P. G., DEAN, A. G., BRONKAMM, G. W., FEORINO, P. & HENLE, W. 1978. Epidemiological evidence for causal relationship between Epstein-Barr virus and Burkitt's lymphoma from Ugandan prospective study. *Nature,* 274**,** 756-61.

DEKATE, J. & CHETTY, R. 2016. Epstein-Barr Virus-Associated Smooth Muscle Tumor. *Arch Pathol Lab Med,* 140**,** 718-22.

DUTTA, D., CHAKRABORTY, S., BANDYOPADHYAY, C., VALIYA VEETTIL, M., ANSARI, M. A., SINGH, V. V. & CHANDRAN, B. 2013. EphrinA2 regulates clathrin mediated KSHV endocytosis in fibroblast cells by coordinating integrin-associated signaling and c-Cbl directed polyubiquitination. *PLoS Pathog,* 9**,** e1003510.

DUUS, K. M., LENTCHITSKY, V., WAGENAAR, T., GROSE, C. & WEBSTER-CYRIAQUE, J. 2004. Wild-type Kaposi's sarcoma-associated herpesvirus isolated from the oropharynx of immune-competent individuals has tropism for cultured oral epithelial cells. *J Virol,* 78**,** 4074-84.

EPSTEIN, M. A., ACHONG, B. G. & BARR, Y. M. 1964. Virus Particles in Cultured Lymphoblasts from Burkitt's Lymphoma. *Lancet,* 1**,** 702-3.

FIELDS, B. N., KNIPE, D. M. & HOWLEY, P. M. 2013. *Fields Virology / editors-in-chief, David M. Knipe, Peter M. Howley; associate editors, Jeffrey I. Cohen [et al.].* United States, Philadelphia: Wolters Kluwer Health/Lippincott, Williams & Wilkins, c2013.

GALLO, R. C., SALAHUDDIN, S. Z., POPOVIC, M., SHEARER, G. M., KAPLAN, M., HAYNES, B. F., PALKER, T. J., REDFIELD, R., OLESKE, J., SAFAI, B. & ET AL. 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science,* 224**,** 500-3.

GILBERT, H. E., ASOKAN, R., HOLERS, V. M. & PERKINS, S. J. 2006. The 15 SCR flexible extracellular domains of human complement receptor type 2 can mediate multiple ligand and antigen interactions. *J Mol Biol,* 362**,** 1132-47.

GISH, W. & STATES, D. J. 1993. Identification of protein coding regions by database similarity search. *Nat Genet,* 3**,** 266-72.

GROSSKOPF, A. K., SCHLAGOWSKI, S., HORNICH, B. F., FRICKE, T., DESROSIERS, R. C. & HAHN, A. S. 2019. EphA7 Functions as Receptor on BJAB Cells for Cell-to-Cell Transmission of the Kaposi's Sarcoma-Associated Herpesvirus and for Cell-Free Infection by the Related Rhesus Monkey Rhadinovirus. *J Virol,* 93, pii: e00064-19. https://doi.org/10.1128/JVI.00064-19.

GUERREIRO-CACAIS, A. O., LI, L., DONATI, D., BEJARANO, M. T., MORGAN, A., MASUCCI, M. G., HUTT-FLETCHER, L. & LEVITSKY, V. 2004. Capacity of Epstein-Barr virus to infect monocytes and inhibit their development into dendritic cells is affected by the cell type supporting virus replication. *J Gen Virol,* 85**,** 2767-78.

HAHN, A., BIRKMANN, A., WIES, E., DORER, D., MAHR, K., STURZL, M., TITGEMEYER, F. & NEIPEL, F. 2009. Kaposi's sarcoma-associated herpesvirus gH/gL: glycoprotein export and interaction with cellular receptors. *J Virol,* 83**,** 396-407.

HAHN, A. S., KAUFMANN, J. K., WIES, E., NASCHBERGER, E., PANTELEEV-IVLEV, J., SCHMIDT, K., HOLZER, A., SCHMIDT, M., CHEN, J., KONIG, S., ENSSER, A., MYOUNG, J., BROCKMEYER, N. H., STURZL, M., FLECKENSTEIN, B. & NEIPEL, F. 2012. The ephrin receptor tyrosine kinase A2 is a cellular receptor for Kaposi's sarcoma-associated herpesvirus. *Nat Med,* 18**,** 961-6.

HARRISON, S. C. 2015. Viral membrane fusion. *Virology,* 479-480**,** 498-507.

HASSMAN, L. M., ELLISON, T. J. & KEDES, D. H. 2011. KSHV infects a subset of human tonsillar B cells, driving proliferation and plasmablast differentiation. *J Clin Invest,* 121**,** 752-68.

HELDWEIN, E. E. 2016. gH/gL supercomplexes at early stages of herpesvirus entry. *Curr Opin Virol,* 18**,** 1-8.

HELDWEIN, E. E., LOU, H., BENDER, F. C., COHEN, G. H., EISENBERG, R. J. & HARRISON, S. C. 2006. Crystal structure of glycoprotein B from herpes simplex virus 1. *Science,* 313**,** 217-20.

HENKE-GENDO, C. & SCHULZ, T. F. 2004. Transmission and disease association of Kaposi's sarcoma-associated herpesvirus: recent developments. *Curr Opin Infect Dis,* 17**,** 53-7.

HENLE, G. & HENLE, W. 1966. Immunofluorescence in cells derived from Burkitt's lymphoma. *J Bacteriol,* 91**,** 1248-56.

HENLE, G., HENLE, W. & DIEHL, V. 1968. Relation of Burkitt's tumor-associated herpes-ytpe virus to infectious mononucleosis. *Proc Natl Acad Sci U S A,* 59**,** 94-101.

HOLEN, H. L., SHADIDI, M., NARVHUS, K., KJOSNES, O., TIERENS, A. & AASHEIM, H. C. 2008. Signaling through ephrin-A ligand leads to activation of Src-family kinases, Akt phosphorylation, and inhibition of antigen receptor-induced apoptosis. *J Leukoc Biol,* 84**,** 1183-91.

ISOBE, Y., SUGIMOTO, K., YANG, L., TAMAYOSE, K., EGASHIRA, M., KANEKO, T., TAKADA, K. & OSHIMI, K. 2004. Epstein-Barr virus infection of human natural killer cell lines and peripheral blood natural killer cells. *Cancer Res,* 64**,** 2167-74.

JANZ, A., OEZEL, M., KURZEDER, C., MAUTNER, J., PICH, D., KOST, M., HAMMERSCHMIDT, W. & DELECLUSE, H. J. 2000. Infectious Epstein-Barr virus lacking major glycoprotein BLLF1 (gp350/220) demonstrates the existence of additional viral ligands. *J Virol,* 74**,** 10142-52.

JASIRWAN, C., FURUSAWA, Y., TANG, H., MAEKI, T. & MORI, Y. 2014. Human herpesvirus-6A gQ1 and gQ2 are critical for human CD46 usage. *Microbiol Immunol,* 58**,** 22-30.

JENSON, H. B., LEACH, C. T., MCCLAIN, K. L., JOSHI, V. V., POLLOCK, B. H., PARMLEY, R. T., CHADWICK, E. G. & MURPHY, S. B. 1997. Benign and malignant smooth muscle tumors containing Epstein-Barr virus in children with AIDS. *Leuk Lymphoma,* 27**,** 303-14.

JIANG, R., GU, X., NATHAN, C. O. & HUTT-FLETCHER, L. 2008. Laser-capture microdissection of oropharyngeal epithelium indicates restriction of Epstein-Barr virus receptor/CD21 mRNA to tonsil epithelial cells. *J Oral Pathol Med,* 37**,** 626-33.

JONES, J. F., SHURIN, S., ABRAMOWSKY, C., TUBBS, R. R., SCIOTTO, C. G., WAHL, R., SANDS, J., GOTTMAN, D., KATZ, B. Z. & SKLAR, J. 1988. T-cell lymphomas containing Epstein-Barr viral DNA in patients with chronic Epstein-Barr virus infections. *N Engl J Med,* 318**,** 733-41.

KAPOSI, M. 1872. Idiopathisches multiples Pigmentsarkom der Haut. *Archiv Dermatol Syph.,* 4: 265-73.

KERUR, N., VEETTIL, M. V., SHARMA-WALIA, N., SADAGOPAN, S., BOTTERO, V., PAUL, A. G. & CHANDRAN, B. 2010. Characterization of entry and infection of monocytic THP-1 cells by Kaposi's sarcoma associated herpesvirus (KSHV): role of heparan sulfate, DC-SIGN, integrins and signaling. *Virology,* 406**,** 103-16.

KIKUTA, H., ITAKURA, O., TANEICHI, K. & KOHNO, M. 1997. Tropism of human herpesvirus 8 for peripheral blood lymphocytes in patients with Castleman's disease. *Br J Haematol,* 99**,** 790-3.

KIRSCHNER, A. N., SOREM, J., LONGNECKER, R. & JARDETZKY, T. S. 2009. Structure of Epstein-Barr virus glycoprotein 42 suggests a mechanism for triggering receptor-activated virus entry. *Structure,* 17**,** 223-33.

KRISHNAN, H. H., SHARMA-WALIA, N., STREBLOW, D. N., NARANATT, P. P. & CHANDRAN, B. 2006. Focal adhesion kinase is critical for entry of Kaposi's sarcoma-associated herpesvirus into target cells. *J Virol,* 80**,** 1167-80.

KUMAR, B., ROY, A., VEETTIL, M. V. & CHANDRAN, B. 2018. Insight into the Roles of E3 Ubiquitin Ligase c-Cbl, ESCRT Machinery, and Host Cell Signaling in Kaposi's Sarcoma-Associated Herpesvirus Entry and Trafficking. *J Virol,* 92, https://doi.org/10.1128/JVI.01376-17.

LAICHALK, L. L. & THORLEY-LAWSON, D. A. 2005. Terminal differentiation into plasma cells initiates the replicative cycle of Epstein-Barr virus in vivo. *J Virol,* 79**,** 1296-307.

LONGNECKER, R., KIEFF, E. & COHEN, J. 2013. *Epstein-Barr virus,* United States, Philadelphia: Wolters Kluwer Health/Lippincott, Williams & Wilkins, c2013.

LUKAC, D. M. & YUAN, Y. 2007. Reactivation and lytic replication of KSHV. *In:* ARVIN, A., CAMPADELLI-FIUME, G., MOCARSKI, E., MOORE, P. S., ROIZMAN, B., WHITLEY, R. & YAMANISHI, K. (eds.) *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis.* Cambridge University Press, Cambridge.

MARK, L., LEE, W. H., SPILLER, O. B., VILLOUTREIX, B. O. & BLOM, A. M. 2006. The Kaposi's sarcoma-associated herpesvirus complement control protein (KCP) binds to heparin and cell surfaces via positively charged amino acids in CCP1-2. *Mol Immunol,* 43**,** 1665-75.

MARTIN, J. N., GANEM, D. E., OSMOND, D. H., PAGE-SHAFER, K. A., MACRAE, D. & KEDES, D. H. 1998. Sexual transmission and the natural history of human herpesvirus 8 infection. *N Engl J Med,* 338**,** 948-54.

MATSUURA, H., KIRSCHNER, A. N., LONGNECKER, R. & JARDETZKY, T. S. 2010. 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,* 107**,** 22641-6.

MILLER, N. & HUTT-FLETCHER, L. M. 1992. Epstein-Barr virus enters B cells and epithelial cells by different routes. *J Virol,* 66**,** 3409-14.

MÖHL, B. S., CHEN, J., SATHIYAMOORTHY, K., JARDETZKY, T. S. & LONGNECKER, R. 2016. Structural and Mechanistic Insights into the Tropism of Epstein-Barr Virus. *Mol Cells,* 39**,** 286-91.

MÖHL, B. S., SATHIYAMOORTHY, K., JARDETZKY, T. S. & LONGNECKER, R. 2014. The Conserved Disulfide Bond within Domain II (D-II) of Epstein-Barr Virus (EBV) gH has Divergent Roles in Membrane Fusion with Epithelial Cells and B Cells. *J Virol*. **88**, 2014, 13570–13579.

MÖHL, B. S., SCHRÖTER, C., KLUPP, B. G., FUCHS, W., METTENLEITER, T. C., JARDETZKY, T. S. & LONGNECKER, R. 2015. Comparative mutagenesis of Pseudorabies and Epstein-Barr virus gH identifies a structural determinant within domain III of gH required for surface expression and entry function. *J Virol*. **90**, 2015, 2285–2293.

MORI, Y. 2009. Recent topics related to human herpesvirus 6 cell tropism. *Cell Microbiol,* 11**,** 1001-6.

MUKAI, M., SURUGA, N., SAEKI, N. & OGAWA, K. 2017. EphA receptors and ephrin-A ligands are upregulated by monocytic differentiation/maturation and promote cell adhesion and protrusion formation in HL60 monocytes. *BMC Cell Biol,* 18**,** 28.

MULLEN, M. M., HAAN, K. M., LONGNECKER, R. & JARDETZKY, T. S. 2002. Structure of the Epstein-Barr virus gp42 protein bound to the MHC class II receptor HLA-DR1. *Molecular cell,* 9**,** 375-85.

NAGAE, M., RE, S., MIHARA, E., NOGI, T., SUGITA, Y. & TAKAGI, J. 2012. Crystal structure of alpha5beta1 integrin ectodomain: atomic details of the fibronectin receptor. *J Cell Biol,* 197**,** 131-40.

NARANATT, P. P., AKULA, S. M., ZIEN, C. A., KRISHNAN, H. H. & CHANDRAN, B. 2003. Kaposi's sarcoma-associated herpesvirus induces the phosphatidylinositol 3-kinase-PKC-zeta-MEK-ERK signaling pathway in target cells early during infection: implications for infectivity. *J Virol,* 77**,** 1524-39.

NEMEROW, G. R. & COOPER, N. R. 1984. Early events in the infection of human B lymphocytes by Epstein-Barr virus: the internalization process. *Virology,* 132**,** 186-98.

NILSSON, K., KLEIN, G., HENLE, W. & HENLE, G. 1971. The establishment of lymphoblastoid lines from adult and fetal human lymphoid tissue and its dependence on EBV. *Int J Cancer,* 8**,** 443-50.

NOTREDAME, C., HIGGINS, D. G. & HERINGA, J. 2000. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol,* 302**,** 205-17.

OGEMBO, J. G., KANNAN, L., GHIRAN, I., NICHOLSON-WELLER, A., FINBERG, R. W., TSOKOS, G. C. & FINGEROTH, J. D. 2013. Human complement receptor type 1/CD35 is an Epstein-Barr Virus receptor. *Cell Rep,* 3**,** 371-85.

PAPADOPOULOS, J. S. & AGARWALA, R. 2007. COBALT: constraint-based alignment tool for multiple protein sequences. *Bioinformatics,* 23**,** 1073-9.

PARK, S. & KO, Y. H. 2014. Epstein-Barr virus-associated T/natural killer-cell lymphoproliferative disorders. *J Dermatol,* 41**,** 29-39.

PAUK, J., HUANG, M. L., BRODIE, S. J., WALD, A., KOELLE, D. M., SCHACKER, T., CELUM, C., SELKE, S. & COREY, L. 2000. Mucosal shedding of human herpesvirus 8 in men. *N Engl J Med,* 343**,** 1369-77.

PENN, I. 1979. Kaposi's sarcoma in organ transplant recipients: report of 20 cases. *Transplantation,* 27**,** 8-11.

PERTEL, P. E. 2002. Human herpesvirus 8 glycoprotein B (gB), gH, and gL can mediate cell fusion. *J Virol,* 76**,** 4390-400.

PICA, F. & VOLPI, A. 2007. Transmission of human herpesvirus 8: an update. *Curr Opin Infect Dis,* 20**,** 152-6.

RAGHU, H., SHARMA-WALIA, N., VEETTIL, M. V., SADAGOPAN, S. & CHANDRAN, B. 2009. Kaposi's sarcoma-associated herpesvirus utilizes an actin polymerization-dependent macropinocytic pathway to enter human dermal microvascular endothelial and human umbilical vein endothelial cells. *J Virol,* 83**,** 4895-911.

RAPPOCCIOLO, G., HENSLER, H. R., JAIS, M., REINHART, T. A., PEGU, A., JENKINS, F. J. & RINALDO, C. R. 2008. Human herpesvirus 8 infects and replicates in primary cultures of activated B lymphocytes through DC-SIGN. *J Virol,* 82**,** 4793-806.

REVELLO, M. G. & GERNA, G. 2010. Human cytomegalovirus tropism for endothelial/epithelial cells: scientific background and clinical implications. *Reviews in medical virology,* 20**,** 136-55.

ROBEY, R. C. & BOWER, M. 2015. Facing up to the ongoing challenge of Kaposi's sarcoma. *Curr Opin Infect Dis,* 28**,** 31-40.

SATHIYAMOORTHY, K., CHEN, J., LONGNECKER, R. & JARDETZKY, T. S. 2017. The COMPLEXity in herpesvirus entry. *Curr Opin Virol,* 24**,** 97-104.

SATHIYAMOORTHY, K., HU, Y. X., MOHL, B. S., CHEN, J., LONGNECKER, R. & JARDETZKY, T. S. 2016. Structural basis for Epstein-Barr virus host cell tropism mediated by gp42 and gHgL entry glycoproteins. *Nat Commun,* 7**,** 13557.

SATHIYAMOORTHY, K., JIANG, J., HU, Y. X., ROWE, C. L., MÖHL, B. S., CHEN, J., JIANG, W., MELLINS, E. D., LONGNECKER, R., ZHOU, Z. H. & JARDETZKY, T. S. 2014. Assembly and Architecture of the EBV B Cell Entry Triggering Complex. *PLoS Pathog,* 10**,** e1004309.

SCHÄFER, G., BLUMENTHAL, M. J. & KATZ, A. A. 2015. Interaction of human tumor viruses with host cell surface receptors and cell entry. *Viruses,* 7**,** 2592-617.

SEIRADAKE, E., HARLOS, K., SUTTON, G., ARICESCU, A. R. & JONES, E. Y. 2010. An extracellular steric seeding mechanism for Eph-ephrin signaling platform assembly. *Nat Struct Mol Biol,* 17**,** 398-402.

SHIMAKAGE, M. 2014. Significant role of macrophages in human cancers associated with Epstein-Barr virus (Review). *Oncol Rep,* 32**,** 1763-71.

SIXBEY, J. W., NEDRUD, J. G., RAAB-TRAUB, N., HANES, R. A. & PAGANO, J. S. 1984. Epstein-Barr virus replication in oropharyngeal epithelial cells. *N Engl J Med,* 310**,** 1225-30.

SNIJDER, J., ORTEGO, M. S., WEIDLE, C., STUART, A. B., GRAY, M. D., MCELRATH, M. J., PANCERA, M., VEESLER, D. & MCGUIRE, A. T. 2018. An Antibody Targeting the Fusion Machinery Neutralizes Dual-Tropic Infection and Defines a Site of Vulnerability on Epstein-Barr Virus. *Immunity,* 48**,** 799-811 e9.

SPRUNT, T. & EVANS, F. 1920. Mononuclear leukocytosis in reaction to acute infections ("infectious mononucleosis"). *in Bulletin of the Johns Hopkins Hospital,* 31**,** 410-417, Johns Hopkins Press, United States.

STAMPFER, S. D. & HELDWEIN, E. E. 2012. Stuck in the middle: structural insights into the role of the gH/gL heterodimer in herpesvirus entry. *Curr Opin Virol*. **3**, 2012, 13–19.

SZAKONYI, G., KLEIN, M. G., HANNAN, J. P., YOUNG, K. A., MA, R. Z., ASOKAN, R., HOLERS, V. M. & CHEN, X. S. 2006. Structure of the Epstein-Barr virus major envelope glycoprotein. *Nat Struct Mol Biol,* 13**,** 996-1001.

TANG, H., WANG, J., MAHMOUD, N. F. & MORI, Y. 2014. Detailed study of the interaction between human herpesvirus 6B glycoprotein complex and its cellular receptor, human CD134. *J Virol,* 88**,** 10875-82.

TANNER, J., WEIS, J., FEARON, D., WHANG, Y. & KIEFF, E. 1987. Epstein-Barr virus gp350/220 binding to the B lymphocyte C3d receptor mediates adsorption, capping, and endocytosis. *Cell,* 50**,** 203-13.

TERBUSH, A. A., HAFKAMP, F., LEE, H. J. & COSCOY, L. 2018. A Kaposi's Sarcoma-Associated Herpesvirus Infection Mechanism Is Independent of Integrins alpha3beta1, alphaVbeta3, and alphaVbeta5. *J Virol,* 92, https://doi.org/10.1128/JVI.00803-18.

THAKKER, S. & VERMA, S. C. 2016. Co-infections and Pathogenesis of KSHV-Associated Malignancies. *Front Microbiol,* 7**,** 151.

THORLEY-LAWSON, D. A. & ALLDAY, M. J. 2008. The curious case of the tumour virus: 50 years of Burkitt's lymphoma. *Nat Rev Microbiol,* 6**,** 913-24.

TUGIZOV, S. M., BERLINE, J. W. & PALEFSKY, J. M. 2003. Epstein-Barr virus infection of polarized tongue and nasopharyngeal epithelial cells. *Nat Med,* 9**,** 307-14.

VALLBRACHT, M., BRUN, D., TASSINARI, M., VANEY, M. C., PEHAU-ARNAUDET, G., GUARDADO-CALVO, P., HAOUZ, A., KLUPP, B. G., METTENLEITER, T. C., REY, F. A. & BACKOVIC, M. 2017. Structure-function dissection of the Pseudorabies virus glycoprotein B fusion loops. *J Virol*. **92**, https://doi.org/10.1128/JVI.01203-17.

VEETTIL, M. V., BANDYOPADHYAY, C., DUTTA, D. & CHANDRAN, B. 2014. Interaction of KSHV with host cell surface receptors and cell entry. *Viruses,* 6**,** 4024-46.

VEETTIL, M. V., SHARMA-WALIA, N., SADAGOPAN, S., RAGHU, H., SIVAKUMAR, R., NARANATT, P. P. & CHANDRAN, B. 2006. RhoA-GTPase facilitates entry of Kaposi's sarcoma-associated herpesvirus into adherent target cells in a Src-dependent manner. *J Virol,* 80**,** 11432-46.

VIEIRA, J., HUANG, M. L., KOELLE, D. M. & COREY, L. 1997. Transmissible Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in saliva of men with a history of Kaposi's sarcoma. *J Virol,* 71**,** 7083-7.

WALTER, P. R., PHILIPPE, E., NGUEMBY-MBINA, C. & CHAMLIAN, A. 1984. Kaposi's sarcoma: presence of herpes-type virus particles in a tumor specimen. *Hum Pathol,* 15**,** 1145-6.

WANG, F. Z., AKULA, S. M., PRAMOD, N. P., ZENG, L. & CHANDRAN, B. 2001. Human herpesvirus 8 envelope glycoprotein K8.1A interaction with the target cells involves heparan sulfate. *J Virol,* 75**,** 7517-27.

WANG, F. Z., AKULA, S. M., SHARMA-WALIA, N., ZENG, L. & CHANDRAN, B. 2003. Human herpesvirus 8 envelope glycoprotein B mediates cell adhesion via its RGD sequence. *J Virol,* 77**,** 3131-47.

WANG, X., KENYON, W. J., LI, Q., MULLBERG, J. & HUTT-FLETCHER, L. M. 1998. Epstein-Barr virus uses different complexes of glycoproteins gH and gL to infect B lymphocytes and epithelial cells. *Journal of virology,* 72**,** 5552-8.

WATERHOUSE, A., BERTONI, M., BIENERT, S., STUDER, G., TAURIELLO, G., GUMIENNY, R., HEER, F. T., DE BEER, T. A. P., REMPFER, C., BORDOLI, L., LEPORE, R. & SCHWEDE, T. 2018. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res,* 46**,** W296-W303.

WEBSTER-CYRIAQUE, J., DUUS, K., COOPER, C. & DUNCAN, M. 2006. Oral EBV and KSHV infection in HIV. *Adv Dent Res,* 19**,** 91-5.

WEISS, L. M., MOVAHED, L. A., WARNKE, R. A. & SKLAR, J. 1989. Detection of Epstein-Barr viral genomes in Reed-Sternberg cells of Hodgkin's disease. *N Engl J Med,* 320**,** 502-6.

WU, L., BORZA, C. M. & HUTT-FLETCHER, L. M. 2005. Mutations of Epstein-Barr virus gH that are differentially able to support fusion with B cells or epithelial cells. *Journal of virology,* 79**,** 10923-30.

XIAO, J., PALEFSKY, J. M., HERRERA, R. & TUGIZOV, S. M. 2007. Characterization of the Epstein-Barr virus glycoprotein BMRF-2. *Virology,* 359**,** 382-96.

XING, Y., OLIVER, S. L., NGUYEN, T., CIFERRI, C., NANDI, A., HICKMAN, J., GIOVANI, C., YANG, E., PALLADINO, G., GROSE, C., UEMATSU, Y., LILJA, A. E., ARVIN, A. M. & CARFI, A. 2015. A site of varicella-zoster virus vulnerability identified by structural studies of neutralizing antibodies bound to the glycoprotein complex gHgL. *Proc Natl Acad Sci U S A* **112**, 2015, 6056–6061.

ZHANG, H., LI, Y., WANG, H. B., ZHANG, A., CHEN, M. L., FANG, Z. X., DONG, X. D., LI, S. B., DU, Y., XIONG, D., HE, J. Y., LI, M. Z., LIU, Y. M., ZHOU, A. J., ZHONG, Q., ZENG, Y. X., KIEFF, E., ZHANG, Z., GEWURZ, B. E., ZHAO, B. & ZENG, M. S. 2018. Author Correction: Ephrin receptor A2 is an epithelial cell receptor for Epstein-Barr virus entry. *Nat Microbiol,* 3**,** 1075.

ZHOU, M., LANCHY, J. M. & RYCKMAN, B. J. 2015. Human Cytomegalovirus gH/gL/gO Promotes the Fusion Step of Entry into All Cell Types, whereas gH/gL/UL128-131 Broadens Virus Tropism through a Distinct Mechanism. *J Virol,* 89**,** 8999-9009.

ZIEGLER, J. L., DREW, W. L., MINER, R. C., MINTZ, L., ROSENBAUM, E., GERSHOW, J., LENNETTE, E. T., GREENSPAN, J., SHILLITOE, E., BECKSTEAD, J., CASAVANT, C. & YAMAMOTO, K. 1982. Outbreak of Burkitt's-like lymphoma in homosexual men. *Lancet,* 2**,** 631-3.

ZUR HAUSEN, H., SCHULTE-HOLTHAUSEN, H., KLEIN, G., HENLE, W., HENLE, G., CLIFFORD, P. & SANTESSON, L. 1970. EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. *Nature,* 228**,** 1056-8.

Figure legends

Figure 1 Homology models of KSHV gB, gH and gL. (A) Schematic diagrams of full-length EBV and KSHV gB, gH and gL. The color codes for gB (Connolly et al., 2011) and gH domains (Möhl et al., 2014) are shown as described earlier. Domain I (DI) of gL is colored in grey. N-glycosylation sites are indicated by asterisks (black predicted or red confirmed for EBV). The signal peptide is shown by a grey box, the transmembrane domain by a hatched box, the membrane-proximal region by the label MPR, and the C-terminal domain by CTD. The structure is shown as cartoon for gB (B), gH and gL (C). The color codes correspond to the schema in A. Disulfide bonds are shown as orange spheres and cysteine's as sticks. The glycan's are indicated as grey spheres (confirmed for EBV) or grey sticks (predicted). Cluster of predicted N-glycan's in KSHV gB and gH are encircled showing a location on one side. (B) Structural view of EBV and KSHV gB with a sketch of the stick figure. The FL of EBV gB are shown in magenta, and the according regions in KSHV gB are labeled by amino acids. (C) Structural view of EBV and KSHV gL and gH. The proposed integrin binding (KGD) motif of EBV gH is indicated as red dots. The homology modeling prediction of KSHV gB and gH/gL were generated using SWISS-MODEL (Waterhouse et al., 2018). The structural views of postfusion EBV gB (PDB ID: 3FVC) (Backovic et al., 2009), gH/gL (3PHF) (Matsuura et al., 2010) and KSHV gB as well as gH and gL were modified using the PyMOL Molecular Graphics System, Version 4.3.0 Schrödinger, LLC.

Figure 2 Tropism switch of EBV. (A) The structural view of the epithelial cell-tropism complex gH/gL is shown as cartoon. The color code is used as described in Fig. 1. (B) The structural view of the B cell-tropism complex gH/gL-gp42 is indicated as cartoon for gH (marine) and gL (cyan), and as cartoon with transparent surface presentation for gp42 (red) with the elongated N-terminus wrapping around on one side of gH. The KGD motif is indicated by marine spheres and labeled. The structural views of gH/gL (3PHF) (Matsuura et al., 2010)

and gH/gL-gp42 (5T1D) (Sathiyamoorthy et al., 2016) were modified using PyMOL Molecular Graphics System, Version 4.3.0 Schrödinger, LLC.

Figure 3 Endocytosis entry pathway of EBV into B-lymphocytes. First, gp350/220 (only gp350 with the proposed binding region as spheres in corresponding colors is depicted) interacts with CD21 tethering EBV to the B cell membrane. After tethering, gp42 binds to its receptor HLA class II resulting in a widening of the hydrophobic pocket in gp42. This conformational change may activate gp42 by itself or gH/gL enabling the binding to the prefusion form of gB. Extended rearrangements within gB (extended intermediate) enable insertion of the fusion loops (FL) (magenta) into the target cell membrane and refolding of gB forms postfusion gB which mediates merging of the two bilayers. The structural views are shown as cartoons and the color code of the EBV gB domains is according to (Connolly et al., 2011) as well as the color code for the B cell-tropism complex is used as described in Fig. 2. The structural views of CD21 (2GSX) (Gilbert et al., 2006), EBV gp350 (2H6O) (Szakonyi et al., 2006), the complex of gH/gL (3PHF) (Matsuura et al., 2010) and gp42-HLA-DR1 (1KG0) (Mullen et al., 2002, Sathiyamoorthy et al., 2014), as well as postfusion gB (3FVC) (Backovic et al., 2009) were modified using PyMOL Molecular Graphics System, Version 4.3.0 Schrödinger, LLC.

Figure 4 Entry of EBV via direct fusion into epithelial cells. First, gp350/220 interacts with CD21 and/or BMRF2 with integrin $\alpha5\beta1$ tethering EBV to the epithelial cell membrane. After tethering, gH/gL binds to its high-affinity receptor EphA2 enabling the binding to the prefusion form of gB. Extended rearrangements within gB trigger insertion of the FL into the target cell membrane, followed by refolding of gB to postfusion form and thereby mediates merging of the two bilayers. The structural views of integrin $\alpha5\beta1$ (3VI4) (Nagae et al., 2012), CD21 (2GSX) (Gilbert et al., 2006), EphA2 (2X10) (Seiradake et al., 2010), EBV gp350 (2H6O) (Szakonyi et al., 2006), and gH/gL (3PHF) (Matsuura et al., 2010) as well as postfusion gB (3FVC) (Backovic et al., 2009) were generated using the PyMOL Molecular Graphics System, Version 4.3.0 (Schrödinger, LLC).

Figure 5 Endocytosis entry pathway of KSHV into human target cells. First, gH, gB, gpH8.1A and ORF4 interact with HSPG tethering KSHV to human target cells. Tethering may support interaction with following entry receptors CD98, xCT, integrin and DC-SIGN as well as EphA2 (is shown as representative for EphA4, 5 and 7). DC-SIGN and integrin are likely bound by gB, whereas gH/gL binds to its receptor EphA2 that may enable the binding of gH/gL to the prefusion form of gB. Thereby, activated gB triggers merging of the viral membrane with endosome membrane. The structural view of EphA2 (2X10) (Seiradake et al., 2010) were generated using The PyMOL Molecular Graphics System, Version 4.3.0 (Schrödinger, LLC).

Table 1 Entry proteins and their receptors as well as entry pathways of EBV and KSHV. The predominant target cells are shown in bold.

	Epstein-Barr virus	Kaposi Sarcoma-associated herpesvirus
Entry Protein	gB gH/gL gp42 gp350/gp220 BMRF2	gB gH/gL gpK8.1A ORF4
Receptor	 EphA2 (gH/gL) HLA class II (gp42) CD21/CR2 (gp350/gp220) $\alpha 5\beta 1$ (BMRF1)	HSPG (gB, gpK8.1A, gH, ORF4) EphA2, A4, A5 and A7 (gH/gL) integrins ($\alpha V\beta 3$, $\alpha V\beta 5$ and $\alpha 3\beta 1$ with proposed gB interaction) DC-SIGN (probably gB) CD98/xCT
Target Cell	**Epithelial cells** Lymphocytes (T, NK and **B cells**) Monocytes Macrophages Smooth muscle cells	Epithelial cells **Endothelial cells** Keratinocytes Fibroblasts Lymphocytes (T and **B cells**) Monocytes Macrophages Dendritic cells
Entry Pathway	Fusion at the plasma membrane Endocytosis	 Endocytosis (clathrin/caveolin- or dynamin-mediated) Macropinocytosis (actin-dependent)
Infection Route	passes through the oropharyngeal epithelium (adenoids and tonsils) to infect underlying naïve B cells	passes through the tonsillar epithelium to infect underlying lymphocytes sexual transmission in adults of high risk groups

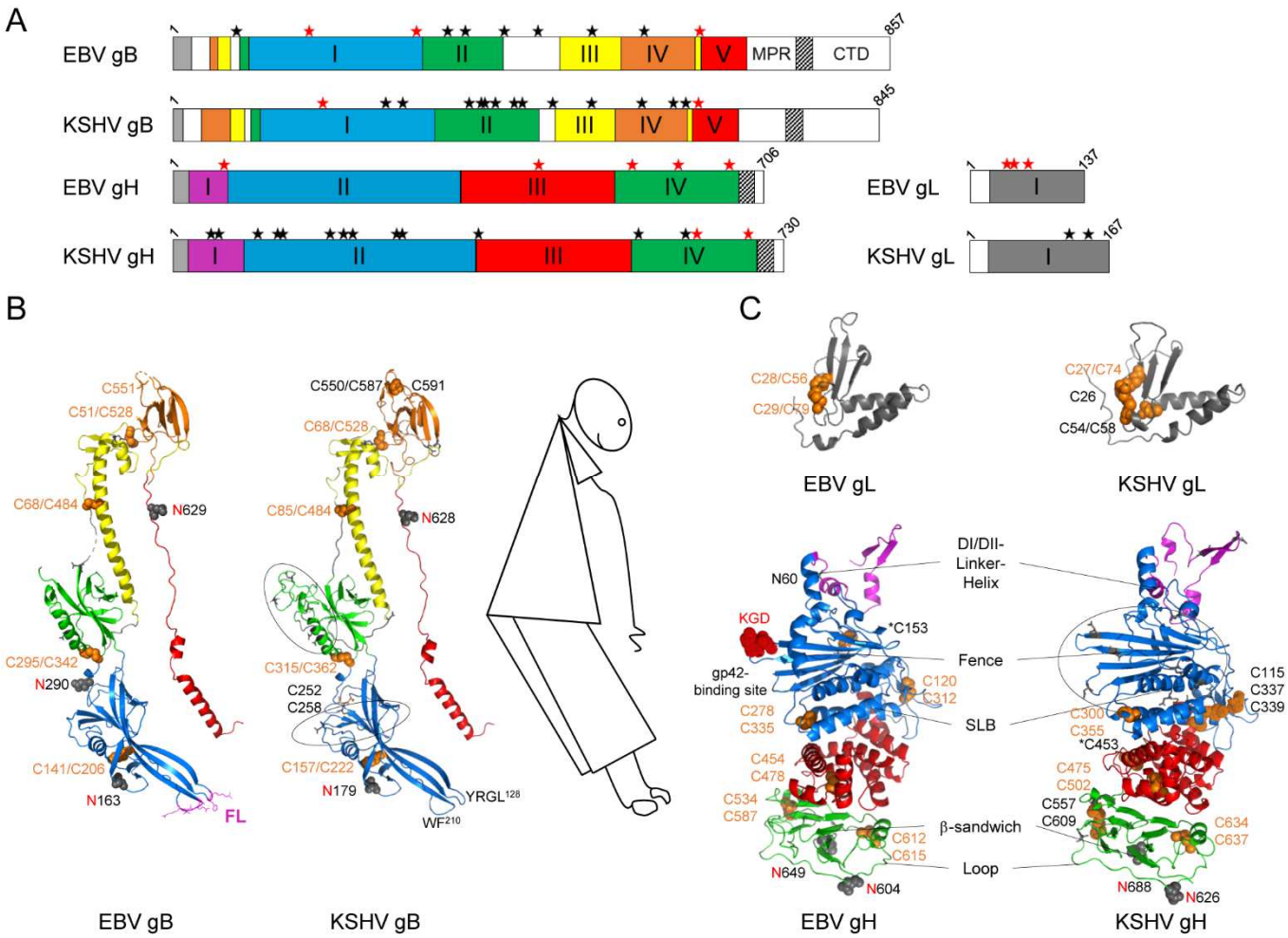

Figure 1 Homology models of KSHV gB, gH and gL. (A) Schematic diagrams of full-length EBV and KSHV gB, gH and gL. The color codes for gB (Connolly et al., 2011) and gH domains (Möhl et al., 2014) are shown as described earlier. Domain I (DI) of gL is colored in grey. N-glycosylation sites are indicated by asterisks (black predicted or red confirmed for EBV). The signal peptide is shown by a grey box, the transmembrane domain by a hatched box, the membrane-proximal region by the label MPR, and the C-terminal domain by CTD. The structure is shown as cartoon for gB (B), gH and gL (C). The color codes correspond to the schema in A. Disulfide bonds are shown as orange spheres and cysteine's as sticks. The glycan's are indicated as grey spheres (confirmed for EBV) or grey sticks (predicted). Cluster of predicted N-glycan's in KSHV gB and gH are encircled showing a location on one side. (B) Structural view of EBV and KSHV gB with a sketch of the stick figure. The FL of EBV gB are shown in magenta, and the according regions in KSHV gB are labeled by amino acids. (C) Structural view of EBV and KSHV gL and gH. The proposed integrin binding (KGD) motif of EBV gH is indicated as red dots. The homology modeling prediction of KSHV gB and gH/gL were generated using SWISS-MODEL (Waterhouse et al., 2018). The structural views of postfusion EBV gB (PDB ID: 3FVC) (Backovic et al., 2009), gH/gL (3PHF) (Matsuura et al., 2010) and KSHV gB as well as gH and gL were modified using the PyMOL Molecular Graphics System, Version 4.3.0 Schrödinger, LLC.

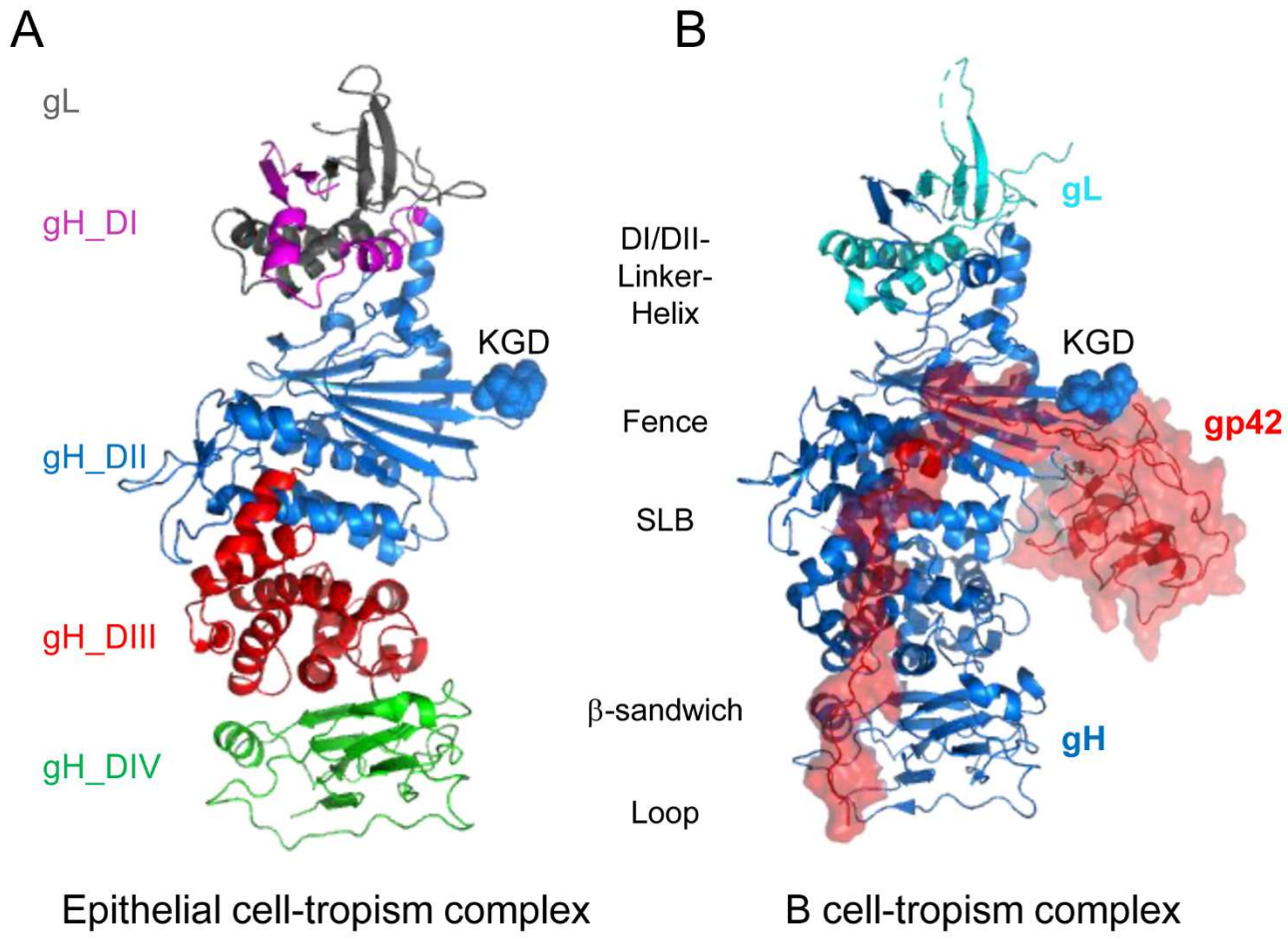

Figure 2 Tropism switch of EBV. (A) The structural view of the epithelial cell-tropism complex gH/gL is shown as cartoon. The color code is used as described in Fig. 1. (B) The structural view of the B cell-tropism complex gH/gL-gp42 is indicated as cartoon for gH (marine) and gL (cyan), and as cartoon with transparent surface presentation for gp42 (red) with the elongated N-terminus wrapping around on one side of gH. The KGD motif is indicated by marine spheres and labeled. The structural views of gH/gL (3PHF) (Matsuura et al., 2010) and gH/gL-gp42 (5T1D) (Sathiyamoorthy et al., 2016) were modified using PyMOL Molecular Graphics System, Version 4.3.0 Schrödinger, LLC.

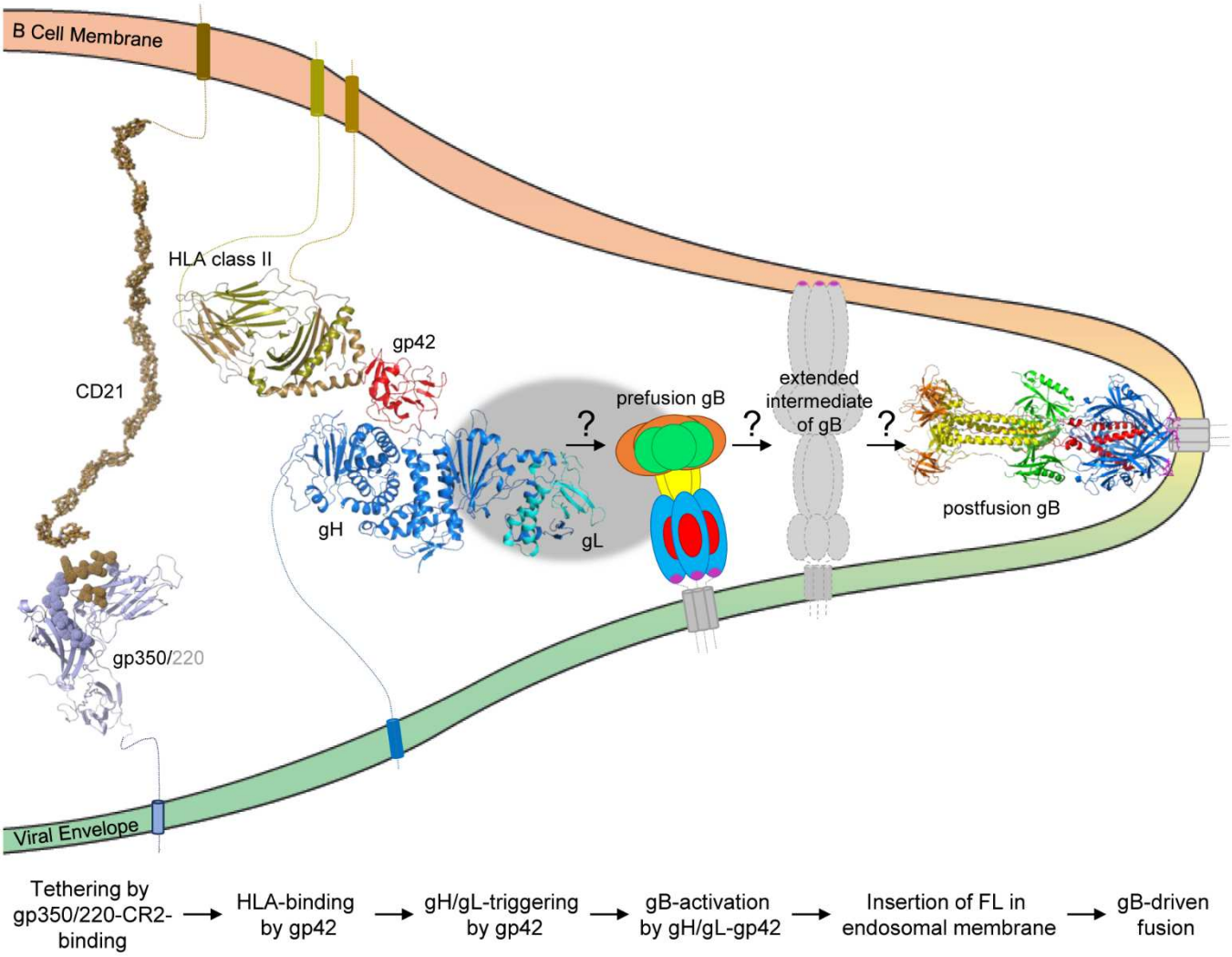

Figure 3 Endocytosis entry pathway of EBV into B-lymphocytes. First, gp350/220 (only gp350 with the proposed binding region as spheres in corresponding colors is depicted) interacts with CD21 tethering EBV to the B cell membrane. After tethering, gp42 binds to its receptor HLA class II resulting in a widening of the hydrophobic pocket in gp42. This conformational change may activate gp42 by itself or gH/gL enabling the binding to the prefusion form of gB. Extended rearrangements within gB (extended intermediate) enable insertion of the fusion loops (FL) (magenta) into the target cell membrane and refolding of gB forms postfusion gB which mediates merging of the two bilayers. The structural views are shown as cartoons and the color code of the EBV gB domains is according to (Connolly et al., 2011) as well as the color code for the B cell-tropism complex is used as described in Fig. 2. The structural views of CD21 (2GSX) (Gilbert et al., 2006), EBV gp350 (2H6O) (Szakonyi et al., 2006), the complex of gH/gL (3PHF) (Matsuura et al., 2010) and gp42-HLA-DR1 (1KG0) (Mullen et al., 2002, Sathiyamoorthy et al., 2014), as well as postfusion gB (3FVC) (Backovic et al., 2009) were modified using PyMOL Molecular Graphics System, Version 4.3.0 Schrödinger, LLC.

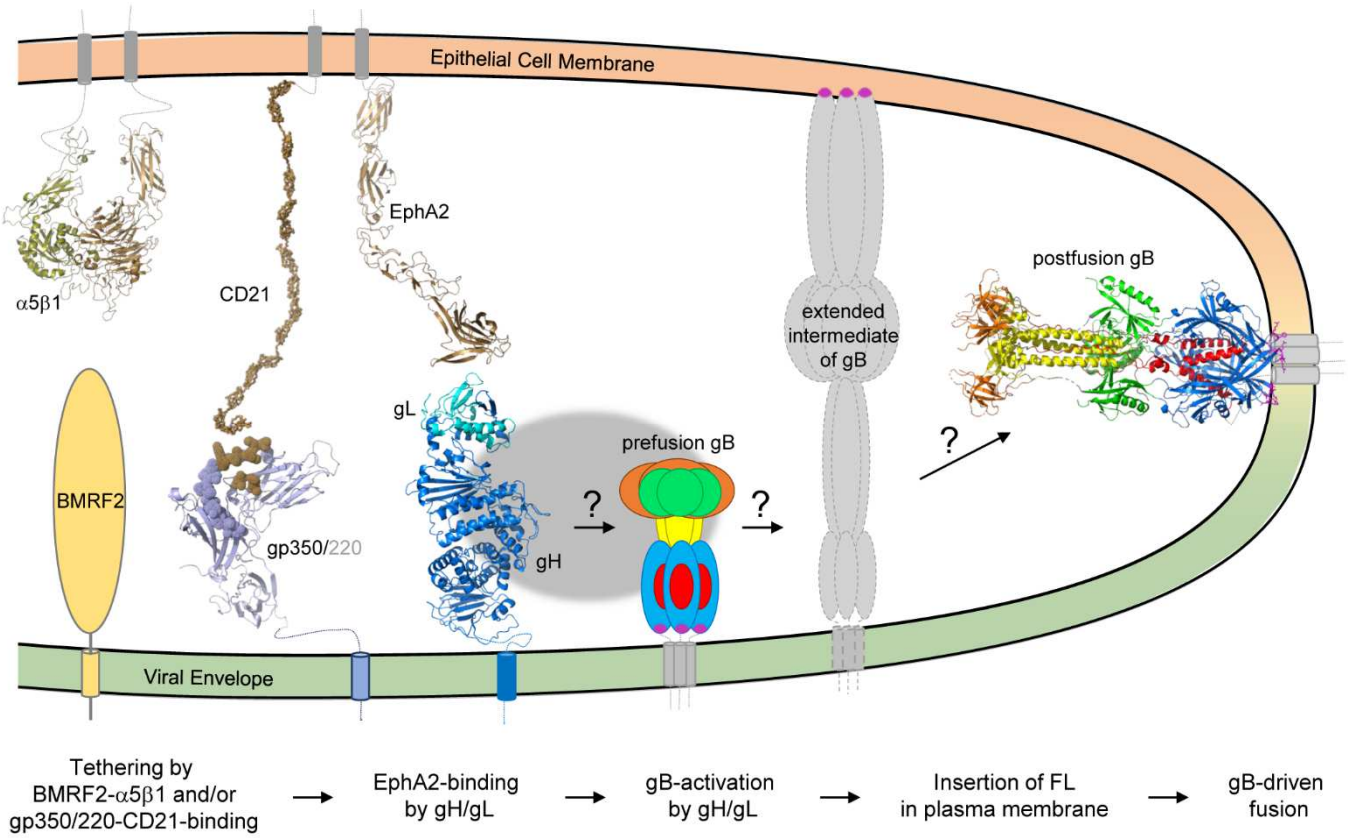

Figure 4 Entry of EBV via direct fusion into epithelial cells. First, gp350/220 interacts with CD21 and/or BMRF2 with integrin α5β1 tethering EBV to the epithelial cell membrane. After tethering, gH/gL binds to its high-affinity receptor EphA2 enabling the binding to the prefusion form of gB. Extended rearrangements within gB trigger insertion of the FL into the target cell membrane, followed by refolding of gB to postfusion form and thereby mediates merging of the two bilayers. The structural views of integrin α5β1 (3VI4) (Nagae et al., 2012), CD21 (2GSX) (Gilbert et al., 2006), EphA2 (2X10) (Seiradake et al., 2010), EBV gp350 (2H6O) (Szakonyi et al., 2006), and gH/gL (3PHF) (Matsuura et al., 2010) as well as postfusion gB (3FVC) (Backovic et al., 2009) were generated using the PyMOL Molecular Graphics System, Version 4.3.0 (Schrödinger, LLC).

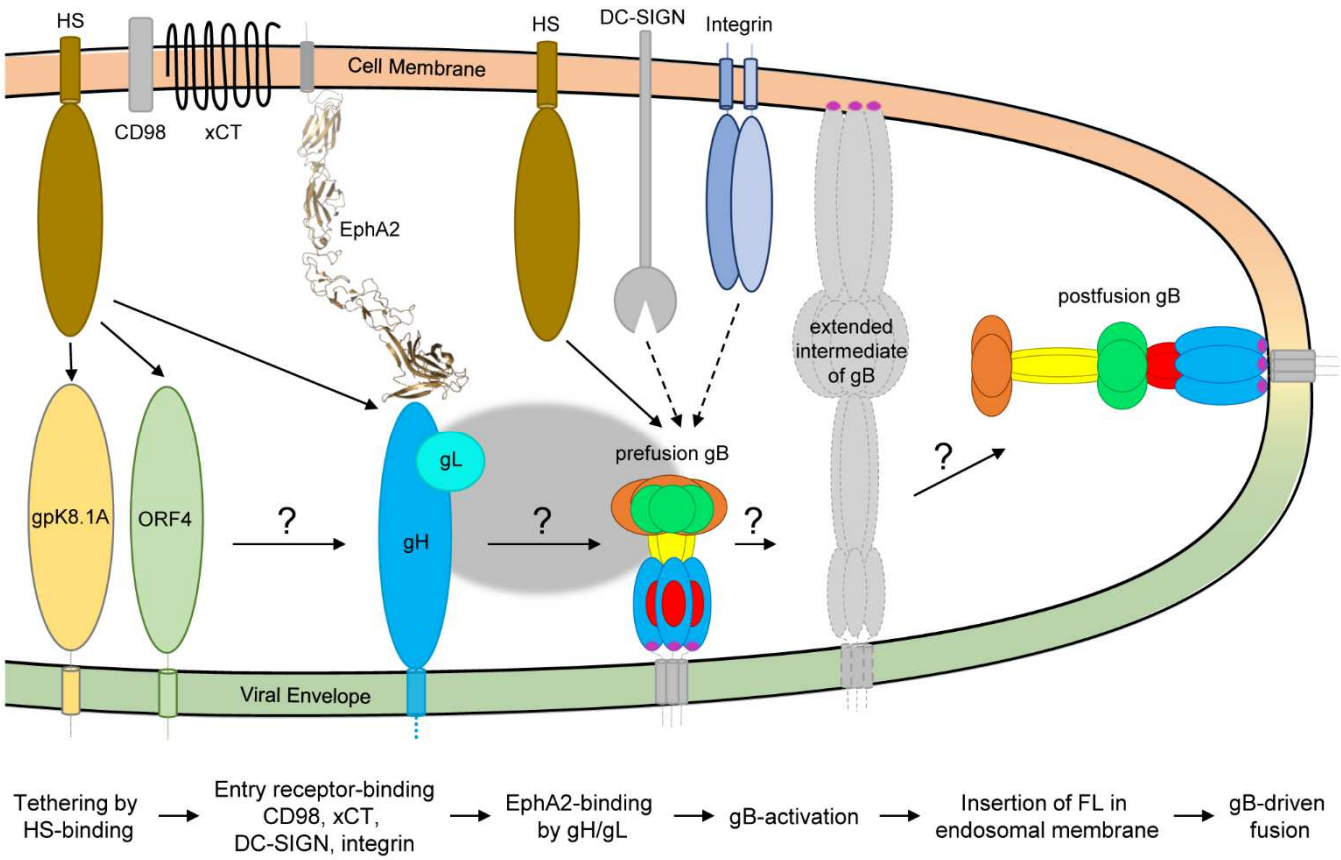

Figure 5 Endocytosis entry pathway of KSHV into human target cells. First, gH, gB, gpH8.1A and ORF4 interact with HSPG tethering KSHV to human target cells. Tethering may support interaction with following entry receptors CD98, xCT, integrin and DC-SIGN as well as EphA2 (is shown as representative for EphA4, 5 and 7). DC-SIGN and integrin are likely bound by gB, whereas gH/gL binds to its receptor EphA2 that may enable the binding of gH/gL to the prefusion form of gB. Thereby, activated gB triggers merging of the viral membrane with endosome membrane. The structural view of EphA2 (2X10) (Seiradake et al., 2010) were generated using The PyMOL Molecular Graphics System, Version 4.3.0 (Schrödinger, LLC).