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Epstein–Barr Virus and Cancer

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Abstract

Epstein–Barr virus (EBV) contributes to about 1.5% of all cases of human cancer worldwide, and viral genes are expressed in the malignant cells. EBV also very efficiently causes the proliferation of infected human B lymphocytes. The functions of the viral proteins and small RNAs that may contribute to EBV-associated cancers are becoming increasingly clear, and a broader understanding of the sequence variation of the virus genome has helped to interpret their roles. The improved understanding of the mechanisms of these cancers means that there are great opportunities for the early diagnosis of treatable stages of EBV-associated cancers and the use of immunotherapy to target EBV-infected cells or overcome immune evasion. There is also scope for preventing disease by immunization and for developing therapeutic agents that target the EBV gene products expressed in the cancers.

BACKGROUND

Epstein–Barr virus (EBV) was discovered in 1964 in cells from a human cancer (1), an African (or endemic) Burkitt's lymphoma (BL), so there was immediate speculation that the virus might be involved in cancer development. Subsequently, it was realized that EBV infection is endemic across the whole world, and it has taken many years to understand more clearly the relationship between EBV and human cancers.

EBV is one of the eight known human herpesviruses (2, 3). Like all herpesviruses, EBV has a relatively large double-stranded DNA genome. The EBV genome is approximately 172 kb in length, and it expresses about 80 proteins and 46 functional small untranslated RNAs. Some of the proteins are involved in replicating the viral genome and producing new viral particles in the viral lytic (productive) cycle. Herpesviruses characteristically also have a second mode of gene expression that allows them to persist long term in the infected host without producing viral particles, and different viral genes are expressed during this latent cycle.

The normal life cycle of EBV involves the infection of B lymphocytes and some epithelial cells (2, 3). The virus is transmitted in saliva, and primary infection usually occurs in the first years of life, with no apparent illness. Most the world's population is thought to become infected by EBV, but the level of virus shed in the saliva varies greatly in different individuals, perhaps by 100-fold (4, 5), and different ethnic groups in developed countries may have quite different infection rates (6). If primary infection is delayed until later in life, infectious mononucleosis (IM; also known as glandular fever) may occur. The symptoms of IM are caused by an excessive immune response to EBV infection. It is not known why some people develop IM upon primary infection, but certain human leukocyte antigen (HLA) types tend to favor IM, and it is possible that receiving a higher initial dose of virus also plays a part. There is also inherent variation in the human population in the innate immune response to EBV. The earliest immune responses to EBV infection in tonsils are probably mediated by natural killer (NK) cells, and there are changes in the NK cell population with age that could be significant for the development of IM (7). In a recent study, about half of the people tested responded with only NK cells, but the remaining people made an NK and $\gamma\delta$ T cell response (8). A genetic basis for the difference in making a $\gamma\delta$ T cell response is not known, but it illustrates the degree of individual variation we may expect in response to EBV infection.

The site of viral persistence is thought to be in B cells, and the viral load in normal carriers of the virus is very low; usually about 0.01% of B lymphocytes are infected (9). In EBV-associated cancers, essentially all of the malignant cells are infected, so the association of the cancer cells with EBV is strong.

Most types of cancer take many years to develop, and the fact that EBV infection persists long term gives the possibility of a sustained contribution to cancer development in some people. In fact, the EBV genes that are expressed during the various stages of latent infection have so many functions that could contribute to cancer and immune evasion, it can be difficult to pick out the functionally important ones; this review explores the mechanisms that are thought to be important in cancers associated with EBV.

EBV CONVERTS HUMAN B CELLS TO LYMPHOBLASTOID CELL LINES IN VITRO

B lymphocytes isolated from blood are mostly resting (nondividing) cells whose normal function is to respond to cognate antigen binding to their cell surface B cell receptor (BCR). Antigen binding to the BCR results in activation into a proliferating lymphoblast, which in vivo may migrate to a lymph node and participate in further development of the immune response. When B cells from peripheral blood are infected with EBV in vitro, the cells are activated to proliferate and grow

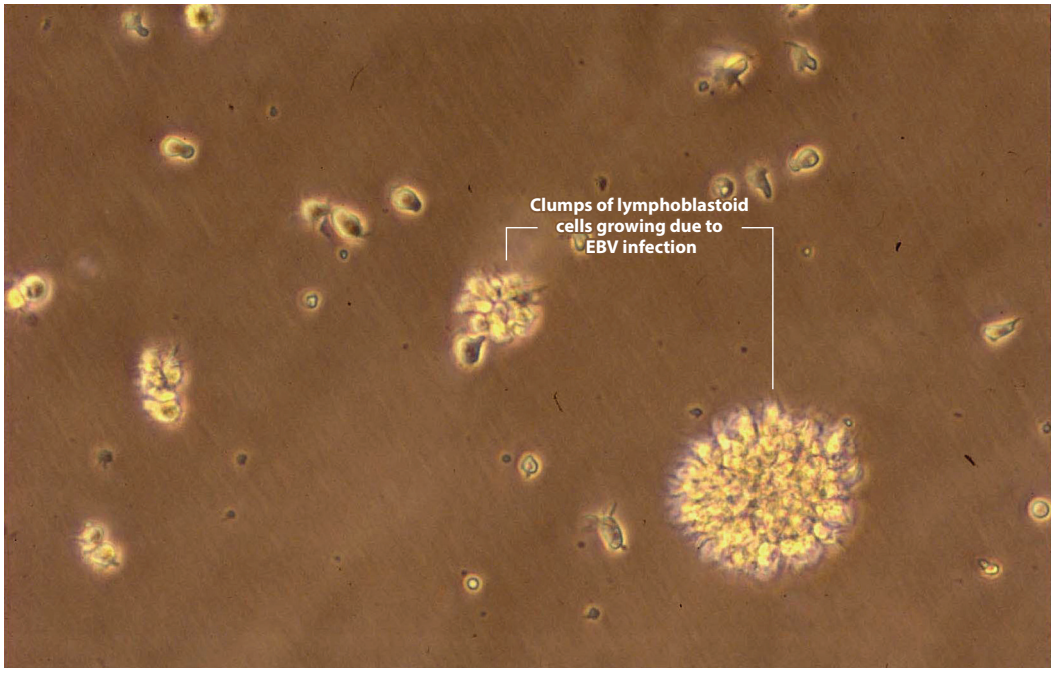

Figure 1

Permanently growing lymphoblastoid cell line made by infecting the author's B lymphocytes with B95-8 Epstein–Barr virus (EBV). The cells tend to grow in clumps because of adhesion molecules on their surface, similar to B cells activated by antigen.

out as lymphoblastoid cell lines (LCLs). This is an efficient process, and the LCLs are similar in appearance (**Figure 1**) and surface markers to B cells that have been activated by antigen, suggesting that normal activation mechanisms have been exploited by the virus (2, 3). The cells are latently infected (no virus particles are produced), and the viral genome is maintained as a circular episome in the cell nucleus, usually with about 10 copies per cell. As the cells grow, the viral genome is also replicated in S phase by the host cell DNA polymerase so that the viral copy number is maintained as the cells divide. Several viral proteins and functional RNAs are expressed, and some of these proteins are clearly responsible for the growth of the host cell (2, 3). The EBNA (Epstein–Barr nuclear antigen) proteins are all nuclear proteins and regulate gene expression and viral DNA replication. LMPs (latent membrane proteins) are in the cytoplasmic and plasma membranes, and they are mainly involved in signal transduction. The positions in the EBV genome of the genes encoding these proteins are shown schematically in **Figure 2**, and **Table 1** contains a summary of their functions (described in detail below). The latency patterns of EBV gene expression in different infections are summarized in **Table 2**; the pattern in LCLs with all of the EBNAs, LMPs, Epstein–Barr small encoded RNAs (EBERs) and micro RNAs (miRNAs) is known as latency III (10).

EBNA1

EBNA1 protein has to be expressed in all EBV-infected proliferating cells because its main function is to ensure the persistence of the viral genome in the cells as they multiply (11). EBNA1 binds with high affinity and specificity through its C terminus as a dimer to the plasmid origin of replication (oriP) region of EBV, which contains repeats of the EBNA1 DNA binding sequence. This binding

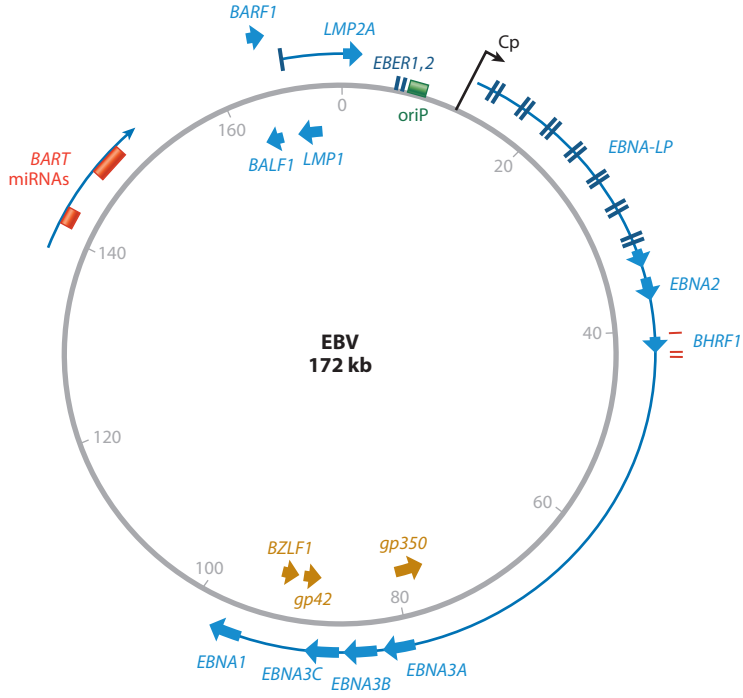

Figure 2

Simplified EBV genome map showing selected genes on the circular form of the viral genome. Precise details of all the EBV genes are in the annotation of the reference sequence (GenBank accession number NC_007605). The mRNAs for the *EBNA* and *BHRF1* genes are all spliced from the long primary transcript as indicated, starting at the Cp. Protein coding parts of mRNAs are shown schematically as filled blue arrows. Regions giving rise to miRNAs are colored red. Abbreviations: Cp, C promoter; EBER, Epstein–Barr small encoded RNA; EBNA, Epstein–Barr nuclear antigen; EBV, Epstein–Barr virus; gp, glycoprotein; LMP, latent membrane protein; LP, leader protein; miRNA, microRNA; oriP, origin of replication.

of EBNA1 and various cell cofactors attracts the cell DNA replication machinery to replicate the EBV genome during each cell cycle. N-terminal regions of the EBNA1 protein loosely attach the EBV genome to apparently random locations on cell chromosomes so that the EBV genomes will be distributed into the daughter cells at cell division and will avoid degradation by the cell as nonchromosomal, erroneously replicated DNA (11). Inhibitors of EBNA1 DNA binding can eliminate EBV genomes from EBV-positive BL cell lines (12) in cell culture, and a fluorescent probe capable of specifically binding to EBNA1 also caused growth inhibition of Raji and C666.1 cell lines, but not of EBV-negative cell lines (13).

In addition to its primary role for the virus in maintaining the viral episome, EBNA1 also influences cells in many other significant ways (11). EBNA1 induces transcription when it binds to the EBV oriP and certain DNA sites in the cell genome, activating the viral LMP1 and C promoter (Cp) of EBV and the expression of cell genes such as *NOX2* (14). EBNA1 also contributes to the altered regulation of telomeres on cell chromosomes in EBV-infected cells (15, 16). The Gly–Ala region of EBNA1 (composed entirely of repeated glycine and alanine residues) serves to make the protein resistant to degradation in the proteasome, and hence, it tends to avoid presentation as peptides to cytotoxic T lymphocytes (CTLs) (17). In addition, translation stress caused by this repetitive sequence can indirectly activate C-MYC expression via a PI3-kinase (PI3K) signaling

Table 1 Summary of Epstein–Barr virus genes expressed in latent infections

Gene	Action
Required for transformation of adult peripheral B cells to LCLs	
EBNA1	Binds to the plasmid origin of replication to allow viral genome replication by cell DNA polymerase
EBNA2	A transcription factor that induces cell and EBV genes to promote cell growth
EBNA3A and *EBNA3C*	Repress BIM and p16; prevent differentiation into plasma cell
LMP1	Constitutively induces NF-κB and is analogous to CD40; prevents apoptosis
BHLF1 or *BALF1*	BCL-2 family proteins that prevent apoptosis
BHRF1 miRNAs	Regulate PTEN, p27, and BHRF1
Also essential for transformation of germinal center B cells to LCLs	
LMP2A	Signals via BCR and PI3-kinase pathways; prevents apoptosis
Also essential for transformation of cord (naive) B cells to LCLs	
EBNA-LP	Cooperates with EBNA2 and overcomes cell innate response to viral genome
Not required for transformation of purified B cells	
EBER1 and *EBER2*	Functional RNAs that may affect innate responses and gene expression
BART miRNAs	Mediate evasion of T and NK cells during infection of B cells in peripheral blood lymphocytes
EBNA3B	Acts as a tumor suppressor gene in a mouse model of DLBCL

Abbreviations: DLBCL, diffuse large B cell lymphoma; LCL, lymphoblastoid cell line; LP, leader protein; miRNA, microRNA; NF-κB, nuclear factor κB.

pathway (18). Indeed, some lymphoma cells containing EBNA1 are sensitive to a specific PI3Kδ inhibitor via this mechanism. There is also evidence that the interaction of EBNA1 with the ubiquitin-specific protease USP7 can alter p53 or MDM2 levels in cells (19) and that EBNA1 provides antiapoptosis activity in BL cell lines (20), possibly via the induction of survivin (21). So the function of EBNA1 extends far beyond simply maintaining the viral genome in EBV-infected cells.

EBNA2 Transcription Factor

EBNA2 is a transcription factor that directly activates viral *LMP* genes and approximately 300 cell genes, including, for example, *MYC* and *RUNX3* (22, 23). Some of the genes activated by EBNA2 and their downstream target genes are required for the proliferation and survival of EBV LCLs. The important roles of many cell genes in LCLs have been defined by systematically knocking them out by targeted CRISPR inactivation (24). EBNA-LP (EBNA leader protein) cooperates with EBNA2 in some gene activation assays, but it may also overcome an innate cell response to viral DNA to allow transcription of the EBV genome upon infection (25).

Table 2 Patterns of latent cycle gene expression in Epstein–Barr virus

Latency pattern	Genes
Latency III[a]	Required for B cell transformation: *EBNA1*, *EBNA2*, *EBNA3A*, *EBNA3C*, *LP*, *LMP1*, *LMP2A*, and *BHRF1* miRNAs Not essential for B cell transformation: *EBNA3B*, *EBER1*, *EBER2*, and *BART* miRNAs
Latency II	*EBNA1*, *LMP1*, *LMP2A*, *EBER1*, *EBER2*, and *BART* miRNAs
Latency I	*EBNA1*, *EBER1*, *EBER2*, and *BART* miRNAs

Abbreviation: miRNA, microRNA.
[a]Latency III has all the latent cycle genes.

EBNA3 Protein Family

The EBNA3 family of proteins comprises EBNA3A, -3B, and -3C. Functionally, EBNA3A and -3C tend to cooperate, whereas the functions of EBNA3B tend to be different (26). EBNA3A and -3C contribute to the EBV transformation of B cells (EBNA3C is essential), but EBNA3B is not required for this. All three proteins act as regulators of gene expression, repressing some genes and activating others. Some key functions of EBNA3A and -3C are to repress the cyclin-dependent kinase inhibitor $p16^{INK4a}$ (27) and the apoptotic protein BIM (BCL2L11) (28). EBNA3A and -3C also have the effect of fixing the EBV LCL in the proliferative lymphoblast stage and preventing differentiation to a plasma cell phenotype (29). The mechanism of EBNA3 function involves cooperation with cell epigenetic control proteins and corepressors, such as Polycomb repressor proteins and possibly histone deacetylase complexes, including the SIN3A complex (30).

Latent Membrane Proteins

Normal T cell help for B cells in the immune response is partly mediated by CD40 ligand on T cells activating CD40 signal transduction in the B cell. EBV LMP1 functions like a constitutively active CD40, but it does not depend on any ligand (31). The signaling from LMP1 via TRADD and TRAF proteins results in activation of the nuclear factor κB (NF-κB) transcription factor, which induces BCL2, which can prevent apoptosis (31). Antiapoptosis activity is also provided by the separate LMP2A protein, which can influence signaling from the B cell receptor and also induce PI3K to activate the AKT pathway, which tends to prevent apoptosis (32).

By making EBV mutants with deletions of individual viral genes, it has been shown that EBNA1, EBNA2, EBNA3C, and LMP1 are essential for converting primary human B cells into LCLs. At least one of the two viral antiapoptosis genes, *BHRF1* or *BALF1*, is also required (33). The mechanisms described above combine to maintain the viral genome and provide the proliferative and antiapoptosis signaling that is required to make an LCL.

Functional RNAs from EBV

In addition to LMPs and the EBNA proteins, EBV also expresses various types of functional RNA upon infecting B cells, the EBER RNAs and many miRNAs. Although most of these are not essential for the transformation of purified B lymphocytes, they help with immune evasion in more physiological conditions (34, 35). The three EBV BHRF1-region miRNAs and the approximately 40 EBV BART-region miRNAs are expressed from different regions of the viral genome (**Figure 2**). They are processed from long primary transcripts, and the miRNAs may be abundant in some infected cells. In some circumstances, EBV miRNAs can be as abundant as highly expressed cell miRNAs. These viral miRNAs inhibit the translation or stability of cell or EBV mRNAs, reducing the expression of specific genes. In addition to their important role in immune evasion during infection of B lymphocytes, many targets of the BART miRNAs have been identified that are potentially relevant to cancer. The BHRF1 miRNAs are required for the efficient transformation of B cells by EBV infection, and they can target PTEN, p27, and EBV BHRF1 (36, 37). In addition to their effects on immune evasion (34, 35), the BART miRNAs can reduce the expression of tumor suppressor genes, such as *DICE1* (38), *PUMA* (39), *PTEN* (40, 41), and *BCL2L11* (*BIM*) (42), among many other targets.

The precise functions of the two EBER RNAs (EBER1 and EBER2) remain uncertain; some studies found a role in B cell transformation or the regulation of interferon responses (43). There is a strong interaction of EBER1 with the cell L22 ribosomal protein; L22 acts as an interface between protein synthesis and p53-dependent apoptosis in some circumstances (44), but no specific

effect on p53-dependent apoptosis in EBV-infected cells has been found (45). An interaction of EBER2 RNA with PAX5 to regulate LMP2 expression has also been proposed (46), and there is some evidence from virus mutants for EBER2 influencing the level of LMP2A RNA (45). Interestingly, EBER2 was found to substitute for the Marek's disease virus telomerase RNA-like viral RNA to maintain the tumorigenic properties of that virus (47), perhaps providing another clue to a mechanism of action. Other studies have suggested that the EBER1 RNA (48) and some EBV miRNAs (49) might be exported from the infected cell in exosomes and could function in surrounding cells.

Thus, functional RNAs comprise a major part of viral gene expression during latent infection and provide many functions without causing an adaptive immune system response.

MEMORY B CELLS NOT LYMPHOBLASTS ARE THE SITE OF EBV PERSISTENCE IN VIVO

Although LCLs are the result of EBV infection in cell culture and reflect part of the physiological infection process, they cannot be the site of latent persistence in vivo because of the effective immune surveillance that develops in all immune-competent infected people. This is mediated primarily by CD8 CTLs that are restricted through major histocompatibility complex (MHC) class I (50). Peptide targets recognized by these T cells have been identified, and many are derived from the same EBV proteins that are required to cause the growth of LCLs, particularly EBNA3 proteins, EBNA2, and LMP1 (50). MHC class I–restricted CD8 CTLs also target peptides from the early lytic cycle proteins, for example BZLF1 (51). There are also CD4 CTL responses, which tend to target EBNA1 (50).

The mechanism of EBV persistence in vivo is now understood in the context of normal B cell development (**Figure 3**). The currently favored model for normal EBV persistence in vivo (9, 52) was deduced by using sensitive polymerase chain reaction (PCR) assays to determine which circulating cells are infected by EBV and then fitting the results to the known properties of the

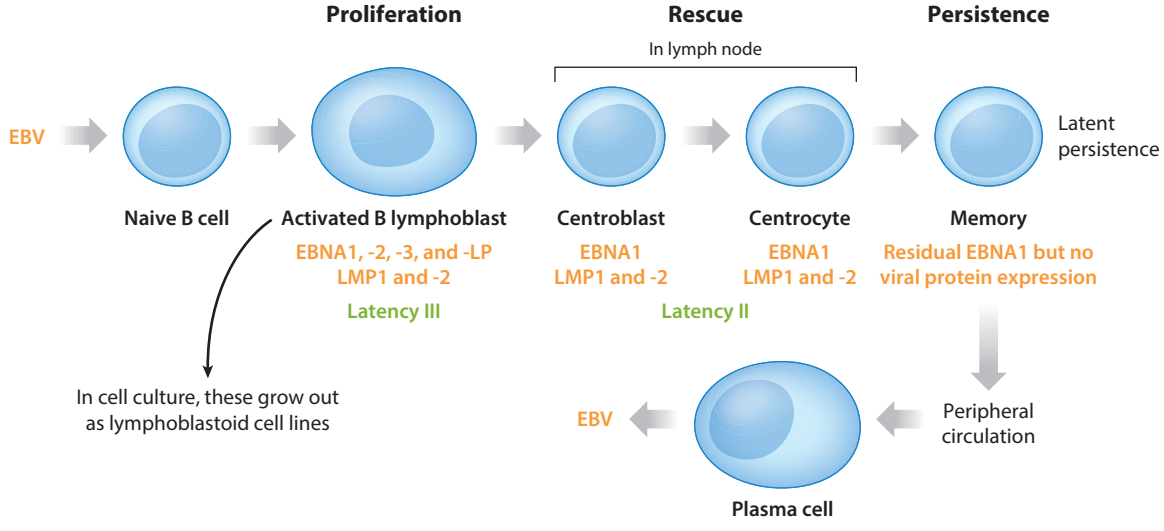

Figure 3

Schematic of the EBV life cycle in B cells (9). Latency pattern (*green*) and EBV protein expression (*orange*) are shown. Abbreviations: EBNA, Epstein–Barr nuclear antigen; EBV, Epstein–Barr virus; LMP, latent membrane protein; LP, leader protein.

various stages of B cell development. It is a widely accepted model, but it should be noted that there is also some evidence for the direct infection of memory cells (53) in addition to the infection of naive B cells described in **Figure 3**. B lymphoblasts activated by EBV infection (with latency III EBV gene expression) are proposed to migrate to lymph nodes where they might be expected to die because the antigen corresponding to their BCR is not necessarily available to rescue them from Fas-mediated cell death. They are saved from this by the continued expression of LMP1 and LMP2 in addition to EBNA1 (latency II), which provide suitable anti-apoptosis cell survival signals. As the infected cells emerge from the lymph node as memory B cells, their EBV gene expression has been reduced to a low level of EBNA1 protein and some EBER RNAs (known as latency 0 if no EBNA1 is expressed). The virus can persist in these relatively long-lived B cells, evading immune detection (9). Intermittently, the memory cells go into the cell cycle to divide, and then EBNA1 is expressed (latency I) to maintain the viral genome. EBV can be activated from these cells into lytic replication and the production of new virus particles by differentiation into plasma cells. These plasma cells are thought to retain homing markers from their original site of infection, which allows them to return to the oropharynx and infect epithelial cells there to shed EBV into the saliva, completing the virus life cycle (9, 52).

So in this context, we now understand the impressive ability of EBV *EBNA* and *LMP* genes to control cell growth and apoptosis as mechanisms that the virus uses to manipulate B cell development to gain access to its site of latent persistence.

CANCERS ASSOCIATED WITH EBV

Most people in the world are infected by EBV and do not develop any disease, but EBV is involved in the development of about 1.5% of all cancers worldwide. Reflecting the cell types that EBV normally infects, EBV-associated cancers (**Table 3**) are mainly lymphomas derived from B cells and carcinomas derived from epithelial cells (3). In addition to African BL, about 30% of Hodgkin's lymphoma (HL) cases, most immunoblastic lymphomas in patients who are immunocompromised, and some NK cell and T cell lymphomas have EBV in the malignant cells of the tumor. Some cases of diffuse large B cell lymphoma (DLBCL) in the elderly also carry EBV in the cancer cells. EBV also infects certain epithelial cells, and epithelial cell cancers (carcinomas) associated with EBV include undifferentiated nasopharyngeal carcinoma (NPC) and gastric carcinoma. Additional much rarer types of cancer that are sometimes associated with EBV include leiomyosarcoma and primary effusion lymphoma.

Table 3 Lymphomas and carcinomas associated with Epstein–Barr virus

Type of cancer	Latency pattern
Lymphoma	
Immunoblastic lymphomas in people who are immunosuppressed	III
Burkitt's lymphoma in areas where malaria is hyperendemic	I
Hodgkin's lymphoma (30% of cases)	II
NK cell and T cell lymphomas	II
Diffuse large B cell lymphoma (5–10% of cases)	II or III
Primary effusion lymphoma	I or II
Carcinoma	
Nasopharyngeal carcinoma	II
Gastric carcinoma	II

Abbreviation: NK, natural killer.

B cells in the lymph node are naturally subject to several highly mutagenic processes (for example, chromosome rearrangement, site specific mutation), and EBV-associated B cell lymphomas can be viewed as aberrations of normal B cell development in EBV-infected cells (54). Chromosome translocations involving the immunoglobulin (Ig) loci may arise through errors in normal Ig gene class switching, and the activation-induced cytidine deaminase (AID) activity that mediates somatic hypermutation may accidentally mutate other genes and additionally cause translocations. In this context, BL cells probably derive from centroblast-like B cells, and HL Reed–Sternberg cells are derived from centrocytes (55).

Immunoblastic Lymphomas

Although lymphoblastoid cells with EBV latency III gene expression are not thought to persist in infected people who are not immunocompromised, they can survive in immunocompromised people and may appear as immunoblastic lymphoma. This is illustrated very clearly in the inherited disease X-linked lymphoproliferative syndrome (56). This rare condition is caused by mutations in the *SH2D1A* gene (encodes the SLAM protein) that result in a specific loss of immune surveillance of EBV-infected B cells. The mutations are recessive, but the *SH2D1A* gene is on the X chromosome, so EBV infection of boys who inherit the mutant allele can result in a potentially lethal EBV-driven B cell lymphoma. Nowadays, families in developed countries are mostly identified, and the affected children may be cured by ablation of their immune systems and hematopoietic stem cell transplantation.

Another immunodeficiency syndrome that results in the failure of T cells and NK cells to control EBV-infected lymphoblasts is X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia (XMEN) and is due to mutations in the *MAGT1* magnesium transporter (57). The *MAGT1* mutations result in a reduced level of free Mg^{2+} ions in T cells that reduces the efficiency of signal transduction to a significant degree. Although XMEN patients are capable of generating EBV-specific CTLs in vivo, their T and NK cells exhibit a killing defect for autologous EBV-immortalized lymphoblastoid B cell lines and other tumor cell line targets. Therefore, XMEN carriers are prone to EBV immunoblastic lymphoma. Remarkably, these patients can be treated by Mg^{2+} supplementation.

People who receive allogeneic transplants require long-term immunosuppression to avoid rejecting the transplant, but this can make them susceptible to virus reactivation and cancers. Approximately 90% of posttransplantation lymphomas are of B cell origin, and many of the early onset cases of posttransplantation lymphoproliferative disease (PTLD) that arise within 2 years after transplantation are immunoblastic lymphomas with latency III EBV gene expression. In some respects these cases are like LCLs that have escaped immune surveillance, but they may have additional oncogenic mutations. The early onset EBV-associated PTLDs frequently regress when immunosuppressive therapy is reduced.

Additional Cancers That May Be Associated with EBV in Immunosuppressed People

The description of PTLD also includes some other B cell lymphomas that may occur after transplantation, most frequently DLBCL or BL (58). The immunosuppression that occurs in AIDS may also promote lymphomas (59), which may be EBV associated. These tend to be immunoblastic lymphoma, BL, or DLBCL. About 45% of the BL cases in AIDS patients in Europe and the United States are EBV-positive.

Kaposi's sarcoma herpesvirus (KSHV) is a close relative of EBV and well known for its role in Kaposi's sarcoma in HIV-infected people. KSHV and EBV occur together in some of the rare

lymphomas present in AIDS patients, particularly primary effusion lymphoma (59). EBV is not the sole viral agent in primary effusion lymphoma, but there may be a synergy between the two viruses, and in a mouse model, there is evidence for KSHV infection increasing the incidence of EBV lymphoma (60). In Kaposi's sarcoma, the KSHV is in the spindle cells, which originate from the endothelium of the interior surface of blood or lymphatic vessels.

EBV may also be found in some rare cases of leiomyosarcoma, a tumor of smooth muscle cells that arises in immunosuppressed people (61), sometimes in the lining of blood vessels but also in other organs and cutaneously. The primary receptor for EBV on lymphocytes is CD21 (CR2), which is bound by the EBV gp350 glycoprotein, and there is also an interaction between EBV gp42 and MHC class II that aids the infection of B cells. The infection of epithelial cells can be mediated by the Ephrin receptor A2 (62, 63). Normal smooth muscle cells can express CD21 (64), but there is no evidence for EBV infection of normal smooth muscle cells in vivo.

EBV GENE EXPRESSION IN CANCERS IN IMMUNE-COMPETENT PEOPLE

Although the key functions of the EBNA and LMP proteins described above are clear in the growth and survival of LCLs, paradoxically some of those essential transforming genes are not expressed in EBV-associated cancers in immune-competent people. This is partly because the proteins are targets for immune surveillance, but it also points to some additional roles of EBV in these cancers.

Burkitt's Lymphoma

BL occurs worldwide at a relatively low level, and in these cases the cancer cells are usually EBV-negative (even though, normally, the patient is infected by EBV). This type of BL is known as sporadic BL (54). In contrast, in sub-Saharan Africa and some other parts of the world where malaria is hyperendemic, there is a much higher incidence of BL, and in these cases the cancer cells usually contain EBV. This is known as endemic BL (54). The roles of chromosome translocation, EBV, and malaria (described in detail below) are summarized in **Figure 4**.

Translocations. All BLs have a chromosome translocation of the *MYC* gene to one of the *Ig* loci; 8:14 is the most frequent, which puts *MYC* close to the *Ig* heavy chain locus and under the control of the heavy chain enhancer. All of these translocations give enhanced and deregulated expression of C-MYC, which is known to drive cell proliferation. The translocations are thought to occur in the lymph node germinal center, where *Ig* gene modifications associated with class switching and somatic hypermutation normally happen in B cells. The AID enzyme mediates the strand breaks for class switching (54). The break point in sporadic (EBV-negative) BLs is usually in the switch region on the *Ig* gene side and in the first (noncoding) exon of *MYC*. This can be rationalized as mistakes in the normal class switching rearrangement, followed by selection of the rearrangements of *MYC* for those that are capable of promoting cell proliferation.

The translocations in EBV-positive (endemic) BLs were at first more difficult to understand. Here, the Ig gene has completed VDJ joining (which normally occurs in the bone marrow), but the Ig gene break point is usually not at a switch junction. Also, the break point on the *MYC* side is not restricted to the first exon and may be many tens of kilobase pairs upstream from the start of the first exon of *C-MYC* (54, 65). Some other VDJ translocations, for example the *BCL2* translocations in follicular lymphoma, occur in the bone marrow (66), but there is no evidence for the *MYC* translocations having arisen there (they cannot be detected in the circulation in the

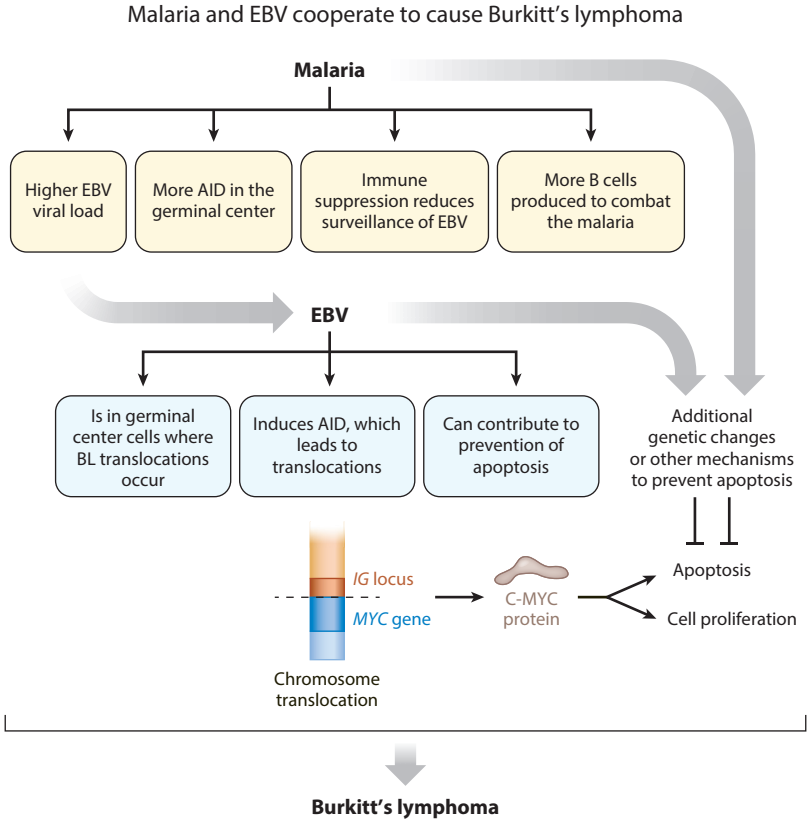

Figure 4

Summary of the mechanisms of Burkitt's lymphoma. Abbreviations: AID, activation-induced cytidine deaminase; BL, Burkitt's lymphoma; EBV, Epstein–Barr virus.

absence of BL). It is now thought that these translocations are also initiated by AID activity, which is induced by EBV and malaria in the lymph node (54). Detailed investigation of enhancers and chromatin looping in BL cell lines and LCLs has suggested that the activation of *MYC* by EBNA2 may increase the susceptibility of upstream enhancer regions to AID activity, contributing to the observed difference in break points between EBV-positive and EBV-negative BLs (65).

Malaria. The endemic regions for EBV-positive BL in Africa and elsewhere are defined by hyperendemic *Plasmodium falciparum* malaria. This results in a higher EBV load (67), higher levels of AID in the lymph node germinal center (68), and immune suppression (69), which may allow transient immune evasion by cells expressing EBV genes. There is also a higher throughput of B cells through the germinal center as the immune system tries to respond to the malaria, which may give more opportunity for the BL translocation to occur. The higher EBV load may be a consequence of reduced immune surveillance and direct induction of EBV replication in some cells by malaria proteins. Similar to the role of Mg^{2+} revealed in XMEN patients, there is also evidence that plasma Mg^{2+} levels are lower in African BL patients than in controls and that low Mg^{2+} plasma levels are linked to higher EBV load (70). These plasma Mg^{2+} effects on viral load are independent of malaria status (70).

Prevention of apoptosis. The overexpression of C-MYC is sufficient to drive cell proliferation, but it also causes apoptosis (71). So *MYC* translocation alone in a cell is not sufficient for cancer to develop. Apart from the *MYC* translocation, an initially confusing array of different genetic and epigenetic changes has been observed in different BL cases, but these can largely be explained as providing any possible way to prevent the apoptosis that would be caused by C-MYC overexpression. C-MYC is able to induce apoptosis both by activating the expression of BIM (BCL2L11) and by inducing ARF, which causes p53 induction by blocking the MDM2 negative regulator of p53. Although there are sometimes mutations in the p53 pathway in BL (72, 73), most evidence points to BIM being the more important mediator of apoptosis in B cell lymphomas driven by C-MYC (74, 75). Consequently, many mechanisms for avoiding BIM expression or BIM-dependent apoptosis may be found in different BLs. Sometimes the C-MYC coding sequence has acquired a point mutation (for example, T58A) so that it does not activate *BIM*; sometimes in EBV-positive BLs, the *BIM* promoter is methylated (76, 77) so that it cannot be induced by C-MYC. Both EBNA3A and -3C EBV proteins repress BIM expression (76), and this can lead to a permanent repressive DNA methylation of the *BIM* promoter and tolerance of high C-MYC levels, even after expression of the EBNA3 proteins is lost (28). Another mechanism (78) is seen in about 10% of endemic BLs, in which there is a deletion in the genome of EBV that removes EBNA2 and increases the expression of the EBV BHRF1 protein (**Figure 2**), which is a close paralog of BCL2. BCL2 or BHRF1 can neutralize the apoptotic function of BIM.

Genome sequencing of BL cases to identify mutations in cell genes that can complement the C-MYC translocation also points to the prevention of apoptosis as a key requirement (79, 80). The most frequent cell gene mutations either activate the TCF3 (E2A) transcription factor or inactivate its negative regulator, ID3 (79). TCF3 activates the PI3K pathway in BL cells and prevents apoptosis via AKT kinase signaling. About 70% of sporadic (EBV-negative) BLs and 40% of EBV-positive endemic BLs have this type of mutation (79). Interestingly, the next most frequent cell gene mutation causes a stabilization of cyclin D3, but this was much more frequent in the sporadic EBV-negative BL cases (38%), in which it can provide an additional proliferative signal via cyclin-dependent kinase. In contrast, only 2% of endemic BL cases have this type of mutation in cyclin D3 (79), so this change does not seem to be required in the presence of EBV.

Hodgkin's Lymphoma

HL is characterized by the distinctive Reed–Sternberg cells, which appear to organize the tumor, surrounded by a much larger number of reactive, nonmalignant B cells. EBV is present in all of the Reed–Sternberg cells (81) in about 30% of HL cases, mostly of the mixed cellularity pathology classification of HL. EBV does not appear to be involved in the remaining 70% of cases, even though those patients will normally be infected with EBV. In EBV-positive cases, LMP1 and LMP2 are strongly expressed in the Reed–Sternberg cells. The Reed–Sternberg cells are thought to be derived from lymph node germinal center B cells, but in EBV-positive cases, the Reed–Sternberg cells frequently have defective (or crippled) rearrangements of their surface BCR, which likely prevents normal cell survival signaling from the BCR (66). LMP1 and LMP2 are thought to complement that deficiency and allow the survival of the Reed–Sternberg cells. Presumably, the Reed–Sternberg cells secrete cytokines that attract the reactive normal B cells, but these cytokines have not yet been defined. Additional gene mutations that are present in HL include translocations of *CIITA* (*MHC2TA*) in about 15% of cases (82).

Certain HLA types are more frequent in HL patients than in the general population (*HLA-A*01* is a risk allele and *HLA-A*02* is a protective allele for developing EBV-associated HL) (83), and these HLA types are also more frequent in IM cases (84). So a clinically proven history of

IM results in about a 4-fold increase in the lifetime risk of EBV-associated HL (85); additional genetic loci that provide risk factors for HL have also recently been identified (86).

Natural Killer Cell and T Cell Lymphomas

These lymphomas were originally described in the United States (87), but are now known to be about 10 times more frequent in Asia and South and Central America than in the United States or Europe (88). About 80% of these lymphomas arise in the nose, oropharynx, and nasopharynx, and they always contain episomal EBV. The clonality of the EBV terminal repeats suggests that EBV was present from an early stage in the development of the disease and in some cases there are deletions of parts of the EBV genome. EBV gene expression usually approximates to latency II, with EBNA1, LMP1, LMP2, EBERs, and BART miRNAs. The tumors frequently have a loss of 6q and mutations of *STAT3*, *BCOR*, and *MLL2* (89). The general lack of NK or T cell culture systems that can be infected in the lab with EBV has limited investigation of how the virus contributes to disease in these cells. In Asia, chronic active EBV infection may precede NK cell and T cell lymphomas, and mutations in *DDX3X* and *TP53* are often found in the lymphoma (90). There has been progress in the treatment of NK cell and T cell lymphomas using PD-1 blockade, which caused some partial and complete responses in patients for whom other treatments had failed (91).

The infection of T or NK cells has not generally been thought to be part of the normal EBV life cycle, but some EBV infection of T cells by type 2 EBV in culture (92) and in children in Africa (93) has been reported (the major natural variation in EBV classifies strains into type 1 and type 2, determined by variation in the *EBNA2* and *EBNA3* genes) (94). It will be important to understand whether T cell and NK cell lymphomas reflect aberrant access of EBV to these cell types (maybe in the context of cell gene mutations during cancer development), or perhaps the infection of T or NK cells is more frequent in some circumstance that has not been recognized previously.

Diffuse Large B Cell Lymphoma

DLBCL is the most frequent type of malignant lymphoma, and it is most common in elderly people (95). In Asia, about 10% of DLBCL cases have EBV in the cancer cells, but that percentage falls to only about 5% in Western countries, and the EBV-associated cases are considered to have a worse prognosis (96). DLBCL cases are classified into germinal center B cell–like (GCB) and activated B cell–like (ABC) on the basis of their cell gene expression. The majority of EBV-positive cases are from the ABC group, although there are also GCB cases that are EBV-positive (97).

Although DLBCL is a heterogeneous disease both clinically and genetically, a recent analysis of more than 1,000 cases identified many genetic drivers of the disease and produced a prognostic model to predict clinical outcome (98). Increased BCL2 expression is frequent (98, 99). A separate analysis of 275 cases identified various genetic changes that result in increased NF-κB activity (100), which can induce BCL2 in B cells. Unfortunately, the EBV-associated cases were not identified in these studies, so the interaction with EBV is still poorly understood. However, there are some examples of mutation of EBV *EBNA3B* in DLBCL cases. Consistent with this, EBV with a deletion of *EBNA3B* has been shown to cause a disease similar to DLBCL in mice with a humanized hematopoietic system (101). This would imply that *EBNA3B* can act as a tumor suppressor gene in EBV and might be an example of a mutation of EBV creating a more oncogenic virus.

Nasopharyngeal Carcinoma

Undifferentiated NPC is exceptionally frequent in the Cantonese populations in Southern China and Hong Kong. It also has a high incidence in Malaysia and Indonesia and in parts of North

Africa (3). Although there are large Chinese populations in Malaysia and Indonesia, the high incidence of NPC is not confined to that ethnic group in those countries. NPC virtually always has EBV present in the malignant epithelial cell component, but the tumor characteristically has a substantial lymphocytic infiltrate. The lymphocytes are normally EBV-negative and are not malignant, but they are thought to have important roles in maintaining the cancer, perhaps by providing cytokines or other signals to the epithelial carcinoma cells and in immune evasion (3). There is an intermediate incidence of NPC in some other parts of the world, but the incidence of NPC in Western countries is low, about 50-fold less than in Hong Kong and Guangzhou. Risk factors for NPC are thought to include male sex, HLA type (102), exposure to environmental or dietary carcinogens, and EBV infection (3).

It is possible to infect human epithelial cells in cell culture by coculture (103) or by transfer infection of EBV from lymphocytes (104, 105), but that infection does not make the epithelial cells grow permanently, and the infection is not maintained long term. A convincing productive replication of EBV has been demonstrated in the differentiating layers of raft-cultured human epithelial cells (106), but again this system does not give long-term, persistent infection.

Current ideas about the development of NPC are guided by the observation that knockdown of the cell p16 cyclin-dependent kinase inhibitor or overexpression of cyclin D1 is sufficient to allow persistent infection of an epithelial cell line by EBV (107). It is proposed that years of accumulating mutations in the nasopharyngeal epithelium due to carcinogen exposure allow the cells to become infected and maintain the EBV genome, which then contributes to the development of cancer. In this model, EBV infection of the tumor cells would not be the earliest event in cancer development, but the clonality of the EBV terminal repeat copy number that is found in most NPC biopsy samples (108) suggests that EBV infection must precede some additional clonal selection of the cells that will go on to become the carcinoma. Genome sequencing of carcinoma cells microdissected from NPC biopsy samples indicates that the most frequent change is the mutation of genes in the NF-κB pathway that can result in activation of NF-κB signaling (109, 110). Mutation of p16 is observed only rarely, so the exact changes that allow persistent infection during disease development remain to be understood.

Although EBV is present in essentially all of the malignant carcinoma cells in NPC, the expression of viral genes is quite variable. It is described as EBV latency II, but the expression of LMP1 is often undetectable in many of the cells. It is possible that the acquisition of mutations in the NF-κB pathway complements the role of LMP1 and allows for the loss of LMP1 expression (109). LMP1 and LMP2A have both been shown in vitro to promote the growth or prevent differentiation of epithelial cells, and LMP2A is often present in NPC cells. Also, all of the EBV-infected cells have detectable EBNA1, EBER RNAs, and BART miRNAs. The BART miRNAs are generally expressed at high levels in NPC, and in a mouse model, they have been shown to enhance the tumorigenicity of a gastric cancer cell line (111). They have many functions that might be relevant to NPC, including reducing apoptosis in epithelial cells (112).

Finally, *BARF1*, which is classified as a lytic cycle gene in lymphocytes, is frequently expressed in NPC cells (43). BARF1 is similar to the receptor for colony stimulating factor 1 (CSF1), but is present in a soluble form in NPC. There are several CSF1R monoclonal antibodies in clinical trials, but none has so far been tested in NPC patients.

Gastric Cancer

Research on EBV and gastric cancer took a major step forward when the Cancer Genome Atlas group published an analysis and classification of gastric cancer (113). About 9% of cases of gastric cancer were found to be EBV-associated, and these cases have a distinct molecular phenotype.

The EBV-associated cases tend to be in the proximal part of the stomach and are usually adenocarcinoma (114). EBV gene expression is often latency II, similar to NPC, but in some cases *LMP1* is not detected, and there is also high expression of *BART* miRNAs in gastric carcinoma (115). In addition to the EBV-associated adenocarcinomas, much rarer lymphoepithelioma-like carcinomas of the stomach are frequently EBV-positive.

Notable features in the mutation profile of the EBV-associated adenocarcinomas compared with other gastric cancers are a lack of p53 mutations, relatively frequent PI3K mutations, and a high degree of CpG methylation in the cell genome (113). A widespread methylation of host genes has been observed upon EBV infection in several contexts (116–119), and inactivation of tumor suppressor genes, such as *p73*, may occur in gastric carcinoma by this mechanism (120). The mutation or inactivation of the p53 pathway function seems essential for most types of carcinoma. The well-known exception is in cervical carcinoma, in which *p53* does not need to be mutated because the p53 protein is targeted for degradation by the papillomavirus E6 protein. The lack of mutation of *p53* in EBV-associated gastric carcinoma has led to speculation that EBV can in some way bypass the need for *p53* mutation, but this is not yet resolved. The p53 protein is expressed in EBV-associated gastric cancers (121).

Another puzzle in relation to gastric cancer is how the virus gains access to the gastric tissue when the EBV life cycle is thought to occur in B lymphocytes and the oral epithelium. The most likely explanation lies in the chronic inflammation that frequently precedes gastric cancer (122); this attracts lymphocytes, and some secreted vesicular products of epithelial cells have been shown to induce virus production in EBV-infected B cells (123), perhaps giving rise to infection of the gastric cells. The pericolic lymph nodes may prove to be involved in normal EBV persistence, and it might even be possible that some of the EBV present in saliva, which is constantly ingested, survives the acid conditions in the stomach sufficiently long to permit the infection of tissue exposed at the site of inflammation.

EBV GENOME SEQUENCE VARIATION: GEOGRAPHY- OR DISEASE RELATED?

The unusual geographic distribution of some EBV-associated cancers raises the possibility that natural variation in the EBV genome contributes to disease incidence. Sequence analysis of approximately 200 EBV genomes has so far failed to find any circulating EBV variant that is specifically related to cancer (5, 94), but EBV polymorphisms that result in a higher level of EBV replication and virus production are enriched in some cancers. *EBNA1* sequence variants that were originally proposed to be linked to BL now seem to be typical of Africa rather than BL (5).

However, there is evidence that the EBV circulating in Southern China has some differences in biological properties compared with strains in the West (124, 125), and it is possible that these might turn out to be relevant to the high NPC incidence there. A single nucleotide polymorphism (SNP) (G155391A) in the RPMS1 open reading frame of EBV has been reported (126) to be the EBV marker most strongly linked to Cantonese NPC strains compared with strains in regions in Northern China where there is a lower incidence, and the SNP was shown to alter the protein stability of the RPMS1 polypeptide. A potentially relevant function for RPMS1 has been identified (127), but unfortunately there is no evidence that this protein is actually produced in EBV-infected cells (128), so the significance of this SNP is not understood at present.

There are, however, some specific examples of EBV variants that do seem to be related to disease, particularly the *EBNA2* deletion mutants in BL cases that lead to increased expression of BHRF1 (78), and the DLBCL cases that have a mutation in *EBNA3B* (101). Further work is needed to determine whether these mutant viruses are transmitted or arise de novo in people. Since the

virus is thought to pass through cells in the lymph node, there is clear potential for variants to be generated in that highly mutagenic environment, and even in LCLs, AID and APOBEC can edit the EBV genome (129). There is also evidence for mutations in cell genes in lymphomas caused by the AID enzyme that is induced by EBNA3C (130).

IMMUNE EVASION BY EBV AND IN EBV-ASSOCIATED CANCERS

The EBV latency I gene expression pattern in BL cells avoids immune surveillance by not expressing any EBV proteins apart from EBNA1. As discussed above, this protein (which is required at only a very low level) avoids presentation on MHC because the stabilizing effect of its Gly-Ala sequence avoids the production of peptides that can be presented (17). The low level of EBNA1 mRNA and EBNA1 translation means that few peptides are produced (131), with the accumulation of the stable protein allowing the low functional levels required to be achieved.

The strategy of expressing very few viral proteins to avoid immune surveillance is extended in EBV by using functional RNA to influence the host cell. The clear roles of BART miRNAs in immune evasion during the primary infection of B cells by EBV, discussed earlier (34, 35), likely also contribute to immune evasion in some of the EBV-associated cancers in which miRNAs are expressed, but this has not yet been studied directly.

Other EBV-associated cancers that occur in immune-competent people express LMP proteins and evidently must have immune evasion mechanisms to allow for the survival of those cells. This can involve the downregulation of MHC or expression of PD-L1 or indoleamine 2,3-dioxygenase (IDO), all of which can inactivate T cell surveillance. There is evidence for the induction of PD-L1 by EBV in HL Reed–Sternberg cells (132), and EBV-associated gastric cancers have much more frequent expression of PD-L1 than do EBV-negative gastric cancers (133). IDO has been reported to be induced by EBV infection of B cells (134) and in an NPC tumor cell line (135). Multiple immune evasion strategies are recognized in HL (136), and the tumor microenvironment is thought to play an important part, both in sustaining tumor growth and in avoiding immune surveillance in the EBV-associated cancers that contain mixed cell types.

When EBV replicates to produce new particles, it expresses many immunogenic lytic cycle proteins. In common with other herpesviruses, in its lytic cycle EBV uses several immune evasion proteins to allow for the production of virus. These strategies include inhibition of the function of transporter associated with antigen processing (TAP) by BNLF2a, reduction of class I expression by BILF1 and BDLF3, gp42 binding to MHC class II, a general reduction of host cell gene expression by BGLF5, the immunosuppressive effects of BCRF1 (the viral paralog of interleukin 10), and soluble BARF1 acting as a decoy for macrophage colony-stimulating factor (M-CSF) to reduce macrophage activation (137).

VACCINE AND THERAPEUTIC T CELLS

There is no vaccine for EBV, nor is it known whether normal persistent infection by EBV leads to resistance to superinfection by the virus. The major neutralizing antigen for the infection of B cells is the glycoprotein gp350, and this has been the basis for most studies of a prophylactic vaccine. The most informative trial so far used a slightly truncated, soluble form of gp350 to immunize 183 university students in Belgium who were EBV-negative at the start of the trial (138). They were given 3 doses of vaccine or placebo during 5 months and then followed up for 19 months to measure the effects of EBV exposure through normal social contact. There was a reduction in the cases of IM from nine in the control group to two in the vaccine group, but there was no reduction in the number of asymptomatic infections (138). Although the results did not reach

statistical significance, they suggest that this type of immunization might reduce the incidence of IM, but it is not likely to prevent infection. In fact, no current human herpesvirus vaccine is able to prevent infection.

The vaccine project has been taken up again by the US National Cancer Institute using an improved formulation (139) and targeting not only gp350 but also the gp42 glycoprotein, which also contributes to EBV receptor binding. Trials are expected to begin in 2019. One clear potential application would be to boost the EBV immunity of people receiving transplants. Patients who were previously EBV-negative or were cleared of EBV by the transplantation protocol and are then exposed to EBV from blood in the donor organ are at the greatest risk of PTLD, and it seems likely that the vaccine could help to prevent the disease. If the vaccine is shown to be safe and effective in transplant patients, it may then be possible to test whether it prevents EBV-associated cancers in other populations at risk. Separate identification of an antibody that neutralizes the infection of both B cells and epithelial cells by EBV (recognizing a complex epitope formed by the gH and gL EBV glycoproteins) also provides the possibility of developing alternative approaches to EBV immunization in the future (140).

The argument that cancer development takes many years and so only persistent viruses contribute to cancers, might suggest that the inability of the gp350 vaccine to prevent infection would undermine its potential use to prevent EBV-associated cancers. However, boosting the immune response to lower the viral load might still help, and there is some evidence from a study in Taiwan that high levels of antibody to gp350 are associated with a lower risk of NPC (141).

Although the vaccine side of EBV immunity is only in development, there has been great success in treating EBV-associated lymphomas in transplant patients using T lymphocytes that can target EBV-infected cells (142). To avoid immune rejection of the therapeutic T cells, these were grown in culture using T cells from the same patient into whom they would be infused; they were then reinfused and results were excellent. This is one of the most successful examples of T cell immunotherapy for cancer, and further refinements are being developed to overcome immune evasion (143). It is, however, laborious and costly to prepare T cells for this purpose, so a simpler approach is being developed using a bank of a few hundred T cell donors among whom a reasonable match can be found (144).

EARLY DIAGNOSIS BASED ON DETECTING EBV DNA IN PLASMA OR BY SEROLOGY

In a normal carrier, EBV resides primarily in B cells, so it can be detected by PCR only in blood cells not in blood plasma. A considerable amount of cell death occurs in EBV-associated cancers, and the contents of a small fraction of the cancer cells leaks into the blood, resulting in detectable EBV DNA in the blood plasma. In some centers, tests for EBV DNA in blood plasma are used as part of the clinical management of NPC patients receiving radiotherapy. The blood plasma levels of EBV DNA fall after radiotherapy, but a subsequent rise is an early sign that the tumor is recurring and more therapy should be given (145). A similar approach has been found to be useful in monitoring the treatment of EBV-associated NK or T cell lymphoma (146) and HL (147).

An impressive study in Hong Kong (148) has extended this approach to screen a normal population to identify early-stage NPC before symptoms appear. More than 20,000 asymptomatic people were tested for persistent plasma EBV DNA by PCR. The positive cases were followed up with endoscopic examination and magnetic resonance imaging. A total of 34 people were found to have NPC, mostly stage I or II disease, which can be treated successfully, in contrast to late-stage disease. This corresponds well to the expected incidence of NPC, and the sensitivity and specificity for NPC were both approximately 98%. Thus, there seems to be a strong case for

population screening to prevent late-stage NPC in populations at risk in Southern China and in countries with a high risk of NPC. However, the moderate cost of this testing has to compete with many other health-care priorities, so more economical ways of delivering this type of screening are being investigated. There is also the possibility of using EBV serology for population screening for NPC (149, 150), but this approach seems to be much less specific and less effective than the plasma EBV DNA test.

CONCLUSIONS

By studying the small DNA tumor viruses, early tumor virology developed the idea that viruses may cause cancer when they get into the wrong cell type and cannot complete their life cycle but continue to express oncogenic viral genes. In some respects this may also be true for EBV in the development of gastric carcinoma, leiomyosarcoma, and the NK and T cell lymphomas. However, the EBV-associated B cell lymphomas develop when cell gene mutations or epigenetic changes occur in cell types that are infected by EBV during its normal life cycle.

Great progress has been made in understanding the mechanism of action of the EBV genes expressed in cancers and in identifying many mechanisms by which the virus can contribute to disease. Indeed, there are so many possible ways in which the virus can promote cancers that it becomes challenging to sift out the functionally important ones. The detailed understanding of EBV infection and molecular biology that has been achieved in recent years will allow for improvement in all aspects of clinical care for patients with these diseases. As discussed in this review, there are great opportunities to offer early diagnosis of EBV-associated cancers during the treatable stages of disease, offer immunotherapy to target EBV-infected cells or overcome immune evasion, prevent disease by immunization, and offer treatment with therapeutic agents that target the EBV gene products that are expressed in the cancers.

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LITERATURE CITED

1. Epstein MA, Achong BG, Barr YM. 1964. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* 1:702–3
2. Kieff E, Rickinson AB. 2007. Epstein–Barr virus and its replication. In *Fields Virology*, ed. DM Knipe, PM Howley, pp. 2603–54. Philadelphia: Lippincott, Williams, and Wilkins
3. Rickinson AB, Kieff E. 2007. Epstein–Barr virus. In *Fields Virology*, ed. DM Knipe, PM Howley, pp. 2655–700. Philadelphia: Lippincott, Williams, and Wilkins
4. 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.* 5:e1000496
5. Correia S, Palser A, Elgueta Karstegl C, Middeldorp JM, Ramayanti O, et al. 2017. Natural variation of Epstein–Barr virus genes, proteins, and primary microRNA. *J. Virol.* 91:e00375-17
6. Condon LM, Cederberg LE, Rabinovitch MD, Liebo RV, Go JC, et al. 2014. Age-specific prevalence of Epstein–Barr virus infection among Minnesota children: effects of race/ethnicity and family environment. *Clin. Infect. Dis.* 59:501–8
7. Munz C. 2017. Epstein–Barr virus-specific immune control by innate lymphocytes. *Front. Immunol.* 8:1658
8. Djaoud Z, Guethlein LA, Horowitz A, Azzi T, Nemat-Gorgani N, et al. 2017. Two alternate strategies for innate immunity to Epstein–Barr virus: one using NK cells and the other NK cells and γδ T cells. *J. Exp. Med.* 214:1827–41

9. Babcock GJ, Decker LL, Volk M, Thorley-Lawson DA. 1998. EBV persistence in memory B cells in vivo. *Immunity* 9:395–404
10. Rowe M, Rowe DT, Gregory CD, Young LS, Farrell PJ, et al. 1987. Differences in B cell growth phenotype reflect novel patterns of Epstein–Barr virus latent gene expression in Burkitt's lymphoma cells. *EMBO J.* 6:2743–51
11. Westhoff Smith D, Sugden B. 2013. Potential cellular functions of Epstein–Barr nuclear antigen 1 (EBNA1) of Epstein–Barr virus. *Viruses* 5:226–40
12. Li N, Thompson S, Schultz DC, Zhu W, Jiang H, et al. 2010. Discovery of selective inhibitors against EBNA1 via high throughput in silico virtual screening. *PLOS ONE* 5:e10126
13. Jiang L, Lan R, Huang T, Chan C, Li H, et al. 2017. EBNA1-targeted probe for the imaging and growth inhibition of tumours associated with the Epstein–Barr virus. *Nat. Biomed. Eng.* 1:0042
14. Gruhne B, Sompallae R, Marescotti D, Kamranvar SA, Gastaldello S, Masucci MG. 2009. The Epstein–Barr virus nuclear antigen-1 promotes genomic instability via induction of reactive oxygen species. *PNAS* 106:2313–18
15. Kamranvar SA, Chen X, Masucci MG. 2013. Telomere dysfunction and activation of alternative lengthening of telomeres in B-lymphocytes infected by Epstein–Barr virus. *Oncogene* 32:5522–30
16. Kamranvar SA, Masucci MG. 2017. Regulation of telomere homeostasis during Epstein–Barr virus infection and immortalization. *Viruses* 9:217
17. Levitskaya J, Sharipo A, Leonchiks A, Ciechanover A, Masucci MG. 1997. Inhibition of ubiquitin/proteasome-dependent protein degradation by the Gly-Ala repeat domain of the Epstein–Barr virus nuclear antigen 1. *PNAS* 94:12616–21
18. Gnanasundram SV, Pyndiah S, Daskalogianni C, Armfield K, Nylander K, et al. 2017. PI3Kδ activates E2F1 synthesis in response to mRNA translation stress. *Nat. Commun.* 8:2103
19. Frappier L. 2012. Contributions of Epstein–Barr nuclear antigen 1 (EBNA1) to cell immortalization and survival. *Viruses* 4:1537–47
20. Kennedy G, Komano J, Sugden B. 2003. Epstein–Barr virus provides a survival factor to Burkitt's lymphomas. *PNAS* 100:14269–74
21. Lu J, Murakami M, Verma SC, Cai Q, Haldar S, et al. 2011. Epstein–Barr Virus nuclear antigen 1 (EBNA1) confers resistance to apoptosis in EBV-positive B-lymphoma cells through up-regulation of survivin. *Virology* 410:64–75
22. Spender LC, Lucchesi W, Bodelon G, Bilancio A, Karstegl CE, et al. 2006. Cell target genes of Epstein–Barr virus transcription factor EBNA-2: induction of the p55α regulatory subunit of PI3-kinase and its role in survival of EREB2.5 cells. *J. Gen. Virol.* 87:2859–67
23. Zhao B, Maruo S, Cooper A, Chase MR, Johannsen E, et al. 2006. RNAs induced by Epstein–Barr virus nuclear antigen 2 in lymphoblastoid cell lines. *PNAS* 103:1900–5
24. Ma Y, Walsh MJ, Bernhardt K, Ashbaugh CW, Trudeau SJ, et al. 2017. CRISPR/Cas9 screens reveal Epstein–Barr virus-transformed B cell host dependency factors. *Cell Host Microbe* 21:580–91.e7
25. Szymula A, Palermo RD, Bayoumy A, Groves IJ, Ba Abdullah M, et al. 2018. Epstein–Barr virus nuclear antigen EBNA-LP is essential for transforming naive B cells, and facilitates recruitment of transcription factors to the viral genome. *PLOS Pathog.* 14:e1006890
26. Allday MJ, Bazot Q, White RE. 2015. The EBNA3 family: two oncoproteins and a tumour suppressor that are central to the biology of EBV in B cells. *Curr. Top. Microbiol. Immunol.* 391:61–117
27. Skalska L, White RE, Parker GA, Turro E, Sinclair AJ, et al. 2013. Induction of $p16^{INK4a}$ is the major barrier to proliferation when Epstein–Barr virus (EBV) transforms primary B cells into lymphoblastoid cell lines. *PLOS Pathog.* 9:e1003187
28. Paschos K, Parker GA, Watanatanasup E, White RE, Allday MJ. 2012. BIM promoter directly targeted by EBNA3C in polycomb-mediated repression by EBV. *Nucleic Acids Res.* 40:7233–46
29. Styles CT, Bazot Q, Parker GA, White RE, Paschos K, Allday MJ. 2017. EBV epigenetically suppresses the B cell-to-plasma cell differentiation pathway while establishing long-term latency. *PLOS Biol.* 15:e2001992
30. Styles CT, Paschos K, White RE, Farrell PJ. 2018. The cooperative functions of the EBNA3 proteins are central to EBV persistence and latency. *Pathogens* 7:31

31. Kieser A, Sterz KR. 2015. The latent membrane protein 1 (LMP1). *Curr. Top. Microbiol. Immunol.* 391:119–49
32. Cen O, Longnecker R. 2015. Latent membrane protein 2 (LMP2). *Curr. Top. Microbiol. Immunol.* 391:151–80
33. Altmann M, Hammerschmidt W. 2005. Epstein–Barr virus provides a new paradigm: a requirement for the immediate inhibition of apoptosis. *PLOS Biol.* 3:e404
34. Albanese M, Tagawa T, Bouvet M, Maliqi L, Lutter D, et al. 2016. Epstein–Barr virus microRNAs reduce immune surveillance by virus-specific $CD8^+$ T cells. *PNAS* 113:E6467–75
35. Tagawa T, Albanese M, Bouvet M, Moosmann A, Mautner J, et al. 2016. Epstein–Barr viral miRNAs inhibit antiviral $CD4^+$ T cell responses targeting IL-12 and peptide processing. *J. Exp. Med.* 213:2065–80
36. Bernhardt K, Haar J, Tsai MH, Poirey R, Feederle R, Delecluse HJ. 2016. A viral microRNA cluster regulates the expression of PTEN, p27 and of a bcl-2 homolog. *PLOS Pathog.* 12:e1005405
37. Poling BC, Price AM, Luftig MA, Cullen BR. 2017. The Epstein–Barr virus miR-BHRF1 microRNAs regulate viral gene expression in *cis*. *Virology* 512:113–23
38. Lei T, Yuen KS, Xu R, Tsao SW, Chen H, et al. 2013. Targeting of *DICE1* tumor suppressor by Epstein–Barr virus-encoded miR-BART3* microRNA in nasopharyngeal carcinoma. *Int. J. Cancer* 133:79–87
39. Choy EY, Siu KL, Kok KH, Lung RW, Tsang CM, et al. 2008. An Epstein–Barr virus-encoded microRNA targets PUMA to promote host cell survival. *J. Exp. Med.* 205:2551–60
40. Gottwein E, Corcoran DL, Mukherjee N, Skalsky RL, Hafner M, et al. 2011. Viral microRNA targetome of KSHV-infected primary effusion lymphoma cell lines. *Cell Host Microbe* 10:515–26
41. Cai L, Ye Y, Jiang Q, Chen Y, Lyu X, et al. 2015. Epstein–Barr virus-encoded microRNA BART1 induces tumour metastasis by regulating PTEN-dependent pathways in nasopharyngeal carcinoma. *Nat. Commun.* 6:7353
42. Marquitz AR, Mathur A, Nam CS, Raab-Traub N. 2011. The Epstein–Barr virus BART microRNAs target the pro-apoptotic protein Bim. *Virology* 412:392–400
43. Takada K. 2012. Role of EBER and *BARF1* in nasopharyngeal carcinoma (NPC) tumorigenesis. *Semin. Cancer Biol.* 22:162–65
44. Fahl SP, Harris B, Coffey F, Wiest DL. 2015. Rpl22 loss impairs the development of B lymphocytes by activating a p53-dependent checkpoint. *J. Immunol.* 194:200–9
45. Gregorovic G, Boulden EA, Bosshard R, Elgueta Karstegl C, Skalsky R, et al. 2015. Epstein–Barr viruses (EBVs) deficient in EBV-encoded RNAs have higher levels of latent membrane protein 2 RNA expression in lymphoblastoid cell lines and efficiently establish persistent infections in humanized mice. *J. Virol.* 89:11711–14
46. Lee N, Moss WN, Yario TA, Steitz JA. 2015. EBV noncoding RNA binds nascent RNA to drive host PAX5 to viral DNA. *Cell* 160:607–18
47. Kheimar A, Kaufer BB. 2018. Epstein-Barr virus-encoded RNAs (EBERs) complement the loss of herpesvirus telomerase RNA (vTR) in virus-induced tumor formation. *Sci. Rep.* 8:209
48. Baglio SR, van Eijndhoven MA, Koppers-Lalic D, Berenguer J, Lougheed SM, et al. 2016. Sensing of latent EBV infection through exosomal transfer of 5′pppRNA. *PNAS* 113:E587–96
49. Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, van Eijndhoven MA, Hopmans ES, et al. 2010. Functional delivery of viral miRNAs via exosomes. *PNAS* 107:6328–33
50. Hislop AD, Taylor GS, Sauce D, Rickinson AB. 2007. Cellular responses to viral infection in humans: lessons from Epstein–Barr virus. *Annu. Rev. Immunol.* 25:587–617
51. Pudney VA, Leese AM, Rickinson AB, Hislop AD. 2005. $CD8^+$ immunodominance among Epstein–Barr virus lytic cycle antigens directly reflects the efficiency of antigen presentation in lytically infected cells. *J. Exp. Med.* 201:349–60
52. Souza TA, Stollar BD, Sullivan JL, Luzuriaga K, Thorley-Lawson DA. 2005. Peripheral B cells latently infected with Epstein–Barr virus display molecular hallmarks of classical antigen-selected memory B cells. *PNAS* 102:18093–98
53. Chaganti S, Heath EM, Bergler W, Kuo M, Buettner M, et al. 2009. Epstein–Barr virus colonization of tonsillar and peripheral blood B-cell subsets in primary infection and persistence. *Blood* 113:6372–81
54. Thorley-Lawson DA, Allday MJ. 2008. The curious case of the tumour virus: 50 years of Burkitt's lymphoma. *Nat. Rev. Microbiol.* 6:913–24

55. Kuppers R. 2003. B cells under influence: transformation of B cells by Epstein–Barr virus. *Nat. Rev. Immunol.* 3:801–12
56. Tangye SG. 2014. XLP: clinical features and molecular etiology due to mutations in *SH2D1A* encoding SAP. *J. Clin. Immunol.* 34:772–79
57. Chaigne-Delalande B, Li FY, O'Connor GM, Lukacs MJ, Jiang P, et al. 2013. Mg^{2+} regulates cytotoxic functions of NK and CD8 T cells in chronic EBV infection through NKG2D. *Science* 341:186–91
58. Dharnidharka VR, Webster AC, Martinez OM, Preiksaitis JK, Leblond V, Choquet S. 2016. Post-transplant lymphoproliferative disorders. *Nat. Rev. Dis. Primers* 2:15088
59. Cesarman E. 2013. Pathology of lymphoma in HIV. *Curr. Opin. Oncol.* 25:487–94
60. McHugh D, Caduff N, Barros MHM, Ramer PC, Raykova A, et al. 2017. Persistent KSHV infection increases EBV-associated tumor formation in vivo via enhanced EBV lytic gene expression. *Cell Host Microbe* 22:61–73.e7
61. McClain KL, Leach CT, Jenson HB, Joshi VV, Pollock BH, et al. 1995. Association of Epstein–Barr virus with leiomyosarcomas in young people with AIDS. *N. Engl. J. Med.* 332:12–18
62. Chen J, Sathiyamoorthy K, Zhang X, Schaller S, Perez White BE, et al. 2018. Ephrin receptor A2 is a functional entry receptor for Epstein–Barr virus. *Nat. Microbiol.* 3:172–80
63. Zhang H, Li Y, Wang HB, Zhang A, Chen ML, et al. 2018. Ephrin receptor A2 is an epithelial cell receptor for Epstein–Barr virus entry. *Nat. Microbiol.* 3:1–8
64. Jenson HB, Montalvo EA, McClain KL, Ench Y, Heard P, et al. 1999. Characterization of natural Epstein–Barr virus infection and replication in smooth muscle cells from a leiomyosarcoma. *J. Med. Virol.* 57:36–46
65. Wood CD, Veenstra H, Khasnis S, Gunnell A, Webb HM, et al. 2016. *MYC* activation and *BCL2L11* silencing by a tumour virus through the large-scale reconfiguration of enhancer-promoter hubs. *eLife* 5:e18270
66. Kuppers R. 2005. Mechanisms of B-cell lymphoma pathogenesis. *Nat. Rev. Cancer* 5:251–62
67. Moormann AM, Chelimo K, Sumba OP, Lutzke ML, Ploutz-Snyder R, et al. 2005. Exposure to holoendemic malaria results in elevated Epstein–Barr virus loads in children. *J. Infect. Dis.* 191:1233–38
68. Thorley-Lawson D, Deitsch KW, Duca KA, Torgbor C. 2016. The link between *Plasmodium falciparum* malaria and endemic Burkitt's lymphoma—new insight into a 50-year-old enigma. *PLOS Pathog.* 12:e1005331
69. Moormann AM, Chelimo K, Sumba PO, Tisