FEATURE ARTICLE

Epstein-Barr Virus: Cell Trafficking Is Crucial for Persistence

This virus moves between host lymphoid and epithelial cells, switching its tropism while enhancing persistence

Lindsey Hutt-Fletcher

Herpesviruses, large enveloped viruses with double-stranded linear genomes, are particularly adept at overcoming immune system and other challenges while persisting within host cells. In particular, Epstein-Barr virus (EBV), one of the eight known human herpesviruses, typically persists for life in its hosts by limiting its lethality, evading immune responses, and surviving within particular host cells even while they are dividing. Moreover, it retains the ability to spread within and between hosts.

For EBV, a set of glycoproteins proves important for its capacity to recognize and fuse with specific types of host cells. Additionally, host-cell proteins also play a key role in this process. Moreover, both viral and cellular proteins are crucial for EBV trafficking between its two major target cell host types, and those proteins play into an ingenious strategy for switching tropism and driving viral persistence.

Establishing Persistence

EBV, which is orally transmitted via saliva, targets B cells in lymphoid tissue of the tonsils and adenoids surrounding the pharynx. The linear virus DNA soon circularizes within the host-cell nucleus and, although more than 50% of the genome is transcribed, no new viruses are produced. Instead, a series of splicing events leads to expression of nine latency proteins, several miRNA molecules, and additional nontranslated molecules of RNA. Together, they drive infected B cells to become proliferating blast cells, which are like the lymphoblastoid cell lines that form after EBV is added to B cells in vitro. These events resemble those that occur during antigen stimulation of B cells, according to David Thorley-Lawson of Tufts University School of Medicine in Boston, Mass.

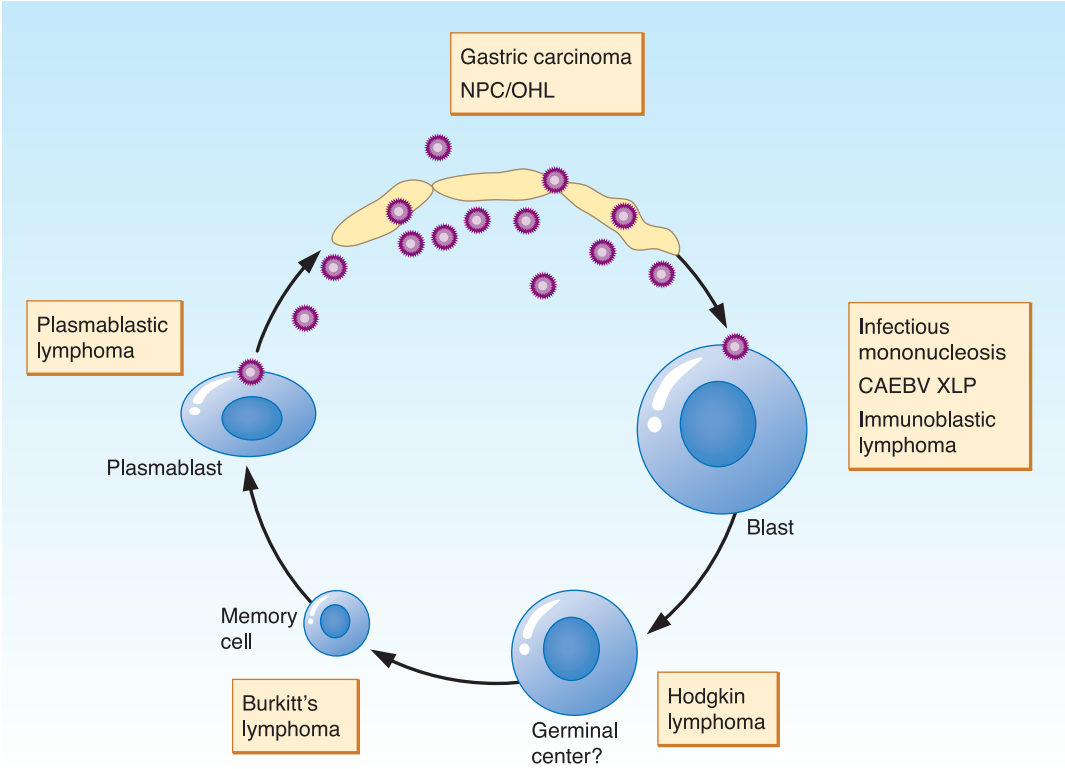
Latently infected B memory cells circulate between the peripheral blood and lymphoid tissues, expressing at most one of the EBV proteins. While the host immune response may eliminate cells in which viral proteins are made, some of the silently infected memory cells escape immune control and persist.

Additionally, some of the EBV-infected cells may differentiate, enabling the virus to enter into its productive lytic cycle and releasing new virus to infect epithelial cells, amplify, and be shed into saliva for transmission to new hosts or to replenish the latently infected B cell reservoir (Fig. 1). When EBV-infected individuals receive the antiviral drug valacyclovir for a long term, latently infected B cells gradually diminish, according to Jeffrey Cohen and his colleagues at the National Institutes of Allergy and Infectious Disease in Bethesda, Md. However, it would take about 11 years for the virus to be eliminated, he estimates.

SUMMARY

- **The Epstein-Barr virus (EBV) infects host lymphoid and epithelial cells in a persistent manner, evading immune responses.**
- **EBV infects 95% of the worldwide adult population, mainly asymptomatically, whereas infections during adolescence can lead to mononucleosis. EBV infections also are associated with several types of malignancies.**
- **EBV requires both an attachment receptor for binding and a coreceptor for fusion and entry of host cells. The coreceptor is critical for trafficking of this virus.**
- **Differential use of two- and three-part protein complexes enables EBV to switch tropism between B and epithelial cells.**

FIGURE 1



The EBV cycle of persistence. Diseases thought to originate from cells at different points in the cycle are indicated in the boxes.

Varied Consequences of EBV Infection

EBV infects more than 95% of the adult population worldwide, albeit without their realizing it. Primary infections are typically asymptomatic, particularly if acquired before the age of about 10 or 12 years. However, primary infections later in life can stimulate a vigorous immune response, leading to the immunopathology we know as infectious mononucleosis. Although most individuals with mononucleosis recover uneventfully, in some exceedingly rare cases, more common in East Asian individuals than those of other heritages, EBV can become chronically active, with a high mortality rate.

Individuals with a mutation in their version of the signaling lymphocytic activation molecule-associated protein, an immunoregulatory protein, develop a disorder called X-linked lymphoproliferative disease. Such individuals typically cannot withstand primary EBV infections and tend to develop a fatal hemophagocytic syndrome. AIDS patients with EBV are at risk for immunoblastic lymphomas, typically in the central nervous system. Such lymphomas are also associated with patients who receive transplants and then receive immunosuppressive drugs.

About 50% of patients with Hodgkin lymphomas are EBV positive. These lymphomas appear to contain EBV-infected B cells with rearranged immunoglobulin genes along with additional mutations. A history of infectious mononucleosis slightly increases the risk of developing Hodgkin lymphoma, possibly because large numbers of infected B cells during acute mononucleosis increase this risk. The association of EBV with only a subset of such tumors is not unique to Hodgkin lymphoma.

EBV is associated with nearly all endemic Burkitt's lymphomas, which carry a *c-myc* translocation and have the hallmarks of memory

B cells. These lymphomas are seen primarily among children in West Africa, where malaria is endemic. The virus and malaria parasites appear to be cofactors in development of such lymphomas, possibly because both are powerful stimulators of B cells.

EBV is also associated with epithelial disorders such as oral hairy leukoplakia (OHL) in late-stage AIDS patients. Moreover, about 10% of gastric cancers and essentially all undifferentiated nasopharyngeal carcinomas (NPC) carry latent EBV. Worldwide, epithelial tumors are the largest EBV-associated tumor burden, with as many as 150,000 to 200,000 new cases diagnosed each year. Latent virus is generally agreed to contribute to the malignant phenotype, either as a tumor initiator or, more probably, as a tumor progressor. Molecular alterations, including chromosome loss, are seen in epithelial tissues in ethnic groups at high risk for NPC. In addition, prospective studies have shown that antibodies to virus lytic replication proteins increase significantly 2–3 years before tumors arise. Trafficking of virus from B cell to epithelial cell and back is then not only important for persistence, but when this balance in healthy individuals is disturbed it may accelerate the development of malignant disease.

Fusion and Trafficking of EBV in B Cells

How the tropism and trafficking between cell types is sustained lies in part with how EBV enters cells. Although this enveloped virus enters cells by fusion, the entry location varies from one cell type to another. For example, fusion with B cells requires endocytosis and occurs in a low-pH compartment, whereas fusion with epithelial cells occurs at neutral pH possibly without endocytosis. Neither process requires a low pH to trigger fusion. Instead, as with other herpesviruses, EBV requires both an attachment receptor for binding and a coreceptor for fusion and entry. The latter is critical for virus trafficking.

EBV fusion with B cells requires at least five proteins, including one host protein, HLA class II and four viral glycoproteins: gp42, gH, gL, and gB. The crystal structures of those four glycoproteins were determined by Theodore Jardetzky, formerly at Northwestern University and now at Stanford University in Stanford, Calif., in collaboration with Richard Longnecker at Northwestern University in Chicago, Ill. The EBV gB structure forms trimers that closely resemble those formed by the gB homolog from herpes simplex virus and the prototypical class III fusion proteins formed by the G protein of vesicular stomatitis virus (VSV).

Unlike the VSV G protein, gB from EBV cannot fuse with cell membranes on its own. Instead, it requires two additional proteins, gH and gL. The solution of the crystal structure of the EBV gHgL dimer revealed a four-domain, rod-like protein in which domain I, furthest away from the virus envelope, is comprised of the N-terminal residues of gH, which is a type 1 membrane protein, and gL, which is a type 2 membrane protein from which the signal peptide is cleaved. Glycoproteins gB and gHgL are considered to be the core fusion machinery, conserved throughout the herpesvirus family. To fuse with a B cell, however, EBV also requires gp42, another cleaved type 2 membrane protein which resembles a C-type lectin. It interacts directly with gH.

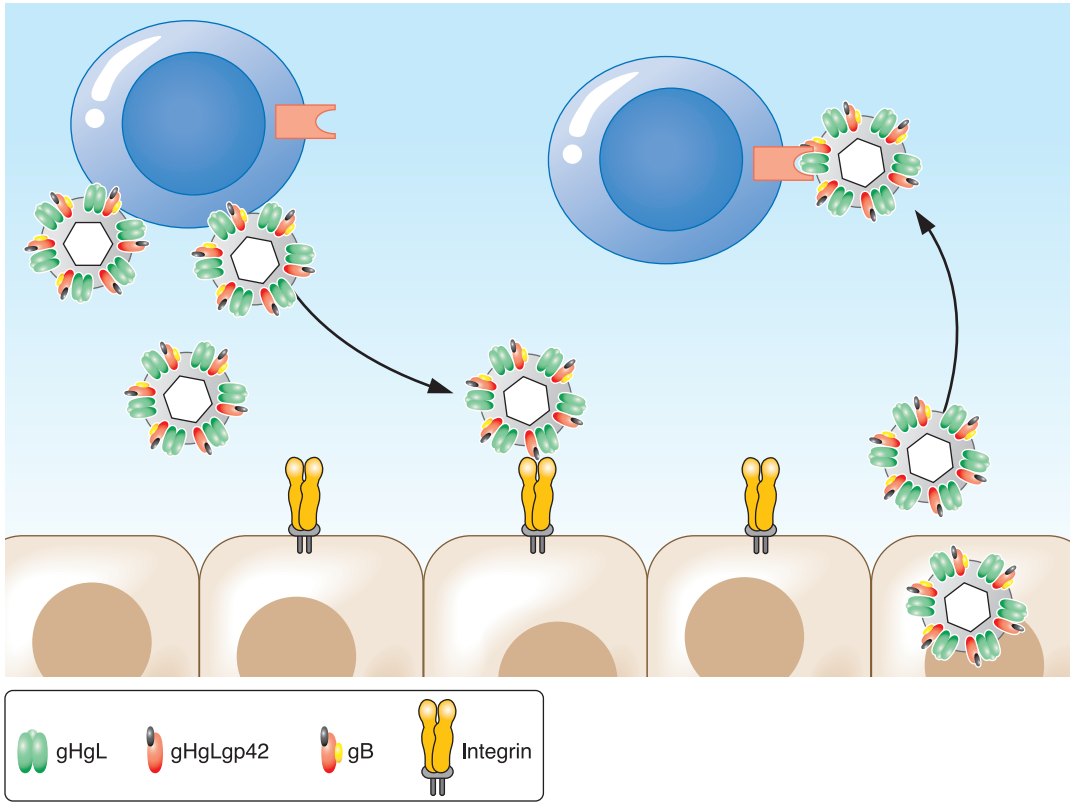
Melanie Spriggs at Immunex Corporation in Seattle, Wash., found that gp42 from EBV interacts with HLA class II. We found that the same soluble version of this protein blocks EBV infection of B cells. An antibody to gp42 that blocks virus fusion with a B cell also blocks gp42 binding to HLA class II protein, and EBV cannot efficiently infect B cells lacking HLA class II. The glycoprotein does not obstruct the peptide groove of HLA class II; instead, it binds to the side of the groove. Other findings by Emmanuel Wertz and colleagues at Leiden University Medical Center suggest that gp42 not only facilitates entry of EBV into a B cell, but also modulates the immune response to a B cell making this virus.

Fusion and Trafficking of EBV in Epithelial Cells

Unlike B cells, epithelial cells do not express HLA class II proteins. EBV fusion with such cells requires only three viral glycoproteins: gB and gHgL. Fusion also requires one cell protein to complete the process—in this case, any one of three epithelial integrins: $\alpha v\beta 5$, $\alpha v\beta 6$, or $\alpha v\beta 8$.

Because a KGD integrin-binding motif is found in the sequence of gH, we suspected that an integrin could be involved in EBV entry into epithelial cells. Although soluble gHgL does not bind to B cells, it binds with high affinity to epithelial cells. Knockdown of integrin αv inhibits

FIGURE 2



The EBV tropism switch. Tripartite gHgLgp42 complexes can be lost as a result of interaction with MHC class II within B cells but are not lost in epithelial cells lacking MHC class II. B cell virus with more bipartite gHgL complexes can slightly better access integrins that trigger fusion with an epithelial cell. Epithelial virus with more tripartite complexes is much better able to access MHC class II molecules which trigger fusion with a B cell.

gHgL binding to epithelial cells and blocks entry of EBV.

Each integrin that is capable of binding gHgL binds with a similar affinity. In the absence of manganese, affinities are on the order of 10^{-9} molar. In its presence, they are in the range of 10^{-12} molar. While soluble gp42 can rescue B cell infection of a gp42-null virus, it blocks infection of an epithelial cell by wild-type EBV. However, EBV carries two different kinds of gHgL complexes—one that is tripartite and includes gp42 and another that is bipartite and includes only gH and gL. These two complexes have a mutually exclusive ability to enter B cells in one case and epithelial cells in the other. If gHgL complexes are converted to gHgLgp42 complexes by adding soluble gp42 in trans, epithelial cell infection is blocked because gHgL cannot access an integrin (Fig. 2).

Differential Complexes Affect EBV Cell Tropism

This differential use of two- and three-part gH complexes allows the virus to switch tropism between B cells and epithelial cells. In a class II positive B cell, some of the three-part complexes interact with class II in the endoplasmic reticulum and are diverted to the peptide loading compartment, which is rich in proteases and degrades them. From such cells, EBV emerges with more two-part than three-part complexes and thus is slightly enhanced for infecting epithelial cells. This enhancement does not occur in a class II-negative epithelial cell, from which EBV emerges with higher levels of three-part complexes. Those EBV viruses are significantly—by about two orders of magnitude—more infectious for B cells than for EBV that emerges from B cells, thus

driving slightly different versions of this virus from one cell type to the other.

Less is known about other details regarding EBV-host cell fusion. Some experts working in this field suspect that coreceptor proteins such as integrins or HLA and gp42 lead gHgL to interact with gB, which is thought to change its conformation and insert into both viral and cell membranes—pulling them together and causing them to fuse. Mutations at both ends of gHgL affect fusion. A neutralizing monoclonal antibody maps to a flap-like structure in domain IV of gHgL, and point mutations there affect fusion in opposite directions in B cells versus epithelial cells. Mutations in residues in a nearby region between domain I and II also affect fusion, suggesting that a domain I/domain II conformational change could be part of the fusion mechanism, according to Jardetzky at Stanford University and Longnecker at Northwestern University.

A single unpaired cysteine residue in gH along the domain I/domain II interface can be labeled with fluorescent probes such as acrylodan or IANBD without affecting function. Binding of soluble integrins to labeled proteins increases fluorescence, consistent with increases in the hydrophobicity of the environment of the probes and providing evidence for the occurrence of a conformational change.

Glycoproteins gB and gHgL mediate fusion when expressed in the same virus or cell membrane. Moreover, soluble integrins can trigger entry of a gHgL null virus into cells expressing gHgL. These findings suggest that the ectodomains of the proteins trigger fusion. One possibility is that, when gHgL interacts with an integrin, it releases enough free energy to convert gB from a metastable to a fusogenic state. Consistent with this analysis, heating cells that express gB alone can trigger at least low levels of fusion.

There is still much to be learned about the entry of EBV into host cells. However, a minimal set of virus glycoproteins that are important to virus fusion has been identified, some of the key cell players have been determined, and uncovering the differential use of virus and cell proteins for the two major target cells of the virus has revealed an ingenious virus strategy for switching tropism and driving the cycle of persistence.

Suggested Reading

Borza, C. M., and L. M. Hutt-Fletcher. 2002. Alternate replication in B cells and epithelial cells switches tropism of Epstein-Barr virus. Nature Med. **8:**594–599.

Chesnokova, L. S., and L. M. Hutt-Fletcher. 2011. 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. **85:**13214–13223.

Chesnokova, L. S., S. Nishimura, and L. Hutt-Fletcher. 2009. Fusion of epithelial cells by Epstein-Barr virus proteins is triggered by binding of viral proteins gHgL to integrins avb6 or avb8. Proc. Natl. Acad. Sci. USA **106:**20464–20469.

Cohen, J. I. 2000. Epstein-Barr virus infection. New Engl. J. Med. **343:**481–492.

Connolly, S. A., J. O. Jackson, T. S. Jardetzky, and R. Longnecker. 2011. Fusing structure and function: a structural view of the herpesvirus entry machinery. Nature Rev. Microbiol. **9:**369–381.

Hutt-Fletcher, L. M. 2007. Epstein-Barr virus entry. J. Virol. **81:**7825–7832.

Kirschner, A. N., J. Sorem, R. Longnecker, and T. S. Jardetzky. 2009. Structure of Epstein-Barr virus glycoprotein gp42 suggests a mechanism for triggering receptor-activated virus entry. Structure **17:**223–233.

Lo, K. W., G. T. Chung, and K. F. To. 2012. Deciphering the molecular genetic basis of NPC through molecular, cytogenetic, and epigenetic approaches. Semin. Cancer. Biol. **22:**79–86.

Longnecker, R. M., E. Kieff, and J. I. Cohen. 2013. Epstein-Barr virus, p. 1898–1959. *In* D. M. Knipe and P. M. Howley (ed.), Fields virology vol. 2. Lippincott Williams & Wilkins, Philadelphia.

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

Thorley-Lawson, D. A., and A. Gross. 2004. Persistence of Epstein-Barr virus and the origins of associated lymphomas. New Engl. J. Med. **350:**1328–1337.