# Viral Entry

**Liudmila S. Chesnokova, Ru Jiang and Lindsey M. Hutt-Fletcher**

**Abstract** Epstein-Barr virus primarily, though not exclusively, infects B cells and epithelial cells. Many of the virus and cell proteins that are involved in entry into these two cell types in vitro have been identified, and their roles in attachment and fusion are being explored. This chapter discusses what is known about entry at the cellular level in vitro and describes what little is known about the process in vivo. It highlights some of the questions that still need to be addressed and considers some models that need further testing.

## Contents



L.S. Chesnokova · R. Jiang · L.M. Hutt-Fletcher (✉)
Department of Microbiology and Immunology, Center for Molecular and Tumor Virology, Feist-Weiller Cancer Center, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130, USA
e-mail: lhuttf@lsuhsc.edu

Present R. Jiang
Department of Clinical Teaching and Training, Tianjin University of Traditional Chinese Medicine, 312 West Anshan Road, 300193 Nankai District, Tianjin, China



C. Münz (ed.), *Epstein Barr Virus Volume 2*, Current Topics in Microbiology and Immunology 391, DOI 10.1007/978-3-319-22834-1_7

## Abbreviations

| | |
|---|---|
| EBV | Epstein-Barr virus |
| NK | Natural killer |
| CR2 | Complement receptor type 2 |
| CR1 | Complement receptor type 1 |
| SCR | Short consensus repeat |
| CTLD | C-type lectin domain |

## 1 Introduction

Epstein-Barr virus (EBV) is shed in the saliva of persistently infected healthy carriers and is generally described as being orally transmitted. It ultimately establishes latency in the memory B cell compartment, but is also thought to replicate productively in epithelial cells, can, at least rarely, infect T cells and natural killer (NK) cells, and is found in the muscle cells of leiomyosarcomas. A full comprehension of virus entry, which encompasses access to vulnerable tissues at the organismal level as well as delivery of virus DNA to the nucleus at the cellular level, is nowhere near a reality. However, progress has been made, particularly in deciphering early events in entry into the two major target cells of the virus, B cells and epithelial cells. This chapter discusses what we know about these early events and starts to put them in the context of the broader picture of infection, transmission, and spread.

## 2 Early Events in B Cell Entry

### *2.1 Proteins Involved in Attachment*

It was recognized early on that EBV is a B lymphotropic virus, and this, together with the fact that human B cells were relatively easily obtained and intensively studied, focused attention on identification of the virus and cell proteins important for B cell entry. Investigations were also simplified and facilitated by the efficient infection of B cells that could be achieved with cell-free virus.

Virus was first shown to attach to B cells as a result of a high-affinity interaction (Moore et al. 1989) between the abundant virion glycoprotein gp350 and complement receptor type 2, CR2 or CD21 (Fingeroth et al. 1984; Nemerow et al. 1987; Tanner et al. 1987). Glycoprotein gp350 is a single-pass, heavily glycosylated, type I membrane protein of 907 amino acids, and the CD21 binding site has been mapped to a glycan-free surface in the membrane-distal amino-terminal domain of the protein (Martin et al. 1991; Szakonyi et al. 2006). It includes a

peptide sequence with similarities to the natural ligand of CD21, the C3dg fragment of complement (Nemerow et al. 1989). The ectodomain of CD21 consists of tandem repeats of modules of 60–75 amino acids known as short consensus repeats (SCRs), and the binding site for gp350 is contained in SCR1 and SCR2 (Martin et al. 1991). These SCRs are those furthest from the cell membrane, and, somewhat problematically, attachment initially positions the virus approximately 50 nm from the cell surface (Nemerow and Cooper 1984). However, the EBV virion also contains a splice variant of gp350, gp220, missing residues 500–757, but retaining the CD21 binding site (Beisel et al. 1985). Whether there is a switch from the use of gp350 to gp220 is not known, but perhaps this, together with the segmental flexibility of CD21 provided by the tandemly repeated SCRs, allows the virus to move closer to the cell following its initial attachment.

On primary B cells CD21 can exist independently, but is additionally found in a trimeric complex with CD19 and CD81 and in a complex with another complement receptor, complement receptor type 1, CR1 or CD35 (Tuveson et al. 1991). CD35 is also a ligand for gp350 that is capable of initiating infection (Ogembo et al. 2013). It possibly binds to the same glycan-free surface as has been shown to interact with CD21, though, since CD35 is lost when B cells are transformed with EBV, its properties as a receptor have only been discovered recently and have not been extensively studied. The trimeric CD21/CD19/CD81 complex is particularly interesting for two reasons. First, CD19 functions as a signal transducer. Cross-linking of CD21 by gp350, as virus attaches, can then activate NF-κB (Sinclair and Farrell 1995; Sugano et al. 1997) and protein kinase C pathways and induce interleukin-6 production (D'Addario et al. 2001; Tanner et al. 1996). Although signaling may have no relevance to entry per se, it may profoundly influence downstream events. Second, CD81 and CD19 can both associate with HLA class II (Reem et al. 2004), a cell protein required for the next step in entry as discussed below.

## *2.2 Proteins Involved in Internalization*

Once attached to the B cell surface via CD21 virus is endocytosed into a low pH compartment from which it must ultimately escape by fusing its envelope with the vesicle membrane (Miller and Hutt-Fletcher 1992). Fusion requires four virus glycoproteins, gH, gL, gp42 and gB, and one cell protein, HLA class II (reviewed in (Hutt-Fletcher 2007). Glycoprotein gH, which has an apparent molecular weight of 85 kilodaltons and in the older literature is referred to as gp85, is, like gp350, a single-pass type I membrane protein. Glycoprotein gL (formerly gp25) is a peripheral membrane protein from which the signal peptide is cleaved and it dimerizes with gH. The crystal structure of gH and gL (Matsuura et al. 2010) reveals a four-domain cylindrical complex in which the membrane-distal globular domain I is comprised of the amino terminus of gH and the entire cleaved gL. Coexpression

of gH and gL, from hereon referred to as a single entity, gHgL, is thus required for the correct folding of the structure.

