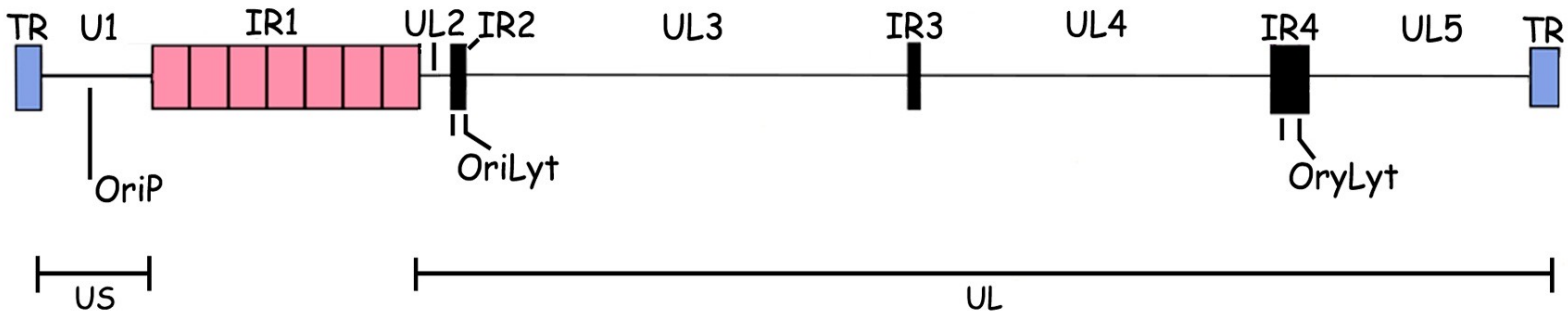
*E*pstein-*B*arr *V*irus (EBV)

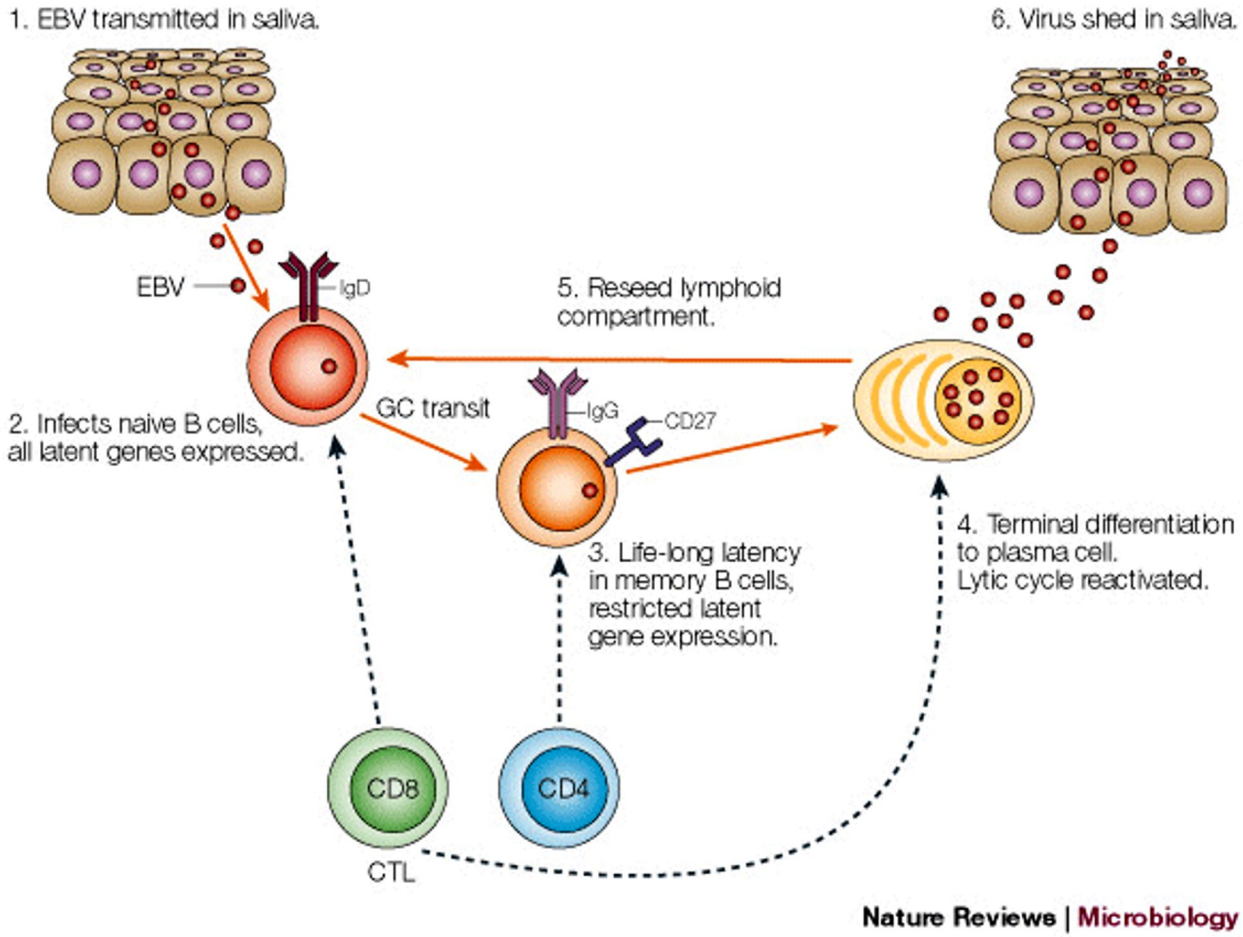
- 1958 Burkitt identified a previously unrecognized form of cancer affecting the jaws of young children in East Africa and he made the crucial insight that the distribution of this tumor appeared to be influenced by climatic factors. Burkitt theorized that the tumor might be due to a mosquito-born virus
- 1964 This observation led Epstein and Barr to examine freshly tumor biopsies for the presence of a virus. Using electron microscopy, they found herpes-like particles in a number of biopsied cells establishing that this was in fact a new virus. EBV was thus identified as the first candidate human tumor virus.
- 1968 EBV is described as the causative agent of infectious mononucleosis
- 1969 EBV immortalizes cells in culture

EBV GENOME

- The EBV genome is a linear dsDNA of about 172 Kbp
- It contains unique sequence (UL, US), internal tandem repeats (IRs) and direct terminal repeats at the ends (TR)

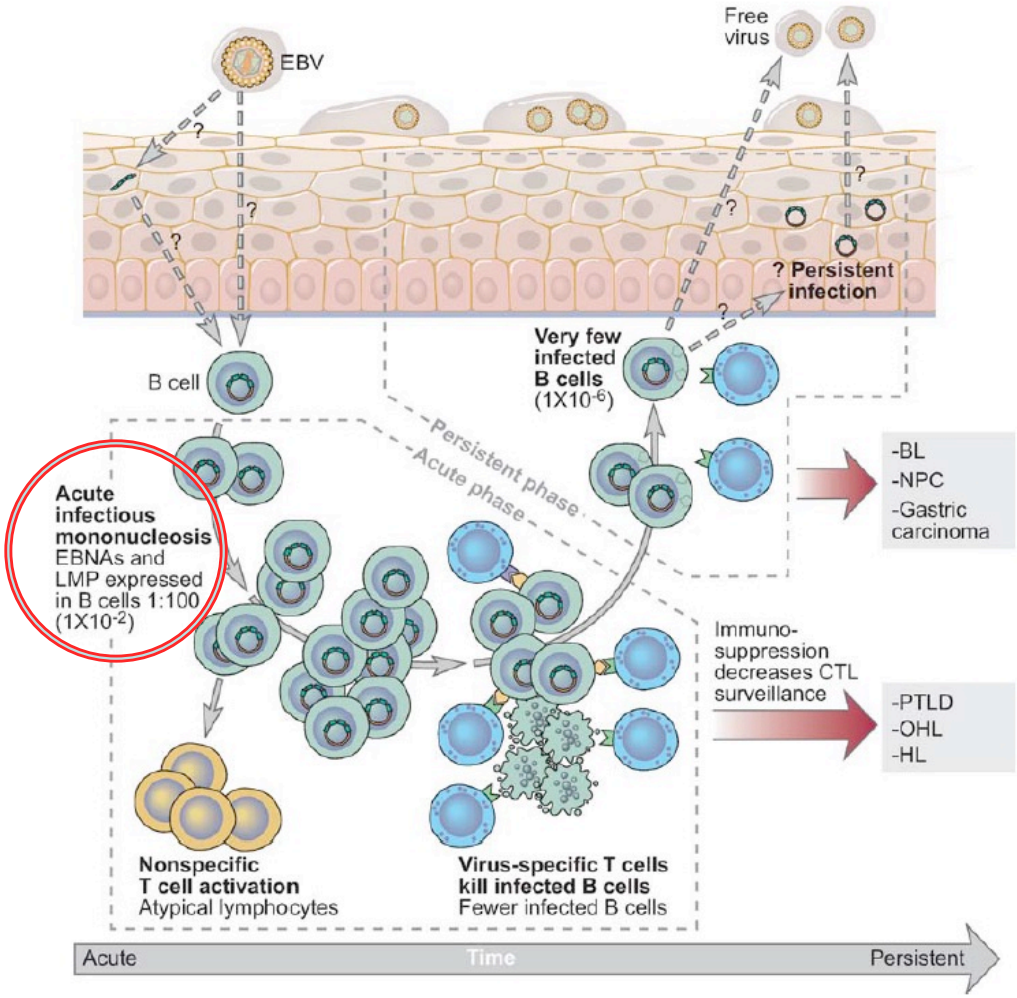


EBV INFECTION



EBV is transmitted in the saliva (1), infects B cells in the oropharyngeal epithelium and enters the lymphoid tissue. Following infection of naive B cells in the lymph node (2), all latent genes are expressed and the viral latent proteins drive the B cell through subsequent proliferation and the germinal centre (GC) reaction. The transit through the germinal centre results in establishment of a life-long infection of memory B cells (3), which are detected in the peripheral circulation. Terminal differentiation to plasmacells results in reactivation of the virus to the lytic cycle, expression of lytic proteins and production of infectious virus (4). The virus can infect B cells within the lymphoid tissue or be shed into the saliva (5,6).

Infectious mononucleosis



Schematic of the major events that occur during acute and persistent infection with Epstein-Barr virus (EBV). Once the target B cell is infected, EBV infection drives B lymphoid proliferation, which stimulates a nonspecific T cell activation. The activated T cells cause the appearance of atypical lymphocytes in the peripheral blood and, if sufficiently vigorous, T cell activation leads to infectious mononucleosis (IM) syndrome. Over time, EBV-specific T cell immunity develops, which reduces the number of infected B lymphocytes. Under normal conditions, the infection is controlled and enters into a latent phase where EBV infection is present in only a very few infected B cells (1 °ø 10−6). Lytic replication, either in oral epithelial cells or B cells near the oral epithelium, results in the shedding of infectious viruses via oral secretions and renewal of the virus life cycle in a new host. Immunosuppression can result in decreased cytotoxic T cell (CTL) surveillance, which leads to an increased risk of developing certain EBV-associated diseases such as post-transplantation lymphoproliferative disorder (PTLD), oral hairy leukoplakia (OHL), and Hodgkin lymphoma (HL). Other EBV-related diseases [Burkitt lymphoma (BL), nasopharyngeal carcinoma (NPC), and gastric carcinoma] are not typically associated with overt immunosuppression, but likely arise as a result of other cofactors acting in concert with persistent EBV infection.

Annu. Rev. Pathol. Mech. Dis. 2006. 1:375–404

Receptors for the virus

Table 1

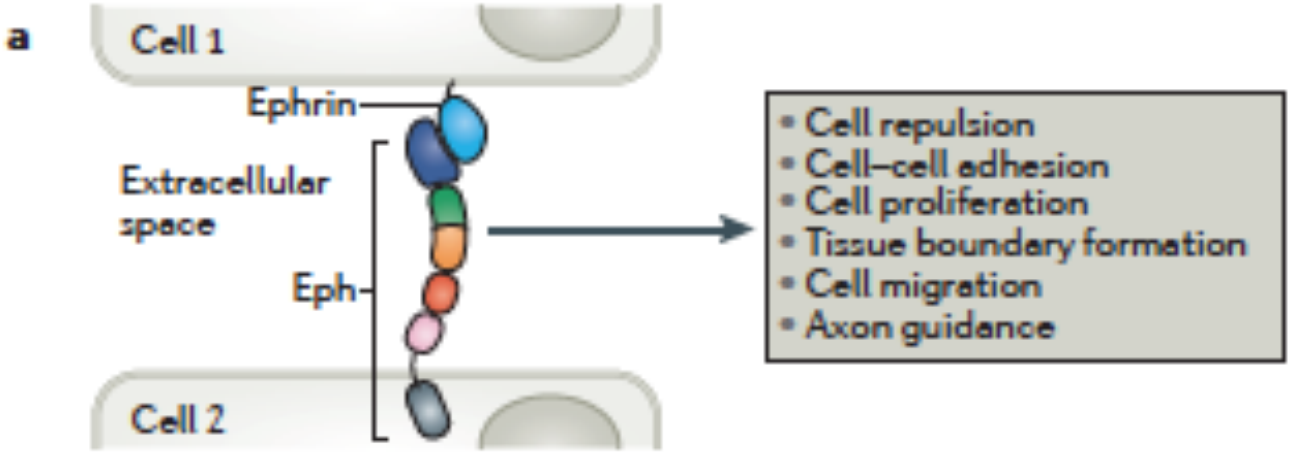
Epstein Barr virus glycoproteins: functions and cellular receptors

Glycoprotein	Gene name	Function	Cellular receptor
gp350	BLLF1	• Binds B cells	• CR2 (CD21) • CR1 (CD35)
gp42	BZLF2	• Binds B cells • Triggers fusion	• HLA class II
gH (gp85)	BDLF3	• Binds epithelial cells • Triggers fusion	• αvβ5, αvβ6 and αvβ8 integrins
gL (gp25)	BKRF2	• Chaperone for gH • Activation/Recruitment of gB	• Unknown
gB (gp110)	BALF4	• Catalyses membrane fusion	• Unknown
BMRF2	BMRF2	• Binds epithelial cells	• β1 integrins

Ephrin receptor A2 is a functional entry receptor for Epstein-Barr virus. Nature Microbiology, Feb. 2018

Ephrin receptor A2 is an epithelial cell receptor for Epstein-Barr virus entry. Nature Microbiology, Feb. 2018

General features of ephrins and Ephs and the concept of bi-directional signaling



Eph Tyr kinase receptors and ephrin ligands are located at the cell membrane and are implicated in short-distance signalling between neighbouring cells. They direct cellular processes such as cell repulsion, cell–cell adhesion, cell proliferation, tissue boundary formation, cell migration and axon guidance, among others.

NATURE REVIEWS | MOLECULAR CELL BIOLOGY VOLUME 17 | APRIL 2016 |

Ephrin receptor A2, the epithelial receptor for Epstein-Barr virus entry, is not available for efficient infection in human gastric organoids
Plos Pathogens feb. 2021 https://doi.org/10.1371/journal.ppat.1009210

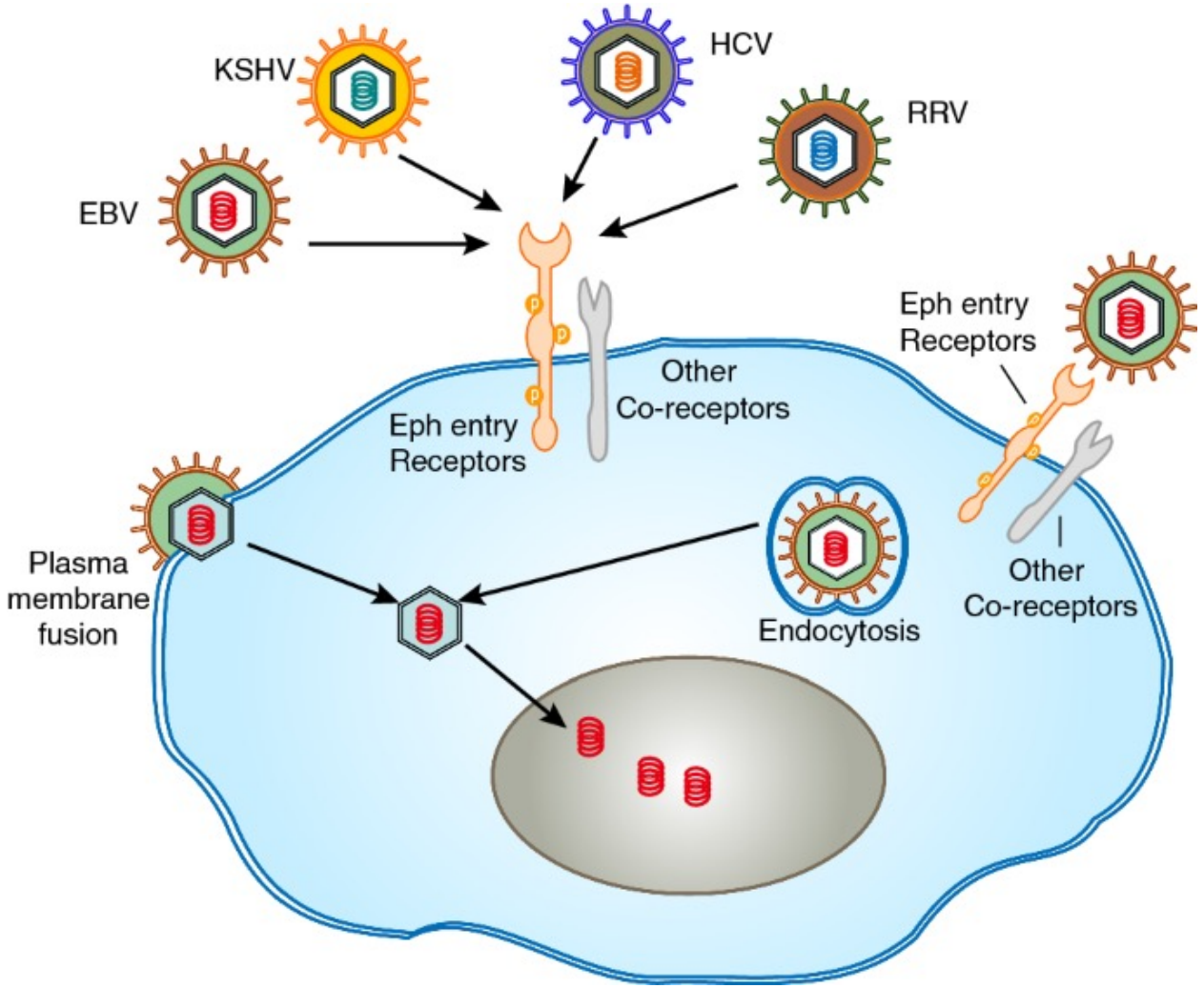
Author summary

Epstein-Barr virus (EBV) is associated with malignancies of lymphoid and epithelial cell lineages, including gastric cancer (GC). Although EBV is only associated with up to 10% of GC, this unique subset is genetically and epigenetically distinct from other forms of GC. However, the sequence of events leading to EBV-associated GC (EBVaGC) remains unclear. Ephrin receptor A2 (EPHA2) was identified as a receptor for EBV entry into epithelial cancer cell lines, yet the physiological relevance of its role in infection of healthy gastric epithelium was not explored. Using human adult healthy stem cell-derived gastric organoids, microscopy showed the EPHA2 receptor was strictly localized to cell-cell junctions and therefore inaccessible to EBV, resulting in poor infection. In contrast, EPHA2 expression was not confined to cell-cell junctions in cancer-derived organoids, rendering it accessible to EBV. Correspondingly, these organoids were more readily infected. Although EBV was not detected in healthy gastric epithelial tissue, immunohistochemical analysis identified EBV in inflamed epithelium. These results suggest viral entry requires initial changes to the gastric epithelium, likely induced by inflammation, to expose the virus receptor and enable efficient infection.

Eph receptors: the bridge linking host and virus

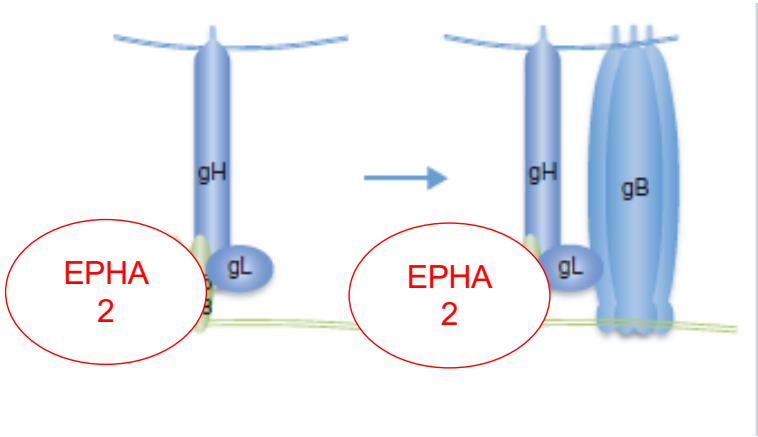
•Jia Wang, Xiang Zheng, Qiu Peng, Xuemei Zhang & Zailong Qin

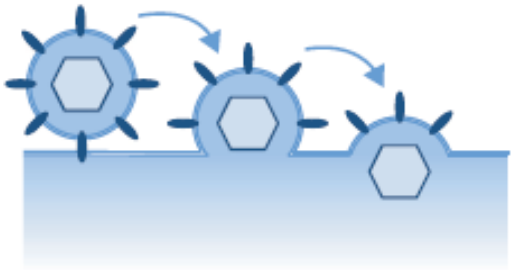
Cellular and Molecular Life Sciences **volume 77**, pages2355-2365(2020)



(RRV-rhesus monkey rhadinovirus)

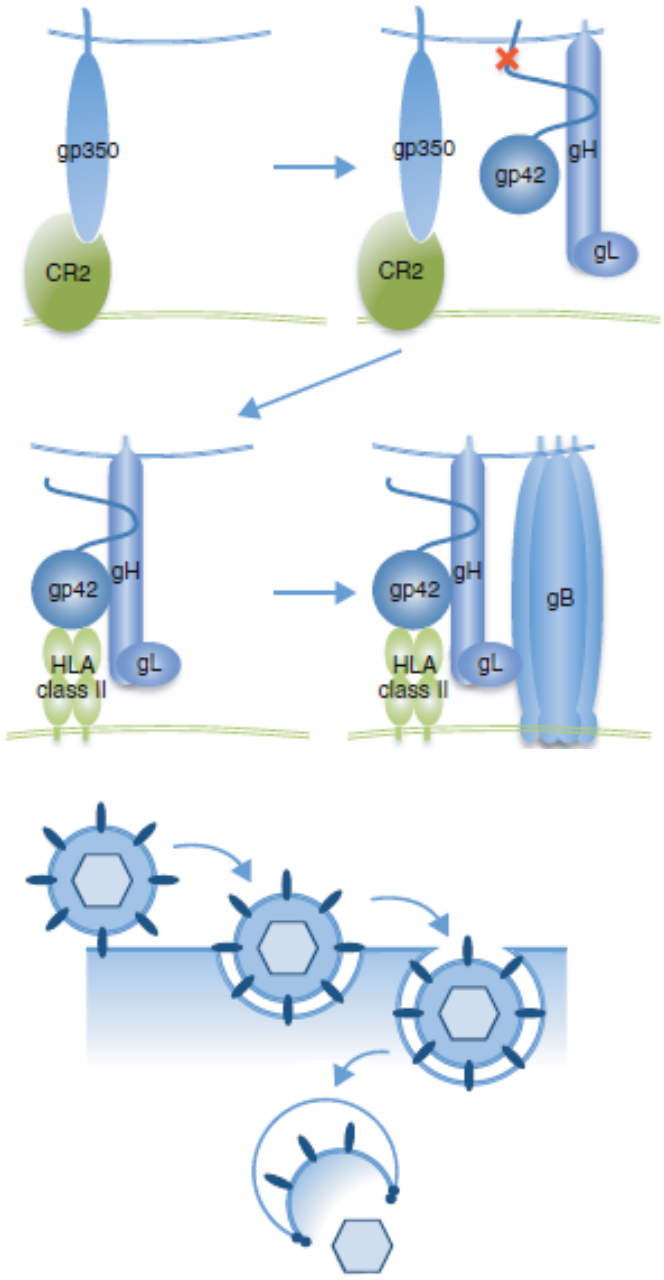
Entry into epithelial cells





EBV entry into epithelial cells appears to involve the action of three glycoproteins and their cellular binding partners. The gH/gL and gB glycoproteins bind to the ephrin type-A receptor 2 (EPHA2). This promotes insertion of the gB binding loops into the plasma membrane. EBV directly fuses with the plasma membrane of the epithelial cell to release the capsid into the cytoplasm.

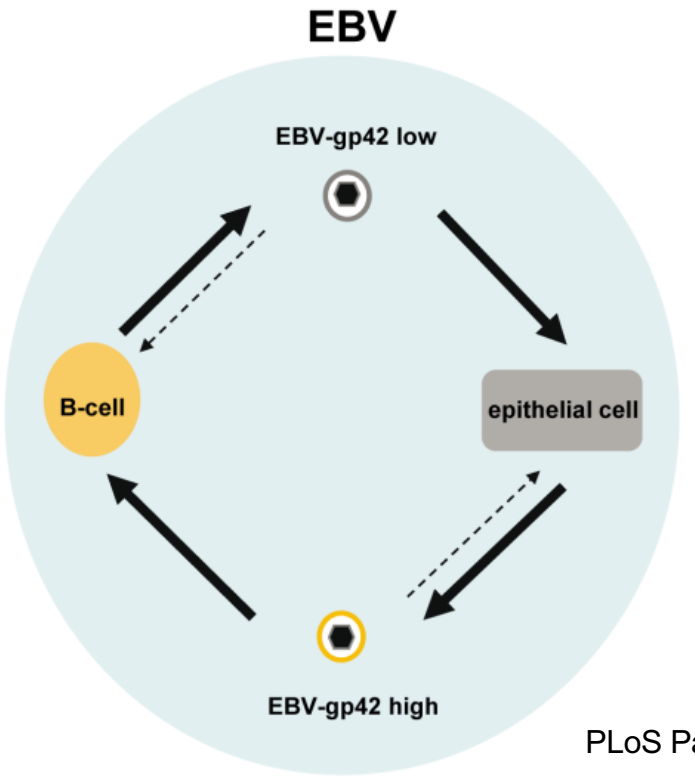
Modified from Current Opinion in Virology 2014, 4:78–84



Entry into B cells

EBV entry into B cells involves the concerted action of at least 5 viral glycoproteins and their cellular binding partners. EBV initially binds to the B cell surface via the viral gp350 interaction with CD21 (or CR2). Gp42 is cleaved near the N-terminal and binds to gH/gL to form the gp42/gH/gL complex. The gp42 then binds to HLA class II on the B cell surface, triggering a conformational change, possibly allowing gB interaction. This then triggers the insertion of the gB fusion loops into the B cell membrane resulting in fusion between the viral envelope and the B cell endocytic membrane.

Current Opinion in Virology 2014, 4:78–84



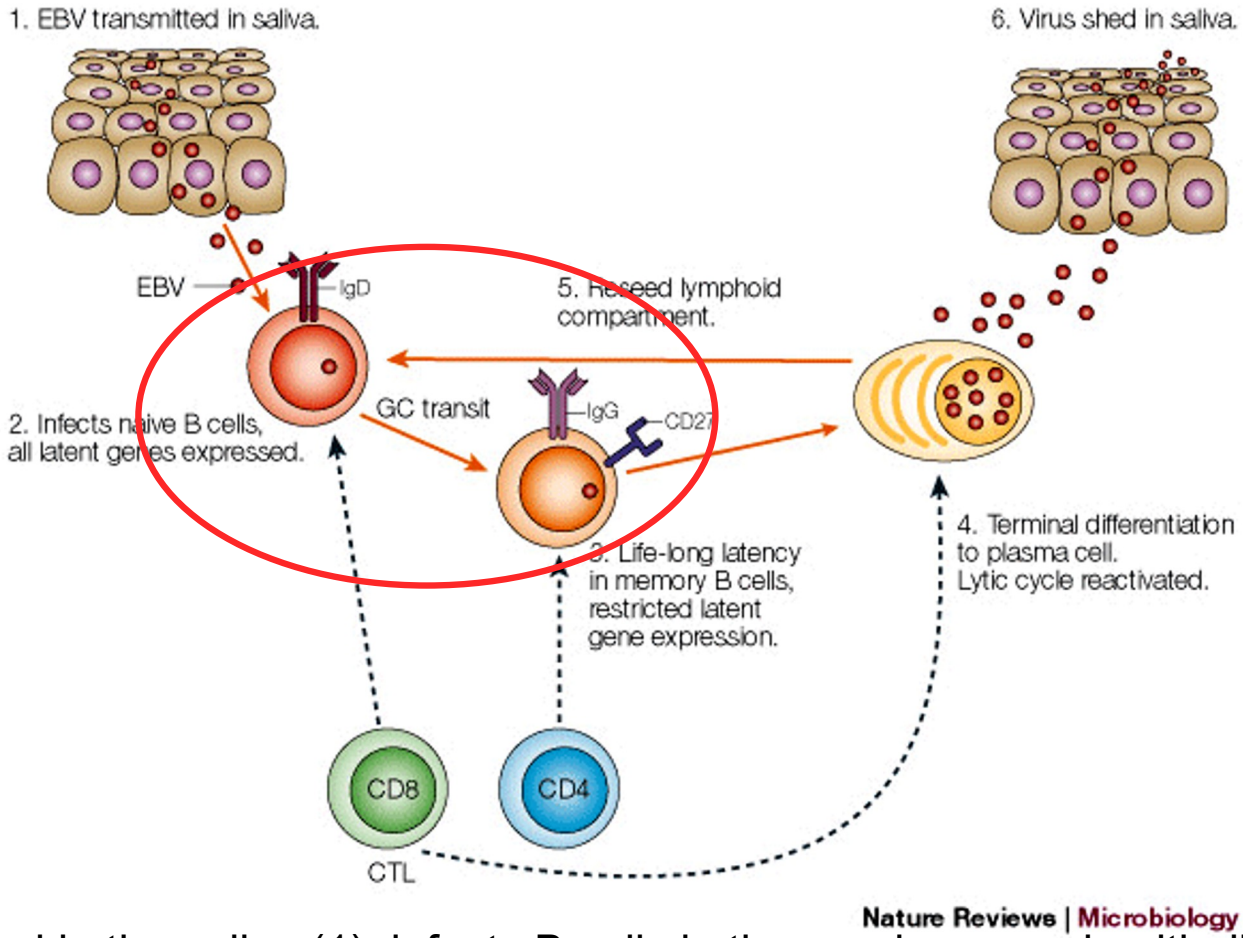
switch of cell tropism

EBV INFECTION
Viral switching of cellular tropism

PLoS Pathogens January 2011 | Volume 7 | Issue 1

By changing the composition of the glycoproteins in the virion, EBV is able to change its cellular tropism, and it does so by alternating the cell type in which it is replicated. In a B cell, the gp42 interacts with immature MHC class II in the endoplasmic reticulum, where it is targeted to the HLA class II trafficking pathway and then at least a proportion is redirected for degradation. Thus, virions produced by B cells have a lower concentration of the gp42/gH/gL complex and a comparatively higher concentration of the gH/gL complex. This potentially makes them more epithelial cell tropic. However, in an HLA class II-negative epithelial cell where gp42 is not redirected for degradation, the virions produced have a higher concentration of the gp42/ gH/gL complex and a lower relative gH/gL concentration, making them more B cell tropic.

EBV INFECTION



EBV is transmitted in the saliva (1), infects B cells in the oropharyngeal epithelium and enters the lymphoid tissue. Following infection of naive B cells in the lymph node (2), all latent genes are expressed and the viral latent proteins drive the B cell through subsequent proliferation and the germinal centre (GC) reaction. The transit through the germinal centre results in establishment of a life-long infection of memory B cells (3), which are detected in the peripheral circulation. Terminal differentiation to plasmacells results in reactivation of the virus to the lytic cycle, expression of lytic proteins and production of infectious virus (4). The virus can infect B cells within the lymphoid tissue or be shed into the saliva (5,6).

Latent Cycle

Viral genome: **episome, oriP is used for replication by the host DNA polymerase**

Expression of a limited number of viral genes

Six nuclear antigens (EBNA1, EBNA2, EBNA3A,-3B,-3C, EBNA-LP).
Three membrane antigens (LMP1, LMP2A, LMP2B)
Two small non-polyadenylated RNA (EBERS)

EBV can establish four distinct forms of latency:

- Latency 0: no protein expressed
- Latency I: EBNA1, EBERs
- Latency II: EBNA1, LMPs, EBERs
- LatencyIII: EBNAs, LMPs, EBERs

Latent cycle proteins (Epstein-Barr Nuclear Antigens)

- EBNA1: required for tethering of the viral genome to ensure replication and segregation of the viral DNA
- EBNA 2: transcription factor necessary for activating the promoters (Cp, LMP1p and LMP2p) involved in driving gene expression in the growth program. It also promote c-Myc activation.
- EBNA 3s: involved in transition from growth to default transcription program. Negative regulators of EBNA2. Facilitate epigenetic down regulation of Cp, Wp and bim. It acts also as repressor of cellular genes.
- EBNA-LP: cooperates in EBNA2-mediated transactivation.

Latent cycle proteins (Latent Membrane Proteins)

- LMP1: **Viral oncogene**

  acts as a constitutively active receptor CD40,

  induces DNA synthesis in quiescent primary B-lymphocytes, prevents apoptosis (bcl-2, mcl-1, A20), induces changes of the expression of surface antigens, controls the production of cytokines

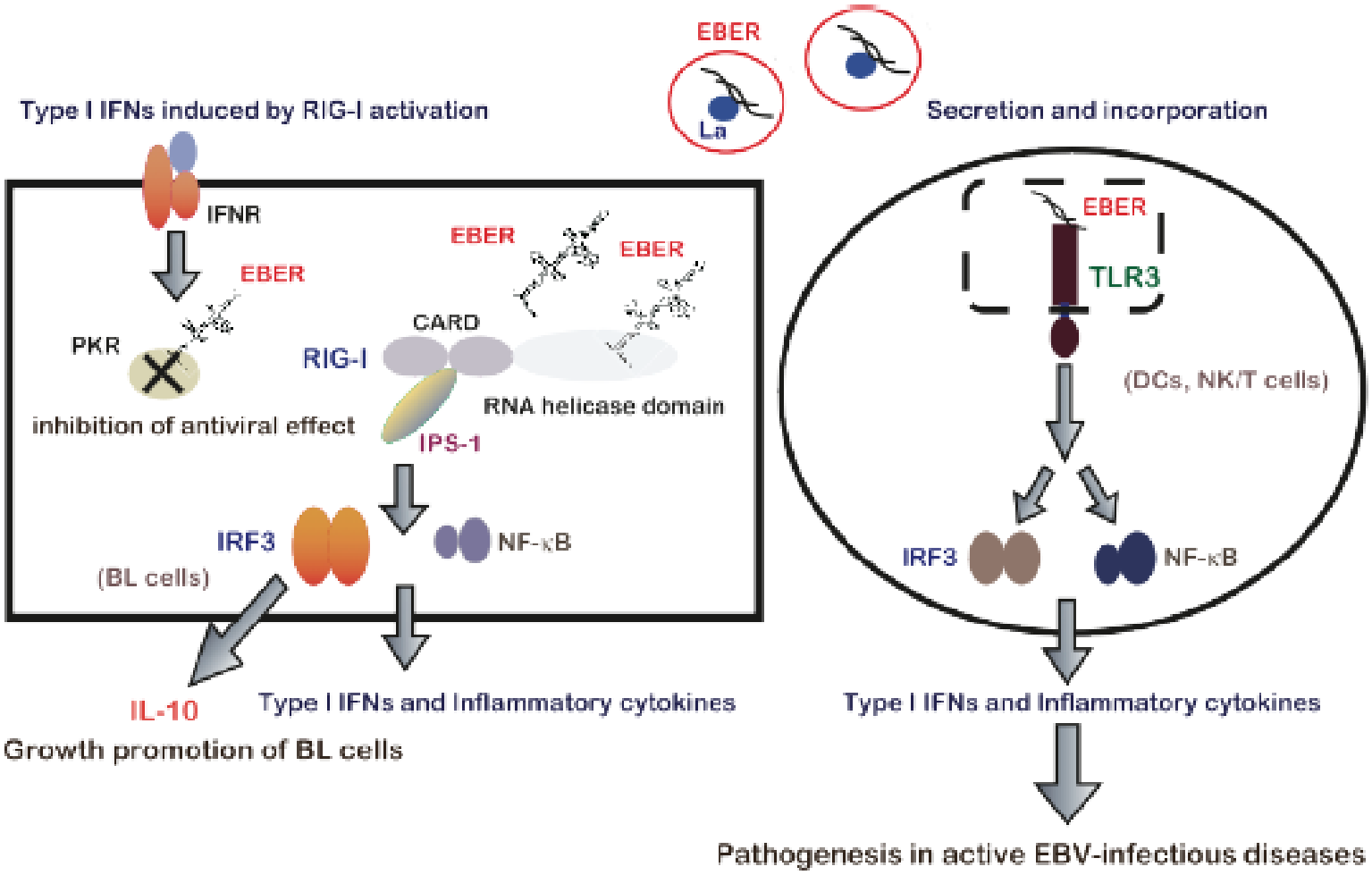
- LMP2a: **Transforming activity**, acts as constitutively active BCR

EBERs

- They are small non-polyadenylated RNA encoded by the virus and present in all forms of latency

- They confer resistance to apoptosis induced by IFNα by inhibiting the activation of PKR

EBERs



La: lupus antigen

Cancers **2014**, *6*(3), 1615-1630; doi:10.3390/cancers6031615

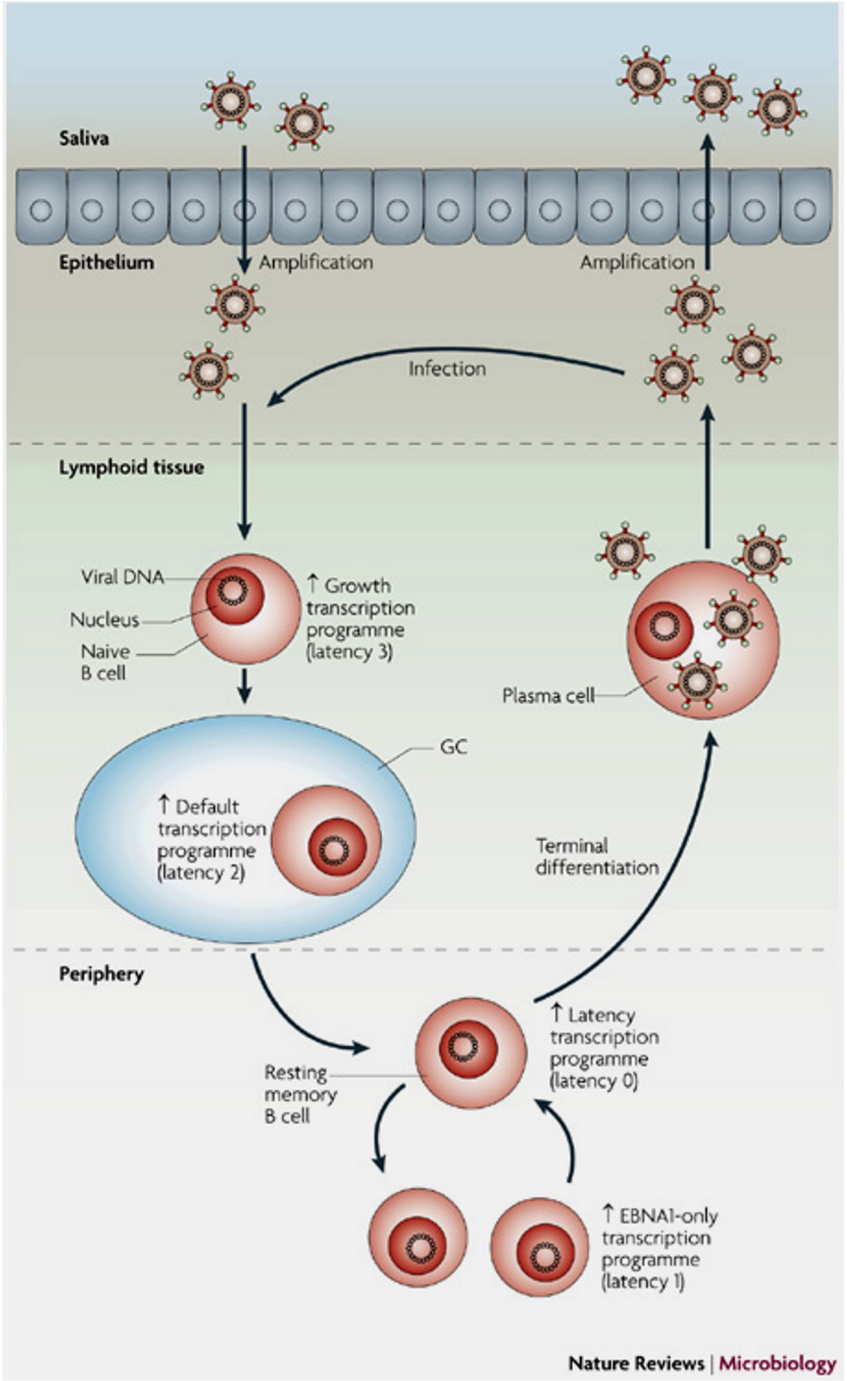
EBER-mediated modulation of innate immune signaling contributes to EBV-mediated pathogenesis. Left, in BL cells, EBERs are recognized by RIG-I via the RNA helicase domain of RIG-I and following recognition, RIG-I associates with the adaptor IPS-1 via its CARD. IPS-1 initiates signaling leading to the activation of IRF3 and NF-κB to induce type I IFNs and inflammatory cytokine expression. EBERs induce the expression of the growth-promoting cytokine IL-10 via RIG-I-mediated IRF3 activation and might support BL development. EBERs also bind to IFN-inducible PKR and block its activity, which is required for the IFN-mediated antiviral effect; therefore, EBV might maintain a latent infection state. Right, activation of innate immunity via TLR3 signaling in response to secreted EBER. During an active EBV-infection, EBER1 is released from EBV-infected lymphocytes primarily in a complex with La. Circulating EBER induces DC maturation via TLR3 signaling and induces type I IFN and inflammatory cytokine production by activating IRF3 and NF-κB. DC activation leads to T cell activation and systemic cytokine release. Furthermore, TLR3-expressing T and NK cells including EBV-infected T or NK cells could be activated by EBER1 through TLR3, thus leading to inflammatory cytokine production. Therefore, immunopathologic diseases caused by active EBV infections including T or NK cell activation and hypercytokinemia, could be attributed to EBER1-induced TLR3-mediated T cell activation and cytokinemia. EBER, Epstein-Barr virus encoded RNA; EBV, Epstein-Barr virus; BL, Burkitt's lymphoma; IPS-1, interferon-β promoter stimulator-1; CARD, caspase recruitment domain; RIG-I, retinoic acid-inducible gene I; DC, dendritic cell; IFN, interferon; NK cell, natural killer cell; IL, interleukin; TLR, Toll-like receptor; PKR, RNA-dependent protein kinase; IRF 3, interferon regulatory factor 3.

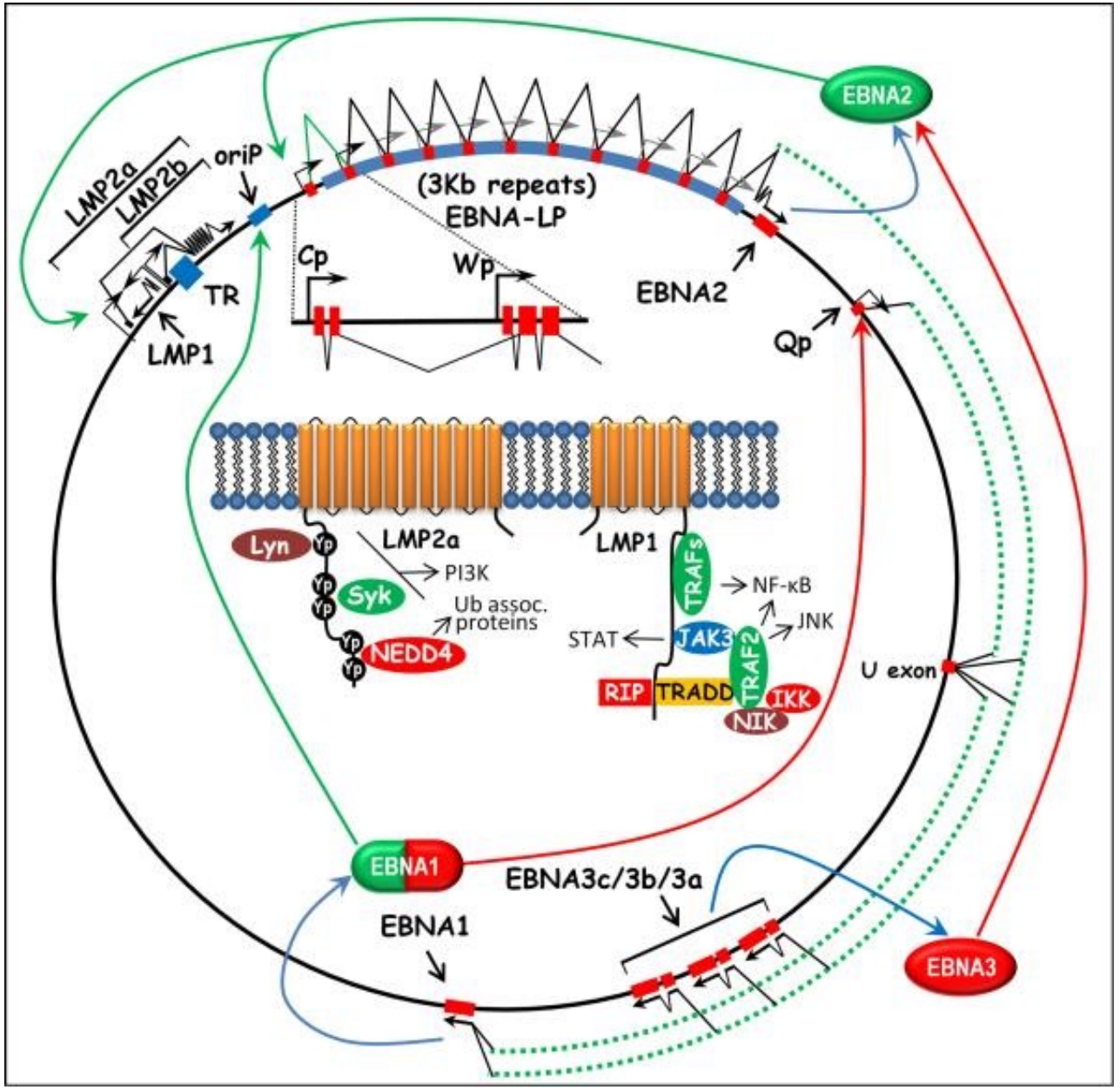
The current model of how EBV establishes and maintains latent infection.

Genome circularizes and Wp promoter is activated, EBNA2 expressed, Cp promoter activated, full growth programm Type 3 latency

Three of the growth-programme proteins (EBNA3A (EBV nuclear antigen 3A), EBNA3B and EBNA3C) negatively autoregulate the growth programme. This allows the cell to migrate into the follicle, initiate a germinal centre (GC) reaction and establish the default transcription programme

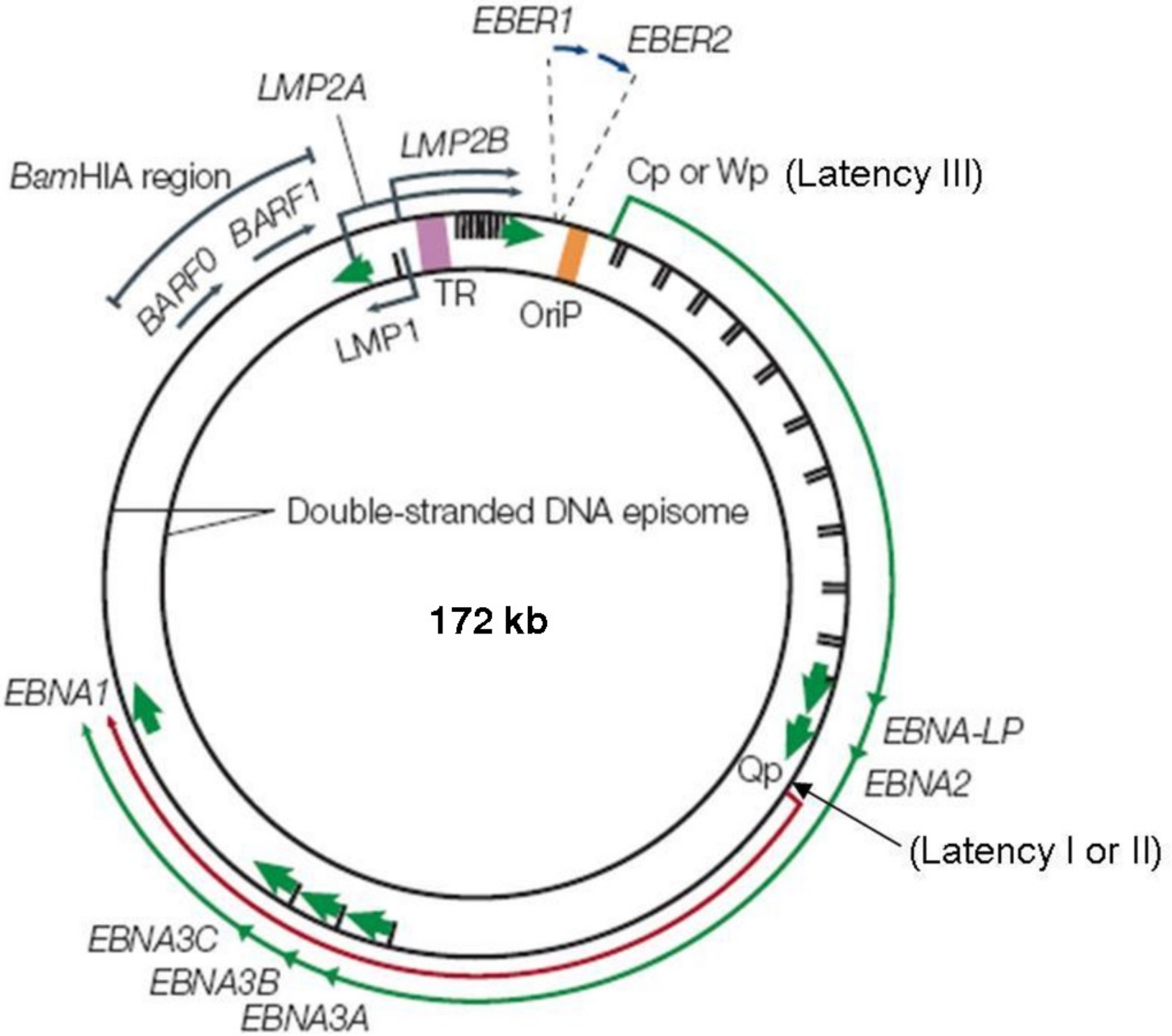
The default programme provides rescue or survival signals that allow the cell to exit the GC as a resting memory B cell. When they occasionally divide they express the EBNA1-only programme.



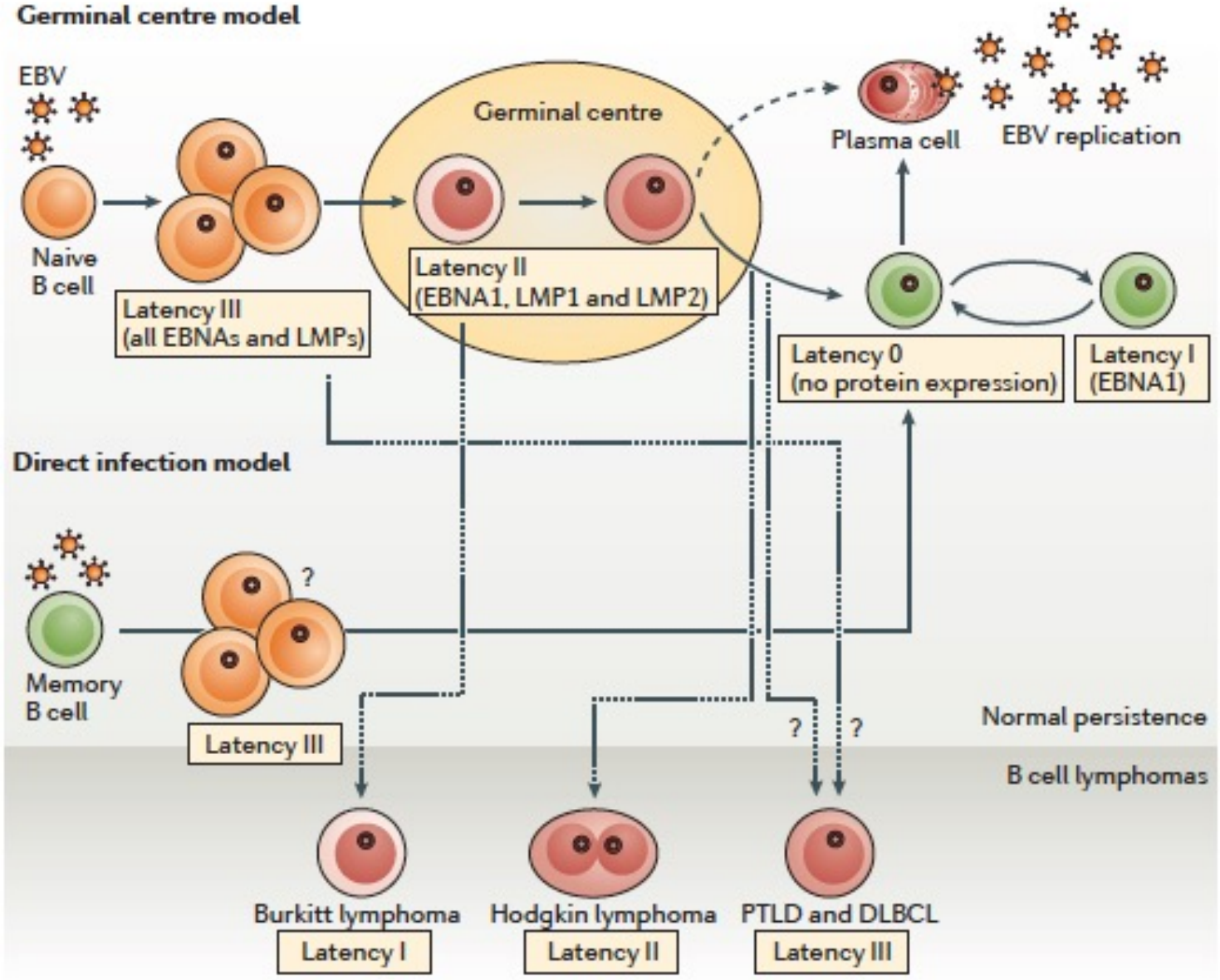


Speck and Ganem, 2010

Schematic illustration of EBV EBNA and LMP gene transcription, and auto-regulation of viral latency-associated gene expression by the EBNA gene products. An exploded view of Cp- and Wp –driven EBNA gene transcription is shown, depicting the organization of exons immediately downstream of each promoter. Cp-initiated transcript contain 2 unique exons, C1 and C2, which splice to a variable number of W1 and W2 exons encoded within the 3.0Kb internal repeats. Wp-initiated transcript contain a single unique exon, W0, which splices to a variable number of W1 and W2 repeat exons. See text for additional details regarding EBV gene expression during different stages of infection. Also shown in the inset is the predicted membrane topology of the LMP-1 and LMP-2a proteins, along with known cellular interacting partners and signaling pathways activated by these proteins.

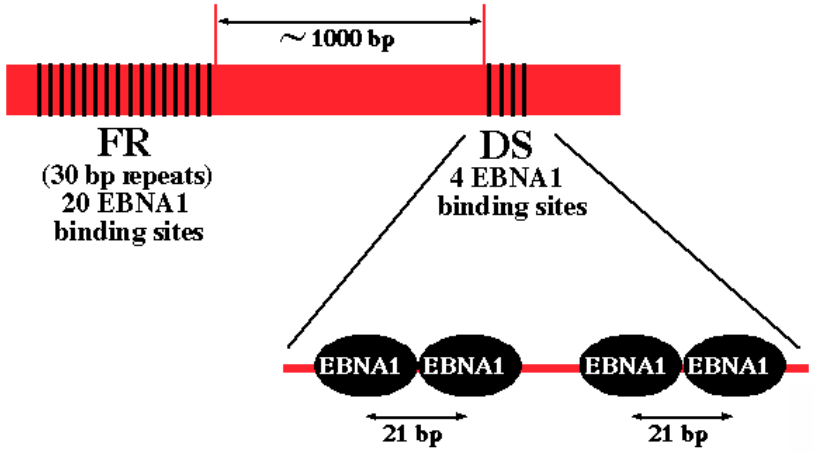

EBER1
EBER2
LMP2A
LMP2B
BamHIA region
BARF1
BARF0
Cp or Wp (Latency III)
TR
OriP
LMP1
Double-stranded DNA episome
172 kb
EBNA1
EBNA-LP
EBNA2
Qp
(Latency I or II)
EBNA3C
EBNA3B
EBNA3A

The Epstein-Barr virus genome. Diagram showing the position and transcription of the latent EBV genes on the double-stranded viral DNA episome. The latent origin of replication (oriP) is depicted in orange. The large green solid arrow heads represent exons encoding the six nuclear antigens (EBNAs 1, 2, 3A, 3B and 3C, and EBNA-LP) and the three latent membrane proteins (LMPs 1, 2A and 2B), the direction of gene transcription is also indicated by the arrow. Transcription of the gene encoding EBNA-LP occurs from an inconsistent number of repetitive exons. LMP2A and LMP2B genes are composed of multiple exons, which are positioned on either side of the terminal repeat (TR) region. The small blue arrows at the top represent the genes encoding the two non-polyadenylated RNAs EBER1 and EBER2, which are consistently expressed during all EBV latencies The long, thin outer green arrow represents EBV transcription during latency III, in which all six EBNAs are transcribed from either the Cp or Wp promoter. Differential splicing of the same long primary RNA transcript generates the individual mRNAs for the different EBNAs. The inner, shorter red arrow represents the Qp promoter-derived EBNA1 transcript during Latencies I and II. The BamHIA region at the top encodes BARF0 and BARF1 and many microRNAs. (Modified from Yang, LS & Rickinson, AB, Nat Rev Cancer, 2004)


Germinal centre model
EBV
Naive B cell
Latency III (all EBNAs and LMPs)
Germinal centre
Latency II (EBNA1, LMP1 and LMP2)
Plasma cell
EBV replication
Latency 0 (no protein expression)
Latency I (EBNA1)
Direct infection model
Memory B cell
?
Latency III
? ?
Normal persistence
B cell lymphomas
Burkitt lymphoma
Latency I
Hodgkin lymphoma
Latency II
PTLD and DLBCL
Latency III

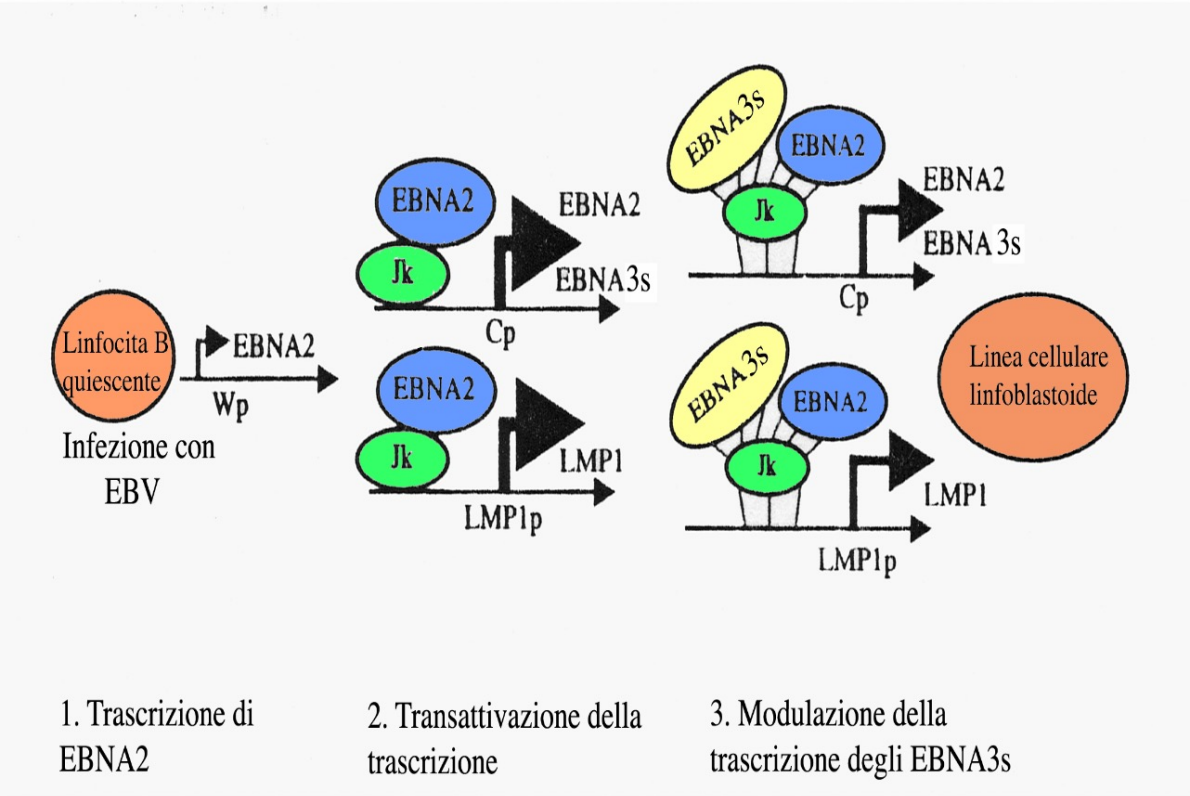
Virus persistence in the B cells of the human host and the origin of EBV-associated B cell lymphomas. **Upper panel: normal persistence**. Epstein–Barr virus (EBV) resides in memory B cells of asymptomatic hosts. There are two models to explain how the virus enters memory B cells. In the germinal centre model, EBV infection of naive B cells leads to a latency III growth programme, in which the proliferation and expansion of the infected B cell pool is driven by expression of all EBV latent genes. Cells then enter the germinal centre and express latency II (default programme), characterized by expression of Epstein–Barr nuclear antigen 1 (EBNA1), latent membrane protein 1 (LMP1) and LMP2. LMP1 provides a CD40-like signal and LMP2 a surrogate B cell receptor-like signal — mimicking the same signals as those provided to the antigen-specific B cell in the normal germinal centre. EBV-infected germinal centre B cells leave and enter the memory B cell pool. Here, EBV protein expression is silenced (latency 0); these cells are replenished by the same signals that induce the proliferation of normal memory B cells. Proliferating EBV-infected memory B cells require EBNA1 expression (latency I) for viral episome segregation. Memory B cells can terminally differentiate into plasma cells (solid arrow), which triggers virus replication. EBV infected germinal centre B cells might also differentiate directly into plasma cells (dashed arrow). **In the direct infection model (shown below)**, EBV accesses memory B cells following the direct infection of these cells, which may involve a latency III intermediary. Lower panel: the origin of the EBV-associated B cell lymphomas. There is uncertainty about the exact stages of differentiation from which the EBV-positive B cell lymphomas arise (as indicated by the black dotted lines), as it cannot be assumed that the pattern of latency observed in the progenitor cell is recapitulated in the corresponding tumour. The figure illustrates the presumed (question marks indicate this uncertainty) cell of origin based on current evidence for post-transplant lymphoproliferative disease (PTLD), diffuse large B cell lymphoma (DLBCL), Hodgkin lymphoma and Burkitt lymphoma.

OriP

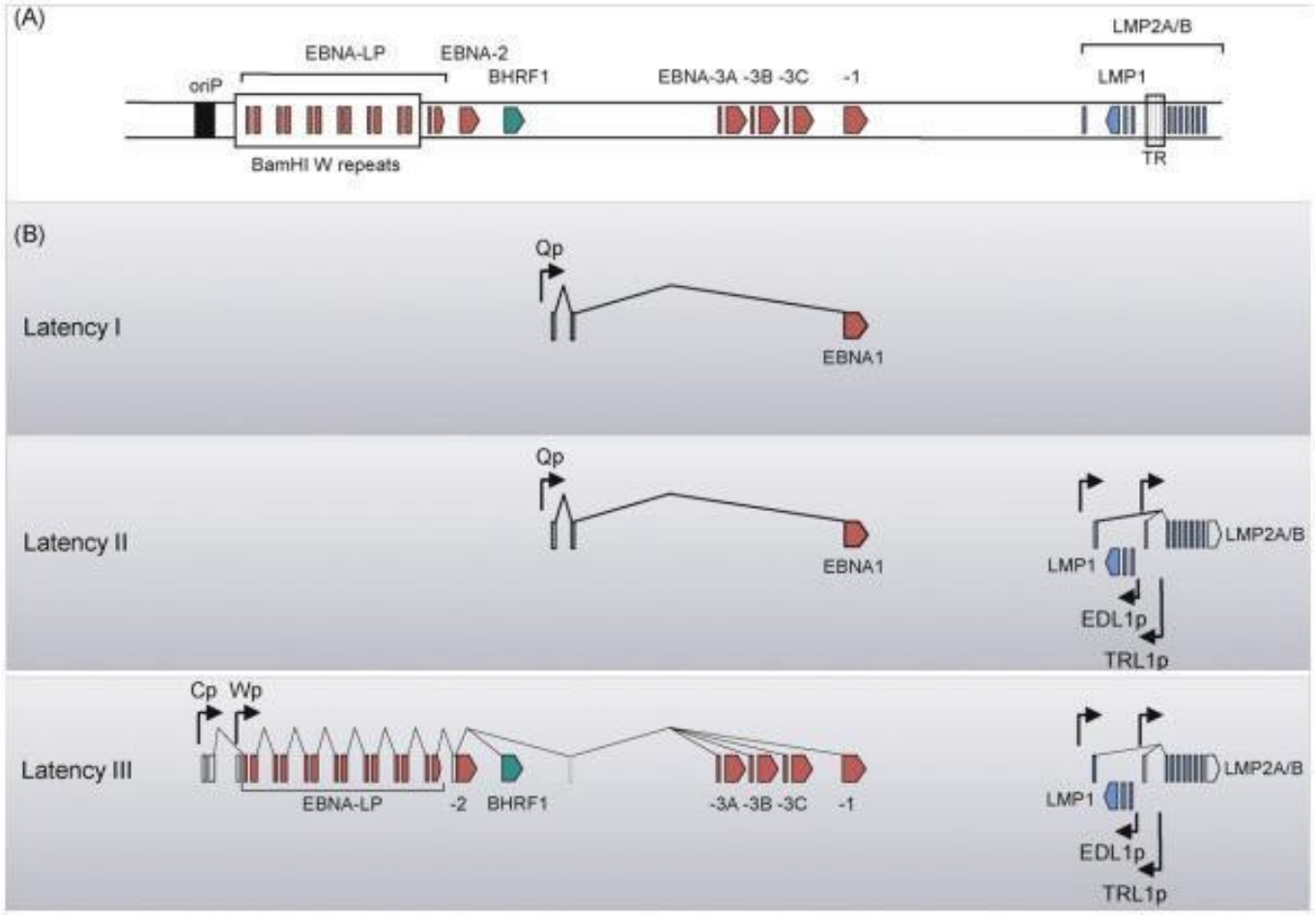


EBNA 1

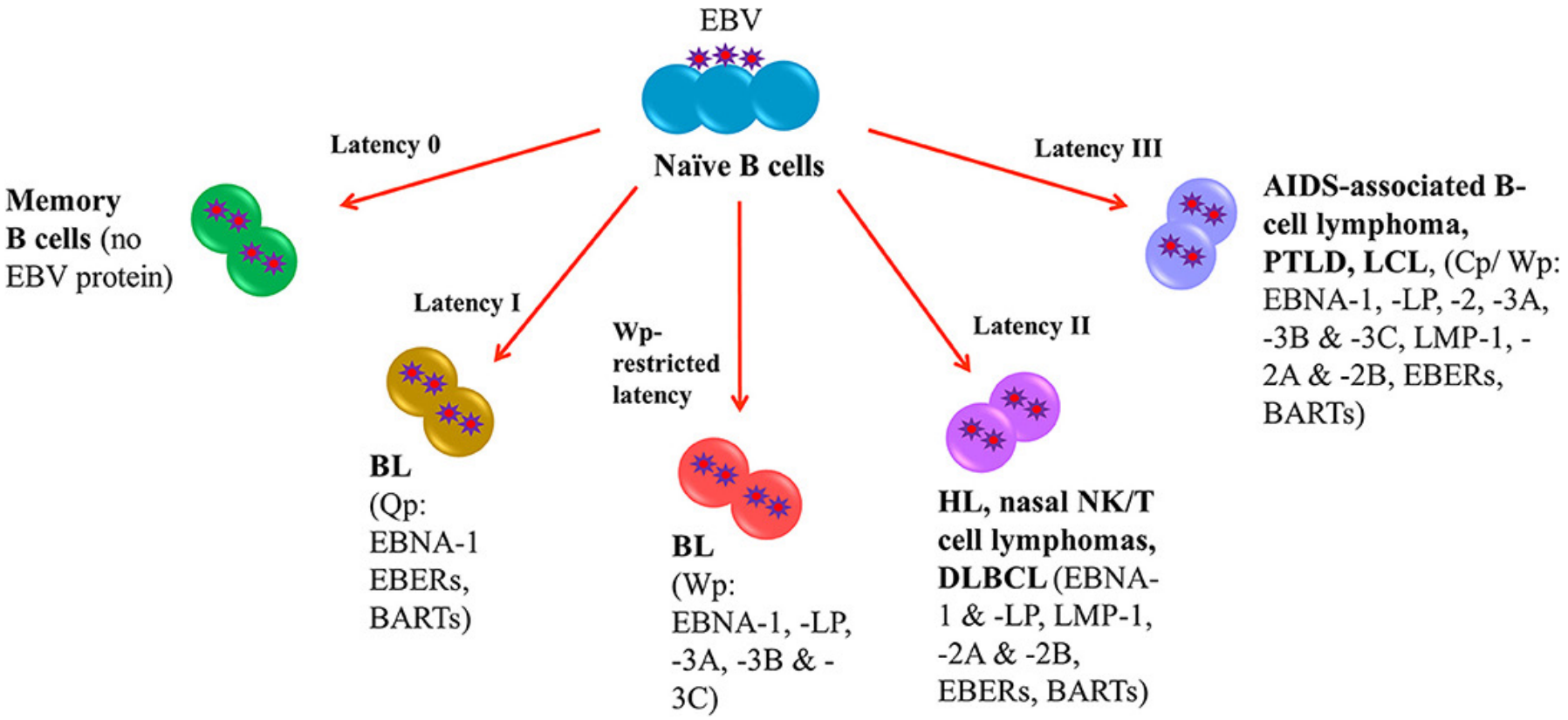
EBNA 2, 3s

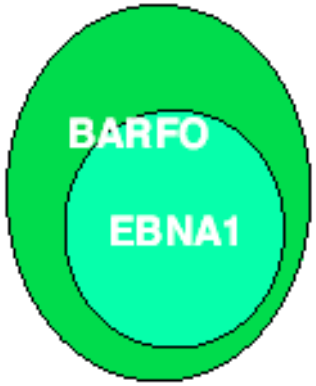
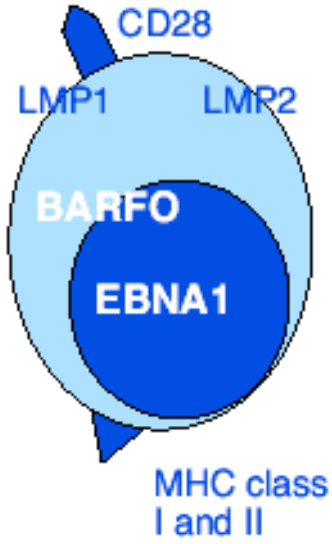
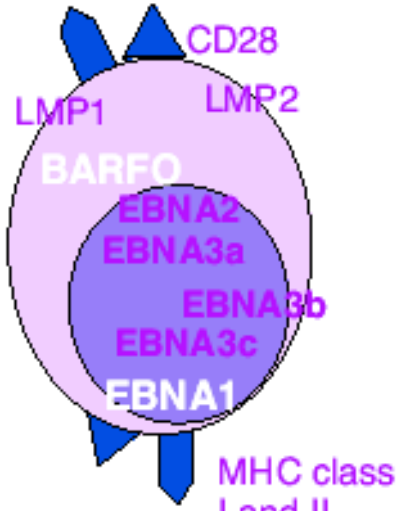


Latency programs



EBV latency in EBV-LPDs.



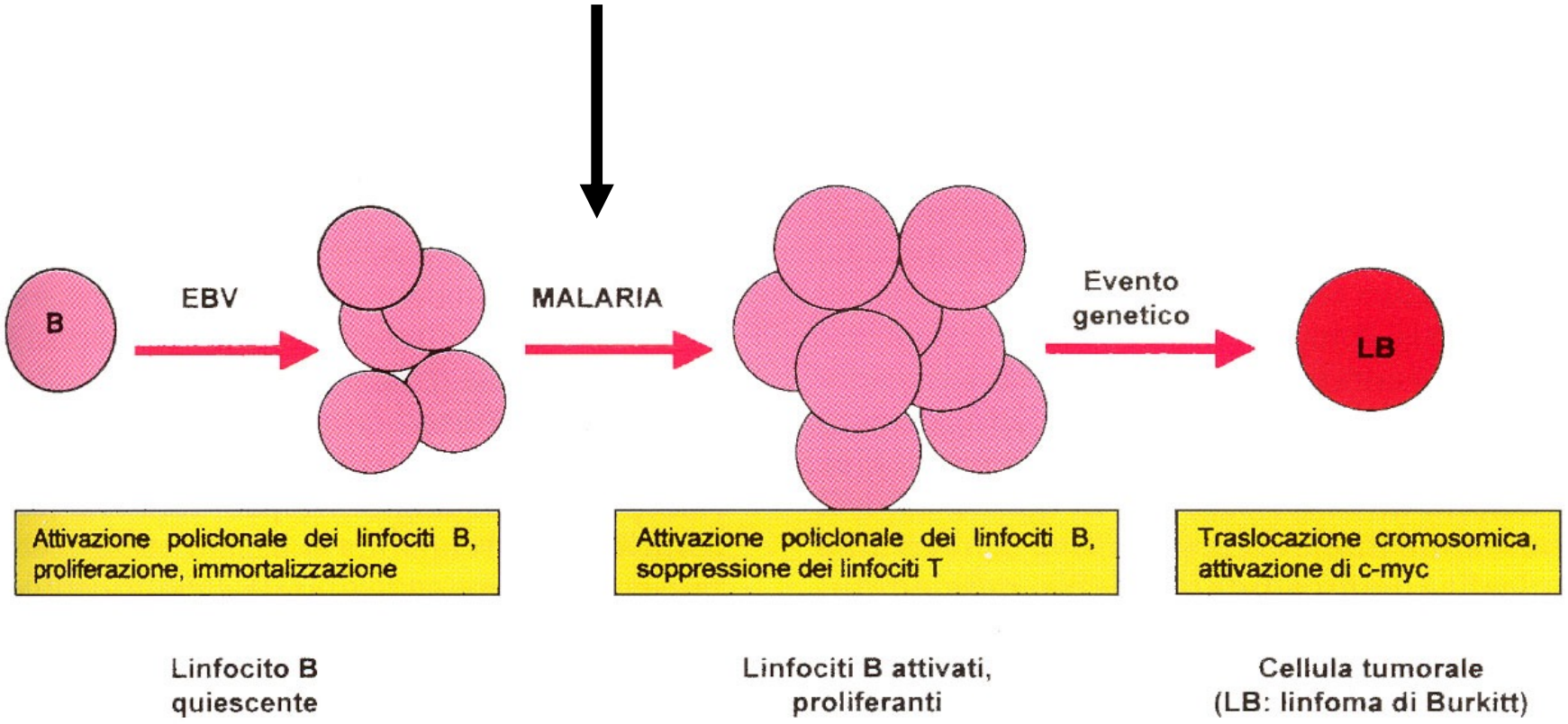
Type I latency	Type II latency	Type III latency
		
Burkitt's lymphoma	Hodgkin's lymphoma Nasopharyngeal carcimona	Lymphoproliferative disease in immuno- compromised, LCL

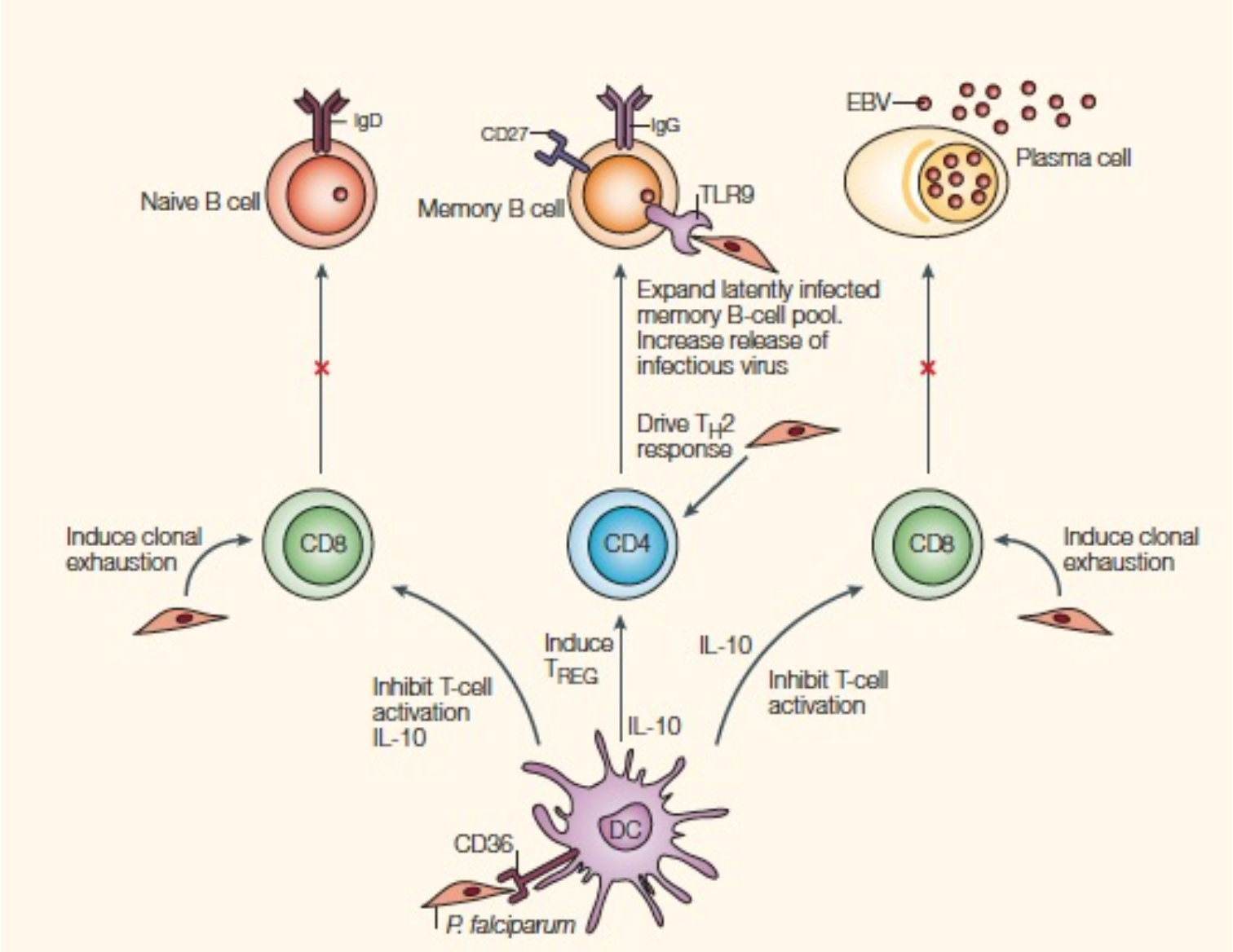
Burkitt Lymphoma was first described in children in equatorial Africa by Denis Burkitt

Table 1 Characteristic features of the different Burkitt lymphoma subtypes

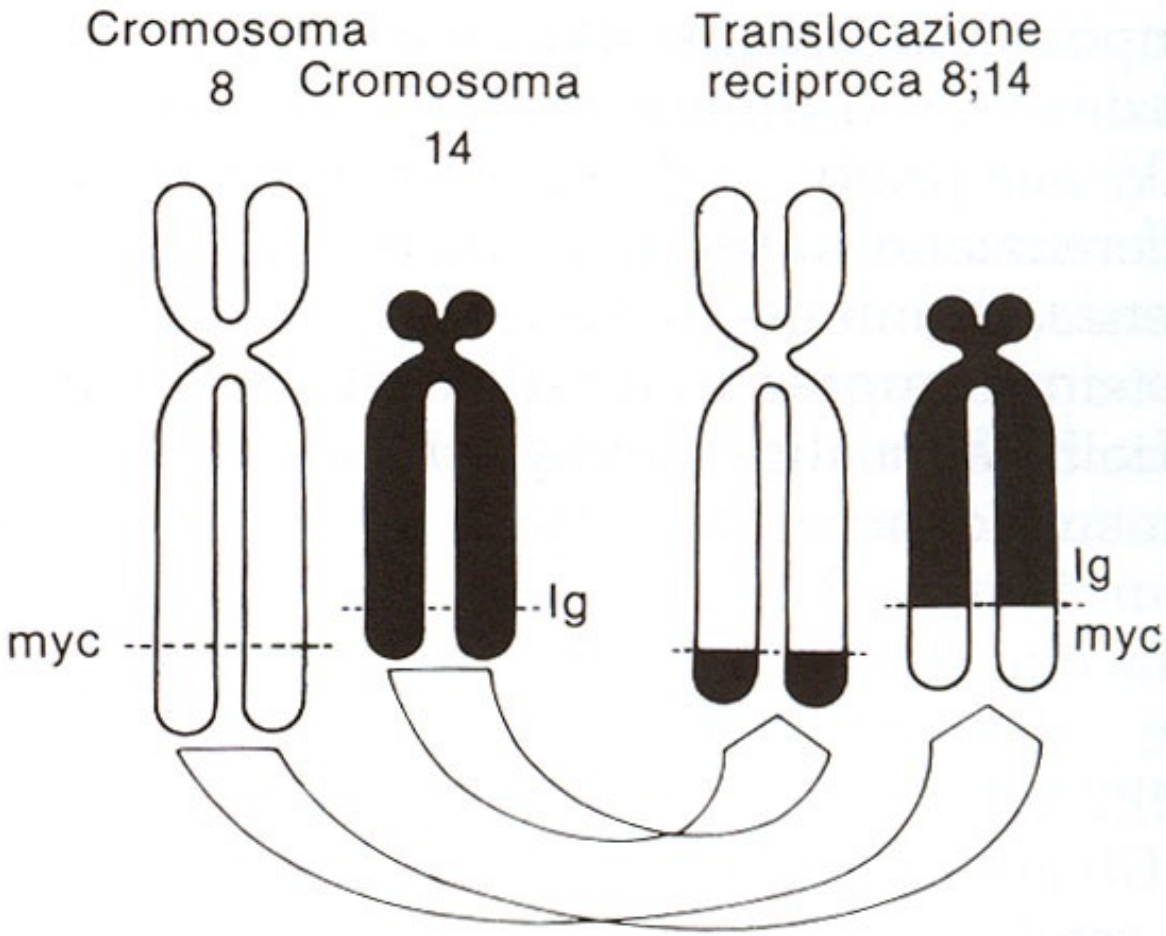
	Endemic	Sporadic	HIV-associated
Distribution	Equatorial belt of Africa and Papua New Guinea	Worldwide	Worldwide
EBV association	98%	5–10%	30–40%
Co-factors	EBV, malaria infection	–	HIV infection
Incidence	5–10/100 000	0.01/100 000	Variable
MYC breakpoint	Often >1 kb upstream from 1st coding exon	Exon 1/intron 1 of *MYC* gene	Exon 1/intron 1 of *MYC* gene
Ig breakpoint	Joining (J) region, switch (S)μ in some cases	Sμ, Sα or J region	Sμ region
Progenitor cell	GC, late GC or memory B cell	GC B cell	GC, late GC or memory B cell
Frequent site of occurrence	Most frequently jaw. Abdomen, kidneys and ovaries may also be involved	Most frequently abdomen. Kidneys, bone marrow and ovaries may also be involved	Lymph nodes, abdomen, bone marrow, CNS

2° evento


B
EBV
MALARIA
Evento genetico
LB
Attivazione policlonale dei linfociti B, proliferazione, immortalizzazione
Attivazione policlonale dei linfociti B, soppressione dei linfociti T
Traslocazione cromosomica, attivazione di c-myc
Linfocito B quiescente
Linfociti B attivati, proliferanti
Cellula tumorale (LB: linfoma di Burkitt)


IgD
Naive B cell
CD27
IgG
Memory B cell
TLR9
EBV
Plasma cell
Expand latently infected memory B-cell pool. Increase release of infectious virus
Drive T_H2 response
Induce clonal exhaustion
CD8
CD4
CD8
Induce clonal exhaustion
Induce T_{REG}
IL-10
IL-10
Inhibit T-cell activation IL-10
Inhibit T-cell activation
DC
CD36
P. falciparum

Plasmodium falciparum interaction with EBV persistence and immunity. Shown are the potential interactions of P. falciparum with the EBV life cycle. P. falciparum could directly interact with EBV-latently infected memory B cells that express Toll-like receptor 9 (TLR9), leading to the expansion of the latently infected memory B-cell pool, and/or drive the differentiation of memory B cells to plasma cells, resulting in release of infectious virus. Engagement of CD36 on dendritic cells (DCs) by P. falciparum erythrocyte membrane protein 1 (PfEMP1) could induce interleukin (IL)-10, resulting in inhibition of EBV-specific CD8+ CTL responses and/or induction of regulatory CD4+ T cells (TREG). The immune response to P. falciparum could result in a dominant T helper cell 2 (TH2) response that would be less effective against emergent BL clones originating from EBV-infected memory B cells. Repeated P. falciparum infections throughout childhood could result in loss of EBV-specific CD8+ CTL responses by the process of clonal exhaustion. Ig, immunoglobulin.



Translocazione 8;14	90% dei casi
Translocazione 2; 8	5% dei casi
Translocazione 8;22	5% dei casi