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EBV glycoproteins: where are we now?

Lindsey M Hutt-Fletcher*

Lindsey M Hutt-Fletcher: lhuttf@lsuhsc.edu

*Department of Microbiology & Immunology, Feist-Weiller Cancer Center and Center for Molecular & Tumor Virology, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130, USA; Tel.: +1 318 675 4948; Fax: +1 318 675 5764

Abstract

Glycoproteins are critical to virus entry, to spread within and between hosts and can modify the behavior of cells. Many viruses carry only a few, most found in the virion envelope. EBV makes more than 12, providing flexibility in how it colonizes its human host. Some are dedicated to getting the virus through the cell membrane and on toward the nucleus of the cell, some help guide the virus back out and on to the next cell in the same or a new host. Yet others undermine host defenses helping the virus persist for a lifetime, maintaining a presence that is mostly tolerated and serves to perpetuate EBV as one of the most common infections of man.

Keywords

assembly; attachment; B cells; egress; epithelial cells; fusion; immune evasion

Background

EBV is a human herpesvirus that is carried by almost all of the adult population of the world [1]. Many primary infections occur in childhood and are asymptomatic. Those occurring after the age of about 12- or 13-year-old are more likely to be accompanied by infectious mononucleosis, a self-limiting, but temporarily debilitating immunopathology. Regardless of whether or not infection is accompanied by disease, the virus persists. It establishes latency in long-lived memory B cells and reactivates sporadically to be amplified in epithelial cells, shed in saliva for oral transmission to a new host or returned to B cells to replenish the B-cell reservoir. The vast majority of people suffer no ill effects from persistence of EBV, but the virus is nevertheless associated, and probably causally associated, with a number of B cell and epithelial cell malignancies, including Burkitt and Hodgkin lymphoma, post-transplant lymphoproliferative disorders and immunoblastic lymphomas of the immunosuppressed, anaplastic nasopharyngeal carcinomas and a subset of gastric carcinomas. It is also linked with autoimmune diseases such as multiple sclerosis.

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The glycoproteins of EBV were among the first of the virus proteins to be studied when the virus was discovered in 1964. Some were highly immunogenic, they provided good diagnostic and seroepidemiologic targets and they were thought likely to be important to the generation of a protective immune response. However, it took almost 45 years to identify all of them and the functions of several are still incompletely understood.

The EBV genome is now thought to encode genes for 13 glycoproteins, 12 of which are expressed only during the productive, lytic replication cycle and one of which may be expressed during latency as well (Table 1). Eleven of them are components of the virion envelope, two are nonstructural proteins. Their nomenclature is somewhat confused as no consistent basis for naming them was developed in the early years. Some are still referred to by their apparent mass when electrophoresed in polyacrylamide gels, for example, gp350 and gp42. Some are called by their gene names, for example, *BILF1* and *BMRF2*. Yet others, which have homologs in all the herpesvirus subfamilies, are, by general agreement, now referred to by the single letter designations originally used to name herpes simplex virus glycoproteins, for example, gH (formerly gp85), gL (formerly gp25) and gB (formerly gp110). In general, the functions of the glycoproteins have put them in three groups, those involved in virus entry and spread, those involved in virus assembly and those that are involved in manipulating the host cell rather than contributing directly to replication, though assignment to any one of these groups does not necessarily imply that a protein has only a single function.

Virus entry

The most abundant of the virion glycoproteins is gp350 [2], which was identified by several groups in the 1980s as the protein responsible for attachment of virus to B lymphocytes [3]. It is a 907-residue, type 1 membrane protein found in the virion as two splice variants, which, when fully glycosylated, have masses of approximately 350 and 220 kDa. The splice maintains the reading frame and both forms of the protein retain the N-terminal attachment site, a glycan-free surface, which tethers the virus to CR2/CD21, or, as has more recently been shown, to CR1/CD35 [4]. Whether there is any functional significance to the maintenance of the two splice variants, or a third recently revealed by RNA-seq that may result in expression of a truncated protein lacking the CR2 binding site is not clear [5]. However, since both CR2 and gp350 have been modeled as extended proteins that initially position the virus at some distance from the cell surface [6], perhaps sequential binding to gp350 and gp220 is relevant to bringing the virus closer to the cell membrane.

The only vaccine for EBV that has to date been tested in a Phase II trial was made from a soluble form of gp350 [7]. It did not prevent infection, but reduced the incidence of infectious mononucleosis in those who received it. A study that used the rhesus lymphocryptovirus, a virus that has approximately 80% homology to EBV and recapitulates EBV pathogenesis in rhesus macaques, found that vaccination with gp350 resulted in a much lower virus load when the animals were challenged 2 years later [8]. This may have significance for the development of disease associated with persistence.

Following attachment to the B-cell surface, EBV, as an enveloped virus, enters the cell via fusion of its envelope with the cell membrane. Fusion, which occurs from within an endocytic vesicle, requires the action of four additional glycoproteins, gB, gHgL and gp42 [3]. Glycoprotein B is a type I membrane protein expressed as a homotrimer [9] and its crystal structure strongly resembles both the structure of herpes simplex virus gB and the postfusion forms of the class III fusion proteins of vesicular stomatitis virus and baculovirus (reviewed in [10]). In all herpesviruses, gB is now generally agreed to be the final executor of fusion. Glycoproteins gHgL and gp42 are viewed as being regulators of the process, though their involvement is essential [11]. The crystal structure of gHgL reveals a cylindrical four domain complex in which domains II, III and IV, which is membrane proximal, are comprised entirely of sequences within the ectodomain of the type I membrane protein gH and the globular domain I is comprised of the N-terminal 65 residues of gH and the entire sequence of soluble gL [12]. Glycoprotein gp42 can be found in cells as a type 2 membrane protein, or as a soluble protein from which the signal sequence is cleaved, though it is as a soluble protein that it functions in fusion [13]. It interacts with gH [14], via a flexible segment in its N-terminal domain [15] and with HLA class II, via a C-type lectin domain at its C-terminus [16,17]. The interaction with HLA class II induces a slight conformational change in gp42 [18], enlarging a hydrophobic pocket found at the canonical lectin-binding site and enabling it to form contacts with the junction of domain II/III of gHgL [19]. It is this change that is thought to relay receptor binding to gHgL and initiate fusion. Exactly how the change is ultimately conveyed to gB is not known, but the final event in fusion is thought to require insertion of fusion loops in the gB trimer into the cell membrane [20] and a conformational change in gB, which pulls cell and virus membranes together [10].

Entry into the other major target cell of EBV, the epithelial cell, involves a slightly different complement of glycoproteins. Some epithelial cells express CD21 at low levels in tissue culture, and cells engineered to express it at high levels for attachment via gp350 can be infected very efficiently [21]. However, *in vivo*, the only normal epithelium found to express CD21 mRNA in the oral cavity, the site to which EBV is transmitted in saliva, is that of tonsils and adenoids [22]. Only when cells that normally do not express CD21 mRNA become dysplastic do they become more likely to become positive, a finding that may have implications for spread of virus under these circumstances [23]. Two glycoprotein complexes, in addition to gp350, have then been implicated in attachment to epithelial cells, gHgL and BMRF2/BDLF2. The gHgL complex binds with very high affinity to integrins $\alpha v \beta 5$, $\alpha v \beta 6$ and $\alpha v \beta 8$ [24,25] via a KGD motif found on an exposed loop in domain II of gHgL [12], but attachment via gHgL compromises infection [21]. BMRF2 is a multispan membrane protein that binds to $\alpha 3 \beta 1$ and $\alpha 5 \beta 1$ integrins via an RGD motif in one of its extra-cellular loops [26]. It associates with BDLF2, a type 2 membrane protein which carries N-linked sugars on its amino terminus and has a long proline-rich luminal domain [27]. In the absence of BMRF2, BDLF2 is not authentically processed and trafficked. The homologous complex in the murine gammaherpesvirus 68 manipulates actin to promote intercellular spread [28] and BMRF2/BDLF2 may do the same [29,30], so whether its primary role in attachment, signaling or cell-to-cell spread is not yet entirely clear. It is also possible that that virus is transferred directly from B cells to epithelial cells [31] or that as

yet unidentified glycoprotein interactions occur. EBV has recently been shown to infect gingival epithelial cells quite readily, if they are grown in raft cultures [32]. The cells do not apparently express CD21 and this culture method may enable better understanding of epithelial attachment.

Fusion of EBV with an epithelial cell, which may occur at the cell surface or following micropinocytosis, depending on the cell type [3,33], also requires gB, but, instead of a trimeric complex of gHgL and gp42, needs a dimeric complex of gHgL alone. Fusion is here initiated by the interaction of gHgL with one of the three α v integrins to which it can bind [25] and the presence of gp42 occludes access of the gHgL KGD motif to an integrin [34,35]. The virus thus carries both complexes of gHgL with gp42 and complexes of gHgL alone in order to access both cell types. In HLA class II-positive cells like B cells, some trimeric complexes bind to HLA class II in the endoplasmic reticulum and traffic with class II to the protease-rich peptide-loading compartment where they are degraded. This does not happen in class II-negative epithelial cells. The relative amounts of gp42-containing complexes thus alternate according to the cell type in which the virus is made, allowing a switch in tropism from one to the other to maintain the cycle of virus persistence [36].

The conformational change in gp42 as it binds HLA class II is mirrored by a conformation change in gHgL as the complex binds an integrin [24]. The change can be detected by an environmentally sensitive fluorescent probe bound to an unpaired cysteine at the domain I/ domain II interface and engineering of a disulfide bond to link domain I and domain II and constrain movement between the domains inhibits epithelial cell, though, interestingly, not B cell fusion [37]. A similar dichotomy between B cell and epithelial cell fusion has been mapped to a flap-like structure in domain IV of gHgL. A monoclonal antibody that maps to this structure blocks epithelial, but not B cell fusion and mutations in the same region can have differential effects on fusion with the two cell types [14,38]. Whether there are parallel differences in usage of gB for entry is not clear. For example, there is evidence for an interaction between gB and neuropilin on nasopharyngeal epithelial cells, which can initiate signaling and impact subsequent events in infection [33]. However, the involvement of gB in fusion itself seems likely to be fundamentally the same in B cells and epithelial cells. Fusion can be mediated by gHgL and gB, if gB is expressed in the same membrane in *cis* or in opposing membranes in *trans* and a virus that lacks gHgL and gp42 can be triggered with a soluble integrin to enter either a B cell or an epithelial cell that expresses gHgL [39]. The proteolytic digestion pattern of gB prior to fusion is different from its pattern after fusion confirming that fusion involves a conformational change in the protein. The same change can be elicited, if fusion is triggered not by an interaction with integrins but by exposure to heat, consistent with energy being needed for the conformational change to take place [39].

Virus assembly

Herpesvirus glycoproteins are important not only to entry but also to assembly and egress of virus. This is an area, however, that has been minimally studied in EBV. Two conserved, non-glycosylated proteins, BFRF1 and BFLF2, are critical for budding into perinuclear space [40] for which purpose they recruit the cellular endosomal sorting complex required for transport (ESCRT) machinery, which is involved in membrane scission and cytoplasmic

budding of many RNA viruses [41]. BFRF1 is a type 2 membrane protein and interacts via its long luminal domain with the soluble BFLF2 [42]. Both proteins are lost with the primary envelope as virus fuses with the outer nuclear membrane to enter the cytoplasm. It is unclear, however, what proteins mediate this fusion event though it is apparently somewhat different from the fusion that occurs during entry. Glycoprotein gH is not essential, since a gH-null virus egresses normally [43]. There are two conflicting reports on the involvement of gB [44,45], but no report currently of whether a virus lacking both gB and gHgL is compromised. Only if both gB and gHgL are missing from herpes simplex virus does the virus have a significant defect in egress from the nucleus [46].

Egress from the cytoplasm into the extracellular space is generally thought, as for all herpesviruses, to occur as a result of tegumented capsids budding back into the secretory compartment for exocytosis. In EBV, the process may require another dimeric glycoprotein complex, which is found in the virion, this time consisting of gM and gN [47,48].

Glycoprotein gM is a multispans, phosphorylated membrane protein with a long proline-rich cytoplasmic tail, whereas gN is very small type 1 membrane protein that carries only O-linked sugar and requires its association with gM in order to traffic from the endoplasmic reticulum to the Golgi apparatus. In the absence of gMgN, virus-producing cells die more rapidly and release primarily nonenveloped virus. The cytoplasmic tail of gM interacts with the cellular, ubiquitously expressed, multifunctional protein p32/gC1qR [49] and the gMgN null phenotype can be recapitulated by targeting p32 with siRNA [Changotra H, Hutt-Fletcher LM, Unpublished Data] However, cellular p32 has been implicated in nuclear egress of human cytomegalovirus and herpes simplex virus [50,51], so whether in the absence of gMgN virus is simply being released by nuclear envelope breakdown and cell lysis is not clear.

Manipulation of the host cell

Many large DNA viruses encode proteins that manipulate the host cell instead of, or in addition to performing more basic replication functions and at least three EBV glycoproteins fall into this category, BILF1, BARF1 and gp42. BILF1 is a constitutively active, heavily glycosylated, seven-transmembrane segment, G-protein-coupled receptor that signals through G α_i , inhibits phosphorylation of PKR and heterodimerizes with CXCR4, impairing its signaling in response to ligand [52–54]. In addition, it contributes to immune evasion by downregulating expression of HLA class I molecules on the cell surface, targeting them for internalization and degradation in the lysosome [55]. The C-terminal cytoplasmic tails of BILF1 and the HLA class I heavy chain interact and are required for the downregulation to occur [56]. BILF1 is expressed primarily early in the lytic cycle, though it may be expressed at low levels in latency as well [53,57].

BARF1 is a small secreted glycoprotein that carries a single N-linked glycan, has O-linked sugar on one threonine residue and is found extracellularly as a hexamer [58]. It is expressed during latency in epithelial cells, but as an early lytic cycle gene in B cells [59]. Many functions have been ascribed to BARF1 in epithelial cells, including inhibition of apoptosis, activation of telomerase, cell cycle dysregulation and malignant transformation. However, most of these effects have been seen following overexpression of the protein in isolation and

sometimes in rodent rather than human cells, so their biological significance is not entirely clear. What is clear, however, is that BARF1 can act as a soluble CSF-1 receptor that blocks CSF-1 signaling, apparently by binding to CSF-1 and locking it in an inactive conformation [60,61]. The potential importance of BARF1 to establishment of persistent infection by EBV has recently been highlighted by examination of the effects of blocking the activity of BARF1 in the rhesus lymphocryptovirus model [62]. Animals infected with a BARF1-mutant rhesus lymphocryptovirus had a much lower set point of virus load than those infected with wild-type virus. The production of BARF1 during epithelial latency may also contribute to immune evasion by nasopharyngeal carcinoma.

Finally, the ability of gp42 to interact with HLA class II not only impacts virus entry but may also affect recognition by CD4⁺ T cells. In cells stably transfected with gp42, the interaction of HLA class II/peptide complexes with T cell receptors is sterically blocked [63].

'Orphan' glycoproteins

The remaining two EBV glycoproteins, BILF2 and BDLF3, have yet to be ascribed any functions. Nothing has been published about BILF2 since its original description as 'gp78/55,' a type 1 membrane protein, found in the virion, which has a protein backbone with a mass of around 28 kDa and a large component of N-linked glycans [64]. There is also relatively little information about BDLF3, or gp150, a mucin-like glycoprotein. A virus lacking expression of BDLF3 has no significant phenotype *in vitro*. It infects epithelial cells slightly better than does wild-type virus, but this may simply reflect loss of charge from the virion envelope [65].

Conclusion

Clearly, there is much left to be learned about the glycoproteins of EBV. We know what they are, we know when they are expressed and, in addition to general information about their location and biosynthesis, we have crystal structures of five and a pseudoatomic structure of the gHgLgp42 trimer together with HLA class II. However, functional information is still very incomplete. We still lack a complete understanding of how the fusion machinery works, we have very little idea of the roles that glycoproteins play in assembly and egress and are we are not even certain of all the players involved. We are beginning to get a picture of how some of the glycoproteins manipulate the cell and its defenses against virus infection, but it is almost certainly not the whole story. It seems likely that the two glycoproteins that as yet have been ascribed no function at all will be found to join the group of 'manipulators', at least BDLF3, since a virus lacking its expression has next to no phenotype *in vitro*. There is plenty of work yet to be done in this field.

Future perspective

If this is where we are now, where might we expect to be in 5 or 10 years? There is general agreement in the field that a vaccine for EBV is needed and that it probably should incorporate glycoprotein gp350 at a minimum [7,66]. Some progress in the development of such a vaccine should be expected and the inclusion of additional glycoproteins such as

gHgL and gp42 might be anticipated. The target populations would presumably be 10- or 11-year-olds in the developed world, before they become susceptible to infectious mononucleosis and attendant sequelae, and much younger infants in southern China, where nasopharyngeal carcinoma is more common and sub-Saharan Africa, where Burkitt lymphoma is a bigger problem and where children are infected very early in life. Whether or not such populations would be considered financially attractive to pharmaceutical companies will presumably determine if the necessary Phase III trials are eventually undertaken.

More basic research may be expected to provide a much better understanding of the events leading up to fusion with both B cells and epithelial cells. The improvements in cryoelectronmicroscopy are already beginning to provide new insights and exciting developments here should certainly be anticipated. The last few years have also seen considerable attention being paid to how all herpesviruses manipulate the host response and EBV is no exception. The functions of BILF2 and BDLF3 will probably be determined fairly soon and there is hope that further use of the rhesus macaque model will help us understand the impact that such proteins may have on pathogenesis.

References

1. Longnecker, RM.; Kieff, E.; Cohen, JI. Epstein-Barr virus. In: Knipe, DM.; Howley, PM., editors. *Fields Virology*. Lippincott Williams & Wilkins; Philadelphia, PA, USA: 2013.
2. Johannsen E, Luftig M, Chase MR, et al. Proteins of purified Epstein-Barr virus. *Proc Natl Acad Sci USA*. 2004; 101:16286–16291. [PubMed: 15534216]
3. Hutt-Fletcher LM. Epstein-Barr virus entry. *J Virol*. 2007; 81:7825–7832. [PubMed: 17459936]
4. Ogembo JG, Kannan L, Ghiran I, Nicholson-Weller A, Finberg R, Fingerhuth JD. Human complement receptor type1/CD35 is an Epstein-Barr virus receptor. *Cell Rep*. 2013; 3:1–15. [PubMed: 23219549]
5. Concha M, Wang X, Cao S, et al. Identification of new viral genes and transcript isoforms during Epstein-Barr virus reactivation using RNA-seq. *J Virol*. 2012; 86:1458–1467. [PubMed: 22090128]
6. Moore MD, Discipio RG, Cooper NR, Nemerow GR. Hydrodynamic, electron microscopic and ligand binding analysis of the Epstein-Barr virus/C3dg receptor (CR2). *J Biol Chem*. 1989; 34:20576–20582. [PubMed: 2555366]
7. Cohen JI. Epstein-Barr virus vaccines. *Clin Transl Immunology*. 2015; 4(1):e32. [PubMed: 25671130]
8. Sashihara J, Hoshino Y, Bowman JJ, et al. Soluble rhesus lymphocryptovirus gp30 protects against infection and reduces viral loads in animals that become infected with virus after challenge. *PLoS Pathog*. 2011; 7:e1002308. [PubMed: 22028652]
9. Backovic M, Longnecker R, Jardetzky TS. Structure of a trimeric variant of the Epstein-Barr virus glycoprotein B. *Proc Natl Acad Sci USA*. 2009; 106:2880–2885. [PubMed: 19196955]
10. Connolly SA, Jackson JO, Jardetzky TS, Longnecker R. Fusing structure and function: a structural view of the herpesvirus entry machinery. *Nat Rev Microbiol*. 2011; 9:369–381. [PubMed: 21478902]
11. Stampfer SD, Heldwein EE. Stuck in the middle: structural insights into the role of gH/gL heterodimer in herpesvirus entry. *Curr Opin Virol*. 2013; 3:13–19. [PubMed: 23107819]
12. Matsuura H, Kirschner AN, Longnecker R, Jardetzky TS. Crystal structure of the Epstein-Barr virus (EBV) glycoprotein H/glycoprotein L (gH/gL) complex. *Proc Natl Acad Sci USA*. 2010; 107:22641–22646. [PubMed: 21149717]
13. Rowe CL, Chen J, Jardetzky TS, Longnecker R. Membrane anchoring of Epstein-Barr virus gp42 inhibits fusion with B cells even with increased flexibility allowed by engineered spacers. *mBio*. 2015; 6:e02285–e02214. [PubMed: 25564465]

14. Wu L, Hutt-Fletcher LM. Point mutations in EBV gH that abrogate or differentially affect B cell and epithelial cell fusion. *Virology*. 2007; 363:148–155. [PubMed: 17307213]
15. Liu F, Marquardt G, Kirchner AN, Longnecker R, Jardetzky TS. Mapping the N-terminal residues of Epstein–Barr virus gp42 that bind gH/gL by using polarization and cell-based fusion assays. *J Virol*. 2010; 84:10375–10385. [PubMed: 20668073]
16. Li QX, Spriggs MK, Kovats S, et al. Epstein–Barr virus uses HLA class II as a cofactor for infection of B lymphocytes. *J Virol*. 1997; 71(6):4657–4662. [PubMed: 9151859]
17. Mullen MM, Haan KM, Longnecker R, Jardetzky TS. Structure of the Epstein–Barr virus gp42 protein bound to the MHC class II receptor HLA-DR1. *Mol Cell*. 2002; 9:375–385. [PubMed: 11864610]
18. Kirchner AN, Sorem J, Longnecker R, Jardetzky TS. Structure of Epstein–Barr virus glycoprotein gp42 suggests a mechanism for triggering receptor-activated virus entry. *Structure*. 2009; 17:223–233. [PubMed: 19217393]
19. Sathiyamoorthy K, Jiang J, Hu YX, et al. Assembly and architecture of the EBV B cell entry triggering complex. *PLoS Pathog*. 2014; 10:e1004309. [PubMed: 25144748]
20. Backovic M, Jardetzky TS, Longnecker R. Hydrophobic residues that form putative fusion loops of Epstein–Barr virus glycoprotein B are critical for fusion activity. *J Virol*. 2007; 81:9596–9600. [PubMed: 17553877]
21. Borza CM, Morgan AJ, Turk SM, Hutt-Fletcher LM. Use of gHgL for attachment of Epstein–Barr virus to epithelial cells compromises infection. *J Virol*. 2004; 78:5007–5014. [PubMed: 15113881]
22. Jiang R, Gu X, Nathan C, Hutt-Fletcher L. Laser-capture microdissection of oropharyngeal epithelium indicates restriction of Epstein–Barr virus receptor/CD21 mRNA to tonsil epithelial cells. *J Oral Path Med*. 2008; 37:626–633. [PubMed: 18710421]
23. Jiang R, Gu X, Moore-Medlin TN, Nathan CA, Hutt-Fletcher LM. Oral dysplasia and squamous cell carcinoma: correlation between increased expression of CD21, Epstein–Barr virus and CK19. *Oral Oncol*. 2012; 48:836–841. [PubMed: 22513207]
24. Chesnokova LS, Hutt-Fletcher LM. Fusion of EBV with epithelial cells can be triggered by $\alpha v \beta 5$ in addition to $\alpha v \beta 6$ and $\alpha v \beta 8$ and integrin binding triggers a conformational change in gHgL. *J Virol*. 2011; 85:13214–13223. [PubMed: 21957301]
25. Chesnokova LS, Nishimura S, Hutt-Fletcher L. Fusion of epithelial cells by Epstein–Barr virus proteins is triggered by binding of viral proteins gHgL to integrins $\alpha v \beta 6$ or $\alpha v \beta 8$. *Proc Natl Acad Sci USA*. 2009; 106:20464–20469. [PubMed: 19920174]
26. Xiao J, Palefsky JM, Herrera R, Berline J, Tugizov SM. The Epstein–Barr virus BMRF-2 protein facilitates attachment to oral epithelial cells. *Virology*. 2008; 370:430–442. [PubMed: 17945327]
27. Gore M, Hutt-Fletcher L. 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*. 2008; 383:162–167. [PubMed: 18995876]
28. May JS, Walker J, Colaco S, Stevenson PG. The murine gammaherpesvirus 68 ORF27 gene product contributes to intercellular viral spread. *J Virol*. 2005; 79:5059–5068. [PubMed: 15795291]
29. Gill MB, Edgar R, May JS, Stevenson PG. A gamma-herpesvirus glycoprotein complex manipulates actin to promote viral spread. *PLoS ONE*. 2008; 3:e1808. [PubMed: 18350146]
30. Xiao J, Palefsky JM, Herrera R, Berline J, Tugizov SM. EBV BMRF-2 facilitates cell-to-cell spread within polarized oral epithelial cells. *Virology*. 2009; 388:335–343. [PubMed: 19394065]
31. Shannon-Lowe C, Rowe M. Epstein–Barr virus infection of polarized epithelial cells via the basolateral surface by memory B cell-mediated transfer infection. *PLoS Pathog*. 2011; 7:e1001338. [PubMed: 21573183]
32. Temple RM, Zhu J, Budgeon LR, Christensen ND, Meyers C, Sample CE. Efficient replication of Epstein–Barr virus in stratified epithelium *in vitro*. *Proc Natl Acad Sci USA*. 2014; 111:16544–16549. [PubMed: 25313069]
33. Wang HB, Zhang H, Zhang JP, et al. Neuropilin 1 is an entry factor that promotes EBV infection of nasopharyngeal epithelial cells. *Nat Commun*. 2015; 6:6240. [PubMed: 25670642]

34. Wang X, Kenyon WJ, Li QX, Mullberg J, Hutt-Fletcher LM. Epstein–Barr virus uses different complexes of glycoproteins gH and gL to infect B lymphocytes and epithelial cells. *J Virol.* 1998; 72:5552–5558. [PubMed: 9621012]
35. Chen J, Rowe CL, Jardetzky TS, Longnecker R. The KGD motif of Epstein–Barr virus gH/gL is bifunctional, orchestrating infection of B cells and epithelial cells. *mBio.* 2012; 3:e00290. [PubMed: 22215569]
36. Borza CM, Hutt-Fletcher LM. Alternate replication in B cells and epithelial cells switches tropism of Epstein–Barr virus. *Nature Med.* 2002; 8:594–599. [PubMed: 12042810]
37. Chen J, Jardetzky TS, Longnecker R. The large groove found in the gH/gL structure is an important functional domain for Epstein–Barr virus fusion. *J Virol.* 2013; 87:3620–3627. [PubMed: 23325693]
38. Wu L, Borza CM, Hutt-Fletcher LM. Mutations of Epstein–Barr virus gH that are differentially able to support fusion with B cells or epithelial cells. *J Virol.* 2005; 79:10923–10930. [PubMed: 16103144]
39. Chesnokova LS, Ahuja MK, Hutt-Fletcher LM. 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.* 2014; 88(21):12193–12201. [PubMed: 25142593]
40. Farina A, Feederle R, Raffa S, et al. BFRF1 of Epstein–Barr virus is essential for efficient primary viral envelopment and egress. *J Virol.* 2005; 79(6):3703–3712. [PubMed: 15731264]
41. Lee CP, Liu PT, Kung HN, et al. The ESCRT machinery is recruited by the viral BFRF1 protein to the nucleus-associated membrane for the maturation of Epstein–Barr virus. *PLoS Pathog.* 2012; 8:e1002904. [PubMed: 22969426]
42. Lake CM, Hutt-Fletcher LM. The Epstein–Barr virus BFRF1 and BFLF2 proteins interact and coexpression alters their cellular localization. *Virology.* 2004; 320:99–106. [PubMed: 15003866]
43. Molesworth SJ, Lake CM, Borza CM, Turk SM, Hutt-Fletcher LM. Epstein–Barr virus gH is essential for penetration of B cell but also plays a role in attachment of virus to epithelial cells. *J Virol.* 2000; 74:6324–6332. [PubMed: 10864642]
44. Lee SK, Longnecker R. The Epstein–Barr virus glycoprotein 110 carboxy-terminal tail domain is essential for lytic virus replication. *J Virol.* 1997; 71:4092–4097. [PubMed: 9094688]
45. Neuhiel B, Feederle R, Adhikary D, et al. Primary B-cell infection with a deltaBALF4 Epstein–Barr virus comes to a halt in the endosomal compartment yet still elicits a potent CD4-positive cytotoxic T-cell response. *J Virol.* 2009; 83:4616–4623. [PubMed: 19244320]
46. Farnsworth A, Wisner TW, Webb M, et al. Herpes simplex virus glycoproteins gB and gH function in fusion between the virion and the outer nuclear membrane. *Proc Natl Acad Sci USA.* 2007; 104:10187–10192. [PubMed: 17548810]
47. Lake CM, Hutt-Fletcher LM. Epstein–Barr virus that lacks glycoprotein gN is impaired in assembly and infection. *J Virol.* 2000; 74:11162–11172. [PubMed: 11070013]
48. Lake CM, Molesworth SJ, Hutt-Fletcher LM. The Epstein–Barr virus (EBV) gN homolog BLRF1 encodes a 15 kilodalton glycoprotein that cannot be authentically processed unless it is co-expressed with the EBV gM homolog BBRF3. *J Virol.* 1998; 72:5559–5564. [PubMed: 9621013]
49. Ghebrehiwet B, Lim B-L, Kumar R, Feng X, Peerschke EIB. gC1q-R/p33, a member of a new class of multifunctional and multicompartamental cellular proteins, is involved in inflammation and infection. *Immunol Rev.* 2001; 180:65–77. [PubMed: 11414365]
50. Milbradt J, Auerochs S, Sticht H, Marschall M. Cytomegaloviral proteins that associate with the nuclear lamina: components of a postulated nuclear egress complex. *J Gen Virol.* 2009; 90:579–590. [PubMed: 19218202]
51. Wang Y, Yang Y, Wu S, et al. p32 is a novel target for viral protein ICP34.5 of herpes simplex virus type 1 and facilitates nuclear egress. *J Biol Chem.* 2014; 289:35795–35805. [PubMed: 25355318]
52. Paulsen SJ, Rosenkilde MM, Eugen-Olsen J, Kledal TN. Epstein–Barr virus-encoded BILF1 is a constitutively active G protein-coupled receptor. *J Virol.* 2005; 79:536–546. [PubMed: 15596846]
53. Beisser PS, Verzijl D, Gruijthuijsen YK, et al. The Epstein–Barr virus BILF1 gene encodes a G protein-coupled receptor that inhibits phosphorylation of RNA-dependent protein kinase. *J Virol.* 2005; 79:441–449. [PubMed: 15596837]

54. Nijmeijer S, Leurs R, Smit MJ, Vischer HF. The Epstein–Barr virus-encoded G protein-coupled receptor BILF1 hetero-oligomerizes with human CXCR4, scavenges Gai proteins, and constitutively impairs CXCR4 functioning. *J Biol Chem*. 2010; 285:29632–29641. [PubMed: 20622011]
55. Zuo J, Currin A, Griffin BD, et al. The Epstein–Barr virus-encoded BILF1 protein-coupled receptor contributes to immune evasion by targeting MHC class I molecules for degradation. *PLoS Pathog*. 2009; 5:e1000255. [PubMed: 19119421]
56. Griffin BD, Gram AM, Mulder A, et al. EBV BILF1 evolved to downregulate cell surface display of a wide range of HLA class I molecules through their cytoplasmic tail. *J Immunol*. 2013; 190:1672–1684. [PubMed: 23315076]
57. Tierney RJ, Shannon-Lowe CD, Fitzsimmons L, Bell AI, Rowe M. Unexpected patterns of Epstein–Barr virus transcription revealed by a high throughput PCR array for absolute quantification of viral DNA. *Virology*. 2015; 474:117–130. [PubMed: 25463610]
58. Tarbouriech N, Ruggiero F, De Turenne-Tessier M, Ooka T, Burmeister WP. Structure of the Epstein–Barr virus oncogene BARF1. *J Mol Biol*. 2006; 359:667–678. [PubMed: 16647084]
59. Hoebe EK, Le Large TYS, Greijer AE, Middeldorp JM. BamHI-A rightward frame 1, and Epstein–Barr virus-encoded oncogene and immune modulator. *Rev Med Virol*. 2013; 23:367–383. [PubMed: 23996634]
60. Strockbine L, Cohen JI, Farrah T, et al. The Epstein–Barr virus BARF1 gene encodes a novel, soluble colony-stimulating factor-1 receptor. *J Virol*. 1998; 72:4015–4021. [PubMed: 9557689]
61. Elegheert J, Bracke N, Pouliot P, et al. Allosteric competitive inactivation of hematopoietic CSF-1 signaling by the viral decoy receptor BARF1. *Nat Struct Mol Biol*. 2012; 19:938–947. [PubMed: 22902366]
62. Ohashi M, Fogg MH, Orlova N, Quink C, Wang F. An Epstein–Barr virus encoded inhibitor of colony stimulating factor-1 signaling is an important determinant for acute and persistent EBV infection. *PLoS Pathog*. 2012; 8:e1003095. [PubMed: 23300447]
63. Rensing ME, Horst D, Griffin BD, et al. Epstein–Barr virus evasion of CD8(+) and CD4(+) T cell immunity via concerted actions of multiple gene products. *Semin Cancer Biol*. 2008; 18:397–407. [PubMed: 18977445]
64. Mackett M, Conway MJ, Arrand JR, Haddad RS, Hutt-Fletcher LM. Characterization and expression of a glycoprotein encoded by the Epstein–Barr virus Bam HI 1 fragment. *J Virol*. 1990; 64:2545–2552. [PubMed: 2159529]
65. Borza C, Hutt-Fletcher LM. 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*. 1998; 72:7577–7582. [PubMed: 9696856]
66. Cohen JI, Fauci AS, Varmus H, Nabel GJ. Epstein–Barr virus: an important vaccine target. *Sci Transl Med*. 2011; 3(107):107fs7.

EXECUTIVE SUMMARY

Glycoproteins & virus entry

- EBV entry into a B cell requires five virus glycoproteins, gp350, which attaches virus to CD21 or CD35 and gB, gHgL and gp42, which are involved in fusion between the virus envelope and the cell membrane. Fusion is triggered when gp42 interacts with HLA class II.
- EBV entry in an epithelial cell probably involves different attachment proteins, which are not completely defined, and fusion requires only gB and gHgL. Fusion is triggered when gHgL interacts with an integrin.
- Glycoprotein gp350 is viewed as a promising vaccine candidate.

Glycoproteins in virus assembly & egress

- Glycoproteins gM and gN are involved in envelopment of virus.
- It is not known what proteins are required for the primary envelope of the virus, acquired by budding through the inner nuclear membrane, to fuse with the outer nuclear membrane and allow delivery of capsids to the cytoplasm.

Glycoproteins that manipulate the cell

- BILF1 acts as a constitutively active G-protein-coupled receptor and can also cause downregulation of HLA class I.
- BARF1 acts as a soluble colony stimulating factor 1 receptor and blocks CSF-1 signaling.
- Glycoprotein gp42 can block recognition of an HLA class II/peptide complex by the T cell receptor.

'Orphan' glycoproteins

- Two glycoproteins, BDLF3 and BILF2 have as yet been ascribed no functions.

Conclusion

- There is a clear outline of how glycoproteins facilitate B-cell entry, but there is still confusion about how EBV initially accesses epithelial cells. The recent discovery that epithelial raft cultures can be readily infected provides new tools to explore this issue.
- Considerable progress has been made in understanding the involvement of glycoproteins in virus/cell fusion and although much still remains unclear, progress is accelerating.
- The role of glycoproteins in assembly and egress of enveloped particles is very poorly understood and beyond the observation that the gMgN complex is important in some way, even the players involved are undetermined. This is a neglected area of research that needs much more attention and like entry could produce therapeutic targets.

- At least three glycoproteins interfere with the immune response, but it is likely that there are additional glycoproteins that are important, possibly the two, BDLF3 and BILF2, that have not yet been ascribed any function.

Table 1

Summary of EBV glycoproteins.

Gene name	Protein name	Type	Expression	Function
<i>BLLF1</i>	gp350/220	Single pass type 1 membrane	Late lytic/structural	Attachment
<i>BALF4</i>	gB	Single pass type 1 membrane	Late lytic/structural	Fusion
<i>BXLF2</i>	gH	Single pass type 1 membrane	Late lytic/structural	Regulation and triggering of fusion
<i>BKRF2</i>	gL	Soluble associated with gH	Late lytic/structural	Regulation and triggering of fusion
<i>BZLF2</i>	gp42	Single pass type 2 membrane/soluble	Late lytic/structural	Triggering of fusion/immune evasion
<i>BBRF3</i>	gM	Multispanning membrane	Late lytic/structural	Assembly and release
<i>BLRF2</i>	gN	Single pass type 1 membrane	Late lytic/structural	Assembly and release
<i>BMRF2</i>	BMRF2	Multispanning membrane	Late lytic/structural	Epithelial cell attachment and spread
<i>BDLF2</i>	BDLF2	Single pass type 2 membrane	Late lytic/structural	Epithelial spread?
<i>BDLF3</i>	BDLF3	Single pass type 1 membrane	Late lytic/structural	Unknown
<i>BILF2</i>	BILF2	Single pass type 1 membrane	Late lytic/structural	Unknown
<i>BILF1</i>	BILF1	Multispanning membrane	Immediate early/early	G-protein-coupled receptor/immune evasion
<i>BARF1</i>	BARF1	Secreted	Latent and early lytic	CSF1 receptor/immune evasion