Herpesvirus glycoprotein nomenclature has now settled on naming of molecules conserved throughout the entire herpesvirus family by letters of the alphabet. Proteins not found in all subfamilies are referred to by either their apparent molecular weights or their gene names. The beta and gammaherpesviruses, but not apparently the alphaherpesviruses, have proteins that associate with gHgL. The first described was EBV glycoprotein gp42 (Li et al. 1995). Glycoprotein gp42 is a type II membrane protein which exists in virions in a cleaved and an uncleaved form, although the cleaved form is functionally optimal (Sorem et al. 2009). It associates non-covalently with gHgL, probably with domain II and the domain I/domain II interface (Chen et al. 2012; Sathiyamoorthy et al. 2014), through residues 36–82 in its amino-terminal region (Kirschner et al. 2007). It binds to the β1 domain of HLA class II, at the side of the peptide binding groove, via a C-type lectin domain (CTLD) at the carboxyl terminus (Mullen et al. 2002), and the interaction with HLA class II is required for the initiation of fusion (Haan et al. 2000; Li et al. 1997). A hydrophobic pocket within the CTLD domain also makes contact with gHgL at the junction of domains II and III (Sathiyamoorthy et al. 2014).

Like gH and gp350, glycoprotein gB (referred to in the early literature as gp110 or gp125, the glycosylated form carrying complex sugars) is a single-pass type I transmembrane protein. It exists as a trimer, and the crystal structure of gB closely resembles not only that of its HSV counterpart, but also the class III fusion proteins of vesicular stomatitis virus and baculovirus (Backovic et al. 2009). The gB homologs of all herpesviruses are now generally considered to be the final executors of fusion, as discussed below.

## 3 Early Events in Epithelial Cell Entry

### *3.1 Proteins Involved in Attachment*

Attachment of EBV to an epithelial cell is a more complicated issue than B cell attachment as a variety of virus glycoproteins, and cell proteins have been implicated in the process. Some epithelial cells in culture express at least low levels of CD21 (Fingeroth et al. 1999), and both these and cells engineered to express CD21 can be infected at high levels (Borza et al. 2004; Li et al. 1992; Valencia and Hutt-Fletcher 2012). Determination of whether epithelial cells in vivo express CD21 has, however, been confounded by the fact that only a subset of antibodies that recognize CD21 on B cells is reactive with all epithelial cells, reports from different groups using them have not always been consistent (Levine et al. 1990; Niedobitek et al. 1989; Talacko et al. 1991; Thomas and Crawford 1989), and one commonly used antibody cross-reacts with an unrelated epithelial protein (Young et al. 1989). Analysis of expression of transcripts in epithelial cells isolated by laser capture microdissection found CD21 mRNA in tonsil and adenoid

epithelium, but not elsewhere, although tissues that when normal failed to express CD21 became positive as they became dysplastic and expression levels correlated with an increase in the grade of dysplasia (Jiang et al. 2008, 2012). This may have implications for the development of nasopharyngeal carcinoma. Individuals who develop these tumors have increases in antibodies to lytic cycle proteins several years prior to diagnosis (Zeng 1985; Zeng et al. 1985), and the role of EBV has been postulated to be that of a tumor promoter rather than a tumor initiator (Lo et al. 2012).

In the absence of CD21, EBV can use gHgL, which includes a KGD motif, to bind to any one of a subset of αv integrins, αvβ5, αvβ6, or αvβ8 (Chesnokova and Hutt-Fletcher 2011; Chesnokova et al. 2009). However, infection by virus attached via gHgL and an integrin is not at all efficient, for reasons that are not yet clear. One possibility is that the use of gHgL for attachment compromises its ability to function in fusion (Borza et al. 2004). A second is that CD21 does more than simply tether virus to the cell surface. Cross-linking of CD21 on an epithelial cell by virus has been reported to result in an interaction between its cytoplasmic tail and the formin homolog overexpressed in spleen (FHOS/FHOD1) (Gill et al. 2004). Formins are scaffolding proteins that nucleate actin and link signal transduction to actin reorganization, which is required for intracellular transport of EBV to the nucleus. However, both a truncated form of CD21 lacking its cytoplasmic domain (Valencia and Hutt-Fletcher 2012) and a construct in which both the cytoplasmic domain and the transmembrane domain were replaced with those of gH (unpublished) support infection as efficiently as the full-length protein. Whether there are interactions between the ectodomain of CD21 and other epithelial surface molecules needs further exploration. There is precedent for this, for while neither CD35 nor CD19 is found on epithelial cells, and their interactions with CD21 on a B cell can occur through the ectodomains of each (Fearon and Carter 1995).

A second interaction between EBV and an integrin has also been implicated in attachment, this time between an RGD motif in the BMRF2 gene product, a multi-span membrane glycoprotein, and αv, α3, α5, and β1 integrins (Tugizov et al. 2003; Xiao et al. 2008). Its major impact seems to be in infection of polarized cells, although how much the role of pBMRF2 reflects an essential involvement in attachment and how much it reflects effects on cell-to-cell spread (Xiao et al. 2009) are not entirely clear. pBMRF2 forms a dimeric complex with the BDLF2 gene product, a type II membrane protein (Gore and Hutt-Fletcher 2008). Homologs of these two proteins are found in other gammaherpesviruses, and, in the murine gammaherpesvirus, MHV68, they have been implicated in a membrane remodeling that is important to virus spread (Gill et al. 2008).

In immune individuals, virus coated with immunoglobulin A can attach to the basolateral surfaces of epithelial cells via the polymeric IgA receptor (Sixbey and Yao 1992), although in polarized cells this leads to transcytosis rather than infection (Gan et al. 1997). Virus bound to CD21 on the surface of a B cell can also be transferred to a CD21-negative epithelial cell (Shannon-Lowe and Rowe 2011; Shannon-Lowe et al. 2006). Following EBV attachment, CD21 co-caps with and activates adhesion molecules which allow the B cell to form a virologic synapse

with an epithelial cell. Finally, there is a very low affinity, but saturable interaction, possibly involving hydrogen and ionic bonds, between the BDLF3 gene product gp150 and an unknown molecule(s) on the surface of an epithelial cell (unpublished). However, virus lacking gp150, which is a highly glycosylated mucin-like protein, is very slightly more infectious for an epithelial cell than is wild-type virus (Borza and Hutt-Fletcher 1998) so this particular interaction would not appear to be productive.

### *3.2 Virus Proteins Involved in Internalization*

Definitive information about internalization of virus has also been difficult to obtain because of the plasticity of epithelial cells in culture, because of the different behaviors of unpolarized, polarized, and stratified epithelial cells, and because, since it is often not feasible to obtain primary cultures, cancer cell lines have frequently been used. Fusion with primary foreskin epithelial cells occurs at neutral pH and unlike B cell fusion does not require endocytosis (Miller and Hutt-Fletcher 1992). Entry into the SVKCR2 cell line, SV40-transformed keratinocytes engineered to express CD21 (Li et al. 1992), the AGS gastric cancer cell line, and hTERT-immortalized normal oral keratinocytes does not require actin remodeling, which is also consistent with fusion at the cell surface (Valencia and Hutt-Fletcher 2012). However, whether or not entry into polarized or stratified epithelial cells follows the same route has not yet been reported.

It is known, however, that fusion with an epithelial cell requires a complement of virus and cell proteins different from those used for B cell fusion. This has allowed EBV to evolve an elegant strategy for switching tropism and cycling between its two major target cells. Glycoproteins gB and gHgL, sometimes referred to as the "core fusion machinery" of herpesviruses, are necessary for virus fusion with an epithelial cell, as they are for fusion with a B cell, but gp42 is not. Fusion is triggered not by HLA class II, which is not constitutively expressed on an epithelial cell, but by an interaction between gHgL and one of the three αv integrins to which it binds (Chesnokova and Hutt-Fletcher 2011; Hutt-Fletcher and Chesnokova 2010). The interaction between gHgL and an integrin is blocked if gp42 is present, and access to both cell types is only possible because EBV carries both three-part gHgLgp42 complexes and two-part gHgL complexes in the virion envelope (Wang et al. 1998; Chen et al. 2012). Virus lacking gp42 can only infect a B cell if a soluble form of gp42, which can reform trimeric complexes, is added in trans, and the same soluble gp42, if added in saturating amounts to wild-type virus, can block infection of an epithelial cell (Wang et al. 1998). Virus produced in B cells carries a reduced number of the trimeric complexes because some bind to HLA class II in the endoplasmic reticulum and travel with HLA class II to the peptide-loading compartment, which is rich in proteases. This does not happen in HLA class II-negative epithelial cells, which produce virus that is enriched for trimeric complexes and which is as much as a hundred-fold more infectious for B

cells than virus produced by B cells themselves. In turn, B cell virus is about five-fold more infectious for an epithelial cell than virus replicated in an epithelial cell. HLA class II and gp42 thus provide the mechanism for alternating replication in the two cell types (Borza and Hutt-Fletcher 2002).

## 4 Mechanisms of Fusion

Although still incompletely understood, the individual roles of the virus glycoproteins involved in fusion are becoming clearer and models of fusion are being developed and tested. Probably, the major breakthrough came when the crystal structures of first herpes simplex virus gB and then EBV gB were solved (Backovic et al. 2009; Heldwein et al. 2006). The striking resemblance of both proteins to the post-fusion conformations of other class III fusion proteins strongly supported the nascent hypothesis that gB was central and proximal to the event. Class III fusion proteins exist as rod-shaped trimers, and each monomer of gB consists of five domains (Backovic and Jardetzky 2009). Domain I, modeled as closest to the virus membrane, is described as a fusion module and contains two fusion loops which are thought to insert into the cell membrane as fusion progresses. Mutations in the putative fusion loops of EBV gB abrogate fusion (Backovic et al. 2007a), and the same hydrophobic residues enable a truncated form of gB to form rosettes that are also typical of class I and class II fusion proteins in their post-fusion form (Backovic et al. 2007b). Domains II and IV contain β-sheets, and domain IV of EBV gB, which is found at the top of the trimeric spike, is thought to be flexible. Domain III has a long α-helix which in the trimer is part of a central coiled-coil. Domain V is an extended segment that inserts between the other two units in the trimer. The class III fusion protein of vesicular stomatitis virus, which is triggered by exposure to low pH, has been crystallized in both its post-fusion and its pre-fusion conformation and significant, but reversible, refolding, such as is seen in class I fusion proteins, occurs as one transitions to the other (Roche et al. 2008). Exposure of EBV to a triggering integrin leads to a change in its proteolytic digestion pattern, suggesting that a, perhaps analogous, conformational shift occurs during EBV fusion (Chesnokova et al. 2014).

The role of gHgL with or without gp42 is now generally thought to be as a regulator rather than an effector of fusion per se. The structure of gHgL resembles that of no known fusion protein, a gB truncation mutant can mediate some epithelial cell fusion in the absence of gHgL (McShane and Longnecker 2004), and heat can act as a partial surrogate for a gHgL interaction with an integrin, triggering the same change in the proteolytic digestion pattern of gB (Chesnokova et al. 2014). Clearly, however, under normal circumstances, an interaction between gHgLgp42 and HLA class II or an interaction between gHgL and an αv integrin is essential.

The crystal structure of gp42 has been solved in both the presence and absence of HLA class II. Mutational analysis of gp42 had highlighted the functional

importance of a hydrophobic pocket within the CTLD (Silva et al. 2004), and comparison of the liganded and unliganded structures of gp42 indicated a small change in the hydrophobic pocket in the liganded form (Kirschner et al. 2009). It was originally suggested that this change, triggered by HLA class II binding, might affect a second interaction between gp42 and either gB or gHgL. The second interaction is now known to be with gHgL, and the introduction of an N-liked glycosylation site in gHgL at this interface reduces membrane fusion (Sathiyamoorthy et al. 2014). A model is developing in which binding of gp42 to HLA class II causes the hydrophobic pocket in the protein to widen, and this change is transmitted via gHgL to gB. There is evidence from work done with herpes simplex virus and human cytomegalovirus that gHgL interacts with gB under conditions where fusion is possible (Atanasiu et al. 2007; Avitabile et al. 2007; Cairns et al. 2011; Vanarsdall et al. 2008), although whether this interaction occurs before or as fusion is triggered is equivocal. Whether or not EBV gB and gHgL interact has not yet been assessed.

A conformational change in gHgL between the domain I/domain II interface was also suggested when mutations affecting fusion were mapped to the crystal structure (Matsuura et al. 2010). There is a single unpaired cysteine residue in the groove between the two domains which allowed coupling of thiol-reactive, environmentally sensitive, fluorescent probes to a soluble truncated form of gHgL. Addition of a soluble αv integrin, which on its own can trigger virus fusion with an epithelial cell, produced a conformational change that could then be detected by fluorescence spectroscopy (Chesnokova and Hutt-Fletcher 2011). Subsequently, additional mutations were made in gHgL which introduced a novel disulfide bond linking domain I and domain II, presumably constraining such a conformational change (Chen et al. 2013). The mutated protein could still mediate fusion with a B cell, but lost the ability to fuse with an epithelial cell. This parallels other mutations made in domain IV of gHgL which differentially affect fusion with a B cell and an epithelial cell (Wu et al. 2005; Wu and Hutt-Fletcher 2007). Though all mutations have been functionally tested only in the context of cell-based fusion assays, three monoclonal antibodies to gHgL, one which binds to domain IV, one which binds close to the domain I/domain II interface, and one which has not yet been mapped, all neutralize epithelial infection very efficiently, but have little to no effect on B cell infection (Chesnokova and Hutt-Fletcher 2011; Wu et al. 2005) suggesting that the observations are probably relevant to virus entry as well.

The further implication of the observations would seem to be that, as they interact with their cellular partners, the architecture of the two fusion complexes, gB and gHgL or gB and gHgLgp42, the latter of which has been partially described (Sathiyamoorthy et al. 2014), is different. What this means for how gHgL interfaces with gB is unclear, although it seems likely that the final events in fusion are similar for both cell types. Fusion can be achieved if gHgL and gB are expressed in trans as well as in cis, and a virus lacking gHgL can enter an epithelial cell expressing gHgL. Infection at low levels can also be achieved in a B cell expressing gHgL without gp42, if a soluble integrin is added, providing some support for the concept that, once activated for fusion, gB proceeds similarly in both cell types (Chesnokova et al. 2014).

## 5 Transit to the Nucleus

The occurrence of fusion at different sites in a B cell and an epithelial cell, at least in the epithelial cells studied so far, has potential implications for transport of virus into the nucleus. A virus entering by endocytosis avoids the problem of crossing the actin cortex and is in a somewhat protected environment until it fuses out of the vesicle. There is precedent in other virus systems for vesicular transport well into the cell, although where precisely EBV fuses, is not known. Microtubules are required for delivery of virus DNA to the nucleus in both B cells and epithelial cells, the actin cytoskeleton also appears to be needed in epithelial, but not B cells, and it is clear that the process is much more efficient in a B cell than in an epithelial cell (Shannon-Lowe et al. 2009; Valencia and Hutt-Fletcher 2012). Approximately half of the virus delivered into an epithelial cell is degraded within hours. What is perhaps surprising is that although only a small percentage of virus that binds to a cell actually makes it to the nucleus, that small amount that does infect either a B cell or an epithelial cell expressing CR2 is capable of efficient gene expression (Borza et al. 2004; Shannon-Lowe et al. 2005).

## 6 Entry in Vivo

Translating what we know about entry at the cellular level into the reality of what happens in vivo is difficult. Beside the general assumption that virus transmitted in saliva first infects cells somewhere in the oropharynx, there is little or no concrete information on the subject. Both cell-free virus and cell-associated virus, or at least cell-associated virus DNA, are found in saliva (Haque and Crawford 1997), but if the virus is cell-associated, which cell type it is associated with is unclear. There is certainly evidence for virus-producing desquamating epithelial cells in saliva (Lemon et al. 1977; Sixbey et al. 1984), and cell-free virus in saliva of carriers has the characteristics of virus shed from an HLA class II-negative cell, implying that it is being shed from an epithelial cell. It is higher in gp42 than virus made in a B cell from the same individual and binds via gHgL to αv integrins very poorly (Jiang et al. 2006). Modeling studies have also suggested that the levels of virus in saliva can only be accounted for by amplification of virus in epithelial cells (Hadinoto et al. 2009). However, whether or not virus-producing B cells are also present in saliva, or uninfected B cells to which virus is bound, both of which might allow for more efficient infection of mucosal epithelium (Shannon-Lowe et al. 2006; Tugizov et al. 2003), is not known.

Beyond this, there are several questions that can be raised. Does virus replicate its way through an epithelial barrier to reach B cells (Temple et al. 2014), is it transcytosed across epithelium (Tugizov et al. 2013), is it picked up by macrophages or dendritic cells (Tugizov et al. 2007), and does it gain access as a result of breaks in the epithelial barrier, as is assumed for human papillomaviruses?

Given that the only symptomatic primary infection associated with EBV is infectious mononucleosis, which has an estimated incubation period of several weeks, it is likely that we will have to rely on future studies with non-human primate lymphocryptoviruses to provide answers to these questions. Examination of the path of virus reactivated in persistent carriers may, however, be more amenable to study.

Work done on oral hairy leukoplakia in the 1990s reinforced the assumption that, while B cells are the reservoir of latent virus, epithelial cells are normally the site of lytic infection and virus is latent only in malignant or pre-malignant epithelial tissue. Although a model akin to that of human papillomaviruses, where virus establishes latency in basal epithelial cells and replication is differentiation-linked, had been proposed (Allday and Crawford 1988; Sixbey 1989), it was reported that while productive replication was indeed probably differentiation-linked (Young et al. 1991), there was no detectable virus in basal epithelium (Niedobitek et al. 1991). Reexamination by real-time reverse transcriptase PCR of RNA in cells isolated by laser capture microdissection from sections of oral hairy leukoplakia, however, not only confirmed the presence of lytic transcripts in middle and upper layers of epithelium, but also found EBER expression in the absence of lytic transcripts in basal cells (unpublished). Similarly, basal epithelial cells in normal tonsils, identified by faint EBER in situ hybridization staining, expressed EBERs in the absence of lytic transcripts, although, in the sections examined to date, no lytic transcripts have been found in cells in the layers above. This does, however, suggest that perhaps the "papilloma virus" model should be revisited. If virus is shed by a terminally differentiating infected B cell in, for example, the tonsil, then on its path out into saliva, it would plausibly first encounter the basal surface of a basal epithelial cell. B cell-mediated transfer infection of polarized cells occurs only through the basal surface (Shannon-Lowe and Rowe 2011), and the integrins, needed for fusion, are primarily in the basolateral membrane. The virus that is shed from a B cell is also low in gp42 and hence epithelial-tropic. Differentiation of latently infected basal epithelial cells might then sporadically lead to production of virus at the epithelial surface. Virus shed from the epithelial cell is of course highly lymphotropic, and release at the epithelial surface is perhaps more likely to result in shedding than in reinfection at the apical surface. Such a model is most consistent with the idea that access to a new B cell occurs where the epithelial barrier is damaged.

## 7 Conclusions

Progress has clearly been made in understanding virus entry. We know many, though probably not all, of the proteins initially involved at the cellular level, and we are beginning to understand how some of them function. However, we know little about intracellular transport and delivery of DNA to the nucleus, which marks the completion of the successful entry process. Also, most efforts have

focused on just two cell types, when it is clear that EBV can infect more than B lymphocytes and epithelial cells. We notice this principally when things go wrong, when, for example, leiomyosarcomas or NK/T cell lymphomas develop, but does it happen in uneventful infections? If not, what changes make these cells accessible? We need to be continually mindful of the fact that almost all we know comes from the study of virus cell interactions out of context in a tissue culture dish. How representative is this of what happens in vivo? Perhaps, the biggest barrier to better understanding of the biology of EBV is its strict human tropism. At the same time, almost all of us are infected, so the population we have available for study is huge. We need to make better and more imaginative use of it.

## References

Allday MJ, Crawford DH (1988) Role of epithelium in EBV persistence and pathogenesis of B cell tumours. Lancet 1:855–856

Atanasiu D, Whitbeck JC, Cairns TM, Reilly B, Cohen GH, Eisenberg RJ (2007) Bimolecular complementation reveals that glycoproteins gB and gH/gL of herpes simplex virus interact with each other during cell fusion. Proc Natl Acad Sci USA 104:18718–18723

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

Backovic M, Jardetzky TS (2009) Class III viral membrane fusion proteins. Curr Opin Struct Biol 19:189–196

Backovic M, Jardetzky TS, Longnecker R (2007a) Hydrophobic residues that form putative fusion loops of Epstein-Barr virus glycoprotein B are critical for fusion activity. J Virol 81:9596–9600

Backovic M, Leser GP, Lamb RA, Longnecker R, Jardetzky TS (2007b) Characterization of EBV gB indicates properties of both class I and class II fusion proteins. Virology 368:102–103

Backovic M, Longnecker R, Jardetzky TS (2009) Structure of a trimeric variant of the Epstein-Barr virus glycoprotein B. Proc Natl Acad Sci USA 106:2880–2885

Beisel C, Tanner J, Matsuo T, Thorley-Lawson D, Kezdy F, Kieff E (1985) Two major outer envelope glycoproteins of Epstein-Barr virus are encoded by the same gene. J Virol 54:665–674

Borza C, Hutt-Fletcher LM (1998) Epstein-Barr virus recombinant lacking expression of glycoprotein gp150 infects B cells normally but is enhanced for infection of the epithelial line SVKCR2. J Virol 72:7577–7582

Borza CM, Hutt-Fletcher LM (2002) Alternate replication in B cells and epithelial cells switches tropism of Epstein-Barr virus. Nature Med 8:594–599

Borza CM, Morgan AJ, Turk SM, Hutt-Fletcher LM (2004) Use of gHgL for attachment of Epstein-Barr virus to epithelial cells compromises infection. J Virol 78:5007–5014

Cairns TM, Whitbeck JC, Lou H, Heldwein EE, Chowdary TK, Eisenberg RJ, Cohen GH (2011) Capturing the herpes simplex core fusion complex (gB-gH/gL) in an acidic environment. J Virol 85:6175–6184

Chen J, Rowe CL, Jardetzky TS, Longnecker R (2012) The KGD motif of Epstein-Barr virus gH/gL is bifunctional, orchestrating infection of B cells and epithelial cells. MBio. doi:10.1128/mBio.00290-11

Chen J, Jardetzky TS, Longnecker R (2013) The large groove found in the gH/gL structure is an important functional domain for Epstein-Barr virus fusion. J Virol 87:3620–3627

Chesnokova LS, Hutt-Fletcher LM (2011) Fusion of EBV with epithelial cells can be triggered by αvβ5 in addition to αvβ6 and αvβ8 and integrin binding triggers a conformational change in gHgL. J Virol 85:13214–13223

Chesnokova LS, Nishimura S, Hutt-Fletcher L (2009) Fusion of epithelial cells by Epstein-Barr virus proteins is triggered by binding of viral proteins gHgL to integrins αvβ6 or αvβ8. Proc Natl Acad Sci USA 106:20464–20469

Chesnokova LS, Ahuja MK, Hutt-Fletcher LM (2014) Epstein-Barr virus glycoproteins gB and gHgL can mediate fusion and entry in trans; heat can act as a partial surrogate for gHgL and trigger a conformational change. J Virol 88 (in press)

D'Addario M, Libermann TA, Xu J, Ahmad A, Menezes J (2001) Epstein-Barr virus and its glycoprotein-350 upregulate IL-6 in human B cells via CD21, involving activation of NF-κB and different signaling pathways. J Mol Biol 308:501–514

Fearon DT, Carter RH (1995) The CD19/CR2/TAPA-1 complex of B lymphocytes: linking natural to acquired immunity. Ann Rev Immunol 13:127–149

Fingeroth JD, Weis JJ, Tedder TF, Strominger JL, Biro PA, Fearon DT (1984) Epstein-Barr virus receptor of human B lymphocytes is the C3d complement CR2. Proc Natl Acad Sci USA 81:4510–4516

Fingeroth JD, Diamond ME, Sage DR, Hayman J, Yates JL (1999) CD-21 dependent infection of an epithelial cell line, 293, by Epstein-Barr virus. J Virol 73:2115–2125

Gan Y, Chodosh J, Morgan A, Sixbey JW (1997) Epithelial cell polarization is a determinant in the infectious outcome of immunoglobulin A-mediated entry by Epstein-Barr virus. J Virol 71:519–526

Gill MB, Roecklein-Canfield J, Sage DR, Zambela-Soediono M, Longtine N, Uknis M, Fingeroth JD (2004) EBV attachment stimulates FHOS/FHOD1 redistribution and co-aggregation with CD21:formin interactions with the cytoplasmic domain of human CD21. J Cell Sci 117:2709–2720

Gill MB, Edgar R, May JS, Stevenson PG (2008) A gamma-herpesvirus glycoprotein complex manipulates actin to promote viral spread. PLoS ONE 3:e1808

Gore M, Hutt-Fletcher L (2008) The BDLF2 protein of Epstein-Barr virus is a type II glycosylated envelope protein whose processing is dependent on coexpression with the BMRF2 protein. Virology 383:162–167

Haan KM, Kwok WW, Longnecker R, Speck P (2000) Epstein-Barr virus entry utilizing HLA-DP or HLA-DQ as a coreceptor. J Virol 74:2451–2454

Hadinoto V, Shapiro M, Sun CC, Thorley-Lawson DA (2009) The dynamics of EBV shedding implicate a central role for epithelial cells in amplifying viral output. PLoS Pathog 7:e10000496

Haque T, Crawford DH (1997) PCR amplification is more sensitive than tissue culture methods for Epstein-Barr virus detection in clinical material. J Gen Virol 78:3357–3360

Heldwein EE, Lou H, Bender FC, Cohen GH, Eisenberg RJ, Harrison SC (2006) Crystal structure of glycoprotein B from herpes simplex virus 1. Science 313:217–220

Hutt-Fletcher LM (2007) Epstein-Barr virus entry. J Virol 81:7825–7832

Hutt-Fletcher LM, Chesnokova LS (2010) Integrins as triggers of Epstein-Barr virus fusion and epithelial cell infection. Virulence 1:395–398

Jiang R, Scott RS, Hutt-Fletcher LM (2006) Epstein-Barr virus shed in saliva is high in B cell tropic gp42. J Virol 80:7281–7283

Jiang R, Gu X, Nathan C, 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 Path Med 37:626–633

Jiang R, Gu X, Moore-Medlin TN, Nathan C-A, Hutt-Fletcher LM (2012) Oral dysplasia and squamous cell carcinoma: correlation between increased expression of CD21, Epstein-Barr virus and CK19. Oral Oncol 48:836–841

Kirschner AN, Lowrey AS, Longnecker R, Jardetzky TS (2007) Binding site interactions between Epstein-Barr virus fusion proteins gp42 and gH/gL reveal a peptide that inhibits both epithelial and B-cell membrane fusion. J Virol 81:9216–9229

Kirschner AN, Sorem J, Longnecker R, Jardetzky TS (2009) Structure of Epstein-Barr virus glycoprotein gp42 suggests a mechanism for triggering receptor-activated virus entry. Structure 17:223–233

Lemon SM, Hutt LM, Shaw JE, Li J-LH, Pagano JS (1977) Replication of EBV in epithelial cells during infectious mononucleosis. Nature 268:268–270

Levine J, Pflugfelder SC, Yen M, Crouse CA, Atherton SS (1990) Detection of the complement (CD21)/Epstein-Barr virus receptor in human lacrimal gland and ocular surface epithelia. Reg Immunol 3:164–170

Li QX, Young LS, Niedobitek G, Dawson CW, Birkenbach M, Wang F, Rickinson AB (1992) Epstein-Barr virus infection and replication in a human epithelial system. Nature 356:347–350

Li QX, Turk SM, Hutt-Fletcher LM (1995) The Epstein-Barr virus (EBV) BZLF2 gene product associates with the gH and gL homologs of EBV and carries an epitope critical to infection of B cells but not of epithelial cells. J Virol 69:3987–3994

Li QX, Spriggs MK, Kovats S, Turk SM, Comeau MR, Nepom B, Hutt-Fletcher LM (1997) Epstein-Barr virus uses HLA class II as a cofactor for infection of B lymphocytes. J Virol 71:4657–4662

Lo KW, Chung GT, To KF (2012) Deciphering the molecular genetic basis of NPC through molecular, cytogenetic, and epigenetic approaches. Semin Cancer Biol 22:79–86

Martin DR, Yuryev A, Kalli KR, Fearon DT, Ahearn JM (1991) Determination of the structural basis for selective binding of Epstein-Barr virus to human complement receptor type 2. J Exp Med 174:1299–1311

Matsuura H, Kirschner AN, Longnecker R, Jardetzky TS (2010) Crystal structure of the Epstein-Barr virus (EBV) glycoprotein H/glycoprotein L (gH/gL) complex. Proc Natl Acad Sci USA 107:22641–22646

McShane MP, Longnecker R (2004) Cell-surface expression of a mutated Epstein-Barr virus glycoprotein B allows fusion independent of other viral proteins. Proc Natl Acad Sci USA 101:17474–17479

Miller N, Hutt-Fletcher LM (1992) Epstein-Barr virus enters B cells and epithelial cells by different routes. J Virol 66:3409–3414

Moore MD, DiScipio RG, Cooper NR, Nemerow GR (1989) Hydrodynamic, electron microscopic and ligand binding analysis of the Epstein-Barr virus/C3dg receptor (CR2). J Biol Chem 34:20576–20582

Mullen MM, Haan KM, Longnecker R, Jardetzky TS (2002) Structure of the Epstein-Barr virus gp42 protein bound to the MHC class II receptor HLA-DR1. Mol Cell 9:375–385

Nemerow GR, Cooper NR (1984) Early events in the infection of human B lymphocytes by Epstein-Barr virus. Virology 132:186–198

Nemerow GR, Mold C, Schwend VK, Tollefson V, Cooper NR (1987) Identification of gp350 as the viral glycoprotein mediating attachment of Epstein-Barr virus (EBV) to the EBV/C3d receptor of B cells: sequence homology of gp350 and C3 complement fragment C3d. J Virol 61:1416–1420

Nemerow GR, Houghton RA, Moore MD, Cooper NR (1989) Identification of the epitope in the major envelope proteins of Epstein-Barr virus that mediates viral binding to the B lymphocyte EBV receptor (CR2). Cell 56:369–377

Niedobitek G, Herbst H, Stein H (1989) Epstein-Barr virus/complement receptor and epithelial cells. Lancet 2:110

Niedobitek G, Young LS, Lau R, Brooks L, Greenspan D, Greenspan JS, Rickinson AB (1991) Epstein-Barr virus infection in oral hairy leukoplakia: virus replication in the absence of a detectable latent phase. J Gen Virol 72:3035–3146

Ogembo JG, Kannan L, Ghiran I, Nicholson-Weller A, Finberg R, Fingeroth JD (2013) Human complement receptor type1/CD35 is an Epstein-Barr virus receptor. Cell Rep. 3:1–15

Reem A-D, Mooney N, Charron D (2004) MHC class II signaling in antigen-presenting cells. Curr Opin Immunol 16:108–113

Roche S, Albertini AAV, Lepault S, Bressanelli S, Gaudin Y (2008) Structures of vesicular stomatitis virus glycoprotein: membrane fusion revisited. Cell Mol Life Sci 65:1716–1728

Sathiyamoorthy K, Jiang J, Hu YX, Rowe CL, Mohl BS, Chen J, Jiang W, Mellins ED, Longnecker R, Zhou ZH, Jardetzky TS (2014) Assembly and architecture of the EBV B cell entry triggering complex. PLoS Pathog 10:e1004309

Shannon-Lowe C, Rowe M (2011) Epstein-Barr virus infection of polarized epithelial cells via the basolateral surface by memory B cell-mediated transfer infection. PLoS Pathog 7:e1001338

Shannon-Lowe C, Baldwin G, Feederle R, Bell AI, Rickinson A, Delecluse H-J (2005) Epstein-Barr virus induced B-cell transformation: quantitating events from virus binding to cell outgrowth. J Gen Virol 86:3009–3019

Shannon-Lowe CD, Neuhierl B, Baldwin G, Rickinson AB, Delecluse H-J (2006) Resting B cells as a transfer vehicle for Epstein-Barr virus infection of epithelial cells. Proc Natl Acad Sci USA 103:7065–7070

Shannon-Lowe C, Adland A, Bell AI, Delecluse HJ, Rickinson AB, Rowe M (2009) Features distinguishing Epstein-Barr virus infections of epithelial cells and B cells: viral genome expression, genome maintenance, and genome amplification. J Virol 83:7749–7760

Silva AL, Omerovic J, Jardetzky TS, Longnecker R (2004) Mutational analysis of Epstein-Barr virus glycoprotein gp42 reveals functional domains not involved in receptor binding but required for membrane fusion. J Virol 78:5946–5956

Sinclair AJ, Farrell PJ (1995) Host cell requirements for efficient infection of quiescent primary B lymphocytes by Epstein-Barr virus. J Virol 69:5461–5468

Sixbey JW (1989) Epstein-Barr virus and epithelial cells. Adv Viral Oncol 8:187–202

Sixbey JW, Yao Q-Y (1992) Immunoglobulin A-induced shift of Epstein-barr virus tissue tropism. Science 255:1578–1580

Sixbey JW, Nedrud JG, Raab-Traub N, Hanes RA, Pagano JS (1984) Epstein-Barr virus replication in oropharyngeal epithelial cells. New Engl J Med 310:1225–1230

Sorem J, Jardetzky TS, Longnecker R (2009) Cleavage and secretion of Epstein-Barr virus glycoprotein gp42 promote membrane fusion with B lymphocytes. J Virol 83:6664–6672

Sugano N, Chen W, Roberts ML, Cooper NR (1997) Epstein-Barr virus binding to CD21 activates the initial viral promoter via NFκB induction. J Exp Med 186:731–737

Szakonyi G, Klein MG, Hannan JP, Young KA, Ma RZ, Asokan R, Holers VM, Chen XS (2006) Structure of the Epstein-Barr virus major envelope glycoprotein. Nat Struct Mol Biol 13:996–1001

Talacko AA, Teo CG, Griffin BE, Johnson NW (1991) Epstein-Barr virus receptors but not viral DNA are present in normal and malignant oral epithelium. J Oral Path Med 20:20–25

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 50:203–213

Tanner JE, Alfieri C, Chatila TA, Diaz-Mitoma F (1996) Induction of interleukin-6 after stimulation of human B-cell CD21 by Epstein-Barr virus glycoproteins gp350 and gp220. J Virol 70:570–575

Temple RM, Zhu J, Budgeon LR, Christensen ND, Meyers C, Sample CE (2014) Efficient replication of Epstein-Barr virus in stratified epithelium in vitro. Proc Natl Acad Sci USA 111:16544–16549

Thomas JA, Crawford DH (1989) Epstein-Barr virus/complement receptor and epithelial cells. Lancet 2:449–450

Tugizov SM, Berline JW, Palefsky JM (2003) Epstein-Barr virus infection of polarized tongue and nasopharyngeal epithelial cells. Nat Med 9:307–314

Tugizov SM, Herrera R, Veluppillai P, Greenspan J, Greenspan D, Palefsky JM (2007) Epstein-Barr virus (EBV)-infected monocytes facilitate dissemination of EBV within the oral mucosal epithelium. J Virol 81:5484–5496

Tugizov SM, Herrera R, Palefsky JM (2013) Epstein-Barr virus transcytosis through polarized oral epithelial cells. J Virol 87:8179–8194

Tuveson DA, Ahearn JM, Matsumoto AK, Fearon DT (1991) Molecular interactions of complement receptors on B lymphocytes: a CR1/CR2 complex distinct from CR2/CD19 complex. J Exp Med 173:1083–1089

Valencia SM, Hutt-Fletcher LM (2012) Important but differential roles for actin in trafficking of Epstein-Barr virus in B cells and epithelial cells. J Virol 86:2–10

Vanarsdall AL, Ryckman BJ, Chase MC, Johnson DC (2008) Human cytomegalovirus glycoproteins gB and gH/gL mediate epithelial cell-cell fusion when expressed either in cis or in trans. J Virol 82:11837–11850

Wang X, Kenyon WJ, Li QX, Mullberg J, Hutt-Fletcher LM (1998) Epstein-Barr virus uses different complexes of glycoproteins gH and gL to infect B lymphocytes and epithelial cells. J Virol 72:5552–5558

Wu L, Hutt-Fletcher LM (2007) Point mutations in EBV gH that abrogate or differentially affect B cell and epithelial cell fusion. Virology 363:148–155

Wu L, Borza CM, Hutt-Fletcher LM (2005) Mutations of Epstein-Barr virus gH that are differentially able to support fusion with B cells or epithelial cells. J Virol 79:10923–10930

Xiao J, Palefsky JM, Herrera R, Berline J, Tugizov SM (2008) The Epstein-Barr virus BMRF-2 protein facilitates attachment to oral epithelial cells. Virology 370:430–442

Xiao J, Palefsky JM, Herrera R, Berline J, Tugizov SM (2009) EBV BMRF-2 facilitates cell-to-cell spread within polarized oral epithelial cells. Virology 388:335–343

Young LS, Dawson CW, Brown KW, Rickinson AB (1989) Identification of a human epithelial cell surface protein sharing an epitope with the C3d/Epstein-Barr virus receptor molecule of B lymphocytes. Int J Cancer 43:786–794

Young LS, Lau R, Rowe M, Niedobitek G, Packham G, Shanahan F, Rowe DT, Greenspan D, Greenspan JS, Rickinson AB, Farrell PJ (1991) Differentiation-associated expression of the Epstein-Barr virus BZLF1 transactivator protein in oral hairy leukoplakia. J Virol 65:2868–2874

Zeng Y (1985) Seroepidemiological studies on nasopharyngeal carcinoma in China. Adv Cancer Res 44:121–138

Zeng Y, Zhang LG, Wu YC, Huang YS, Huang NQ, Li JY, Wang B, Jiang MK, Fang Z, Meng NN (1985) Prospective studies on nasopharyngeal carcinoma in Epstein-Barr virus IgA/VCA antibody-positive persons in Wuzhou City, China. Int J Cancer 36:545–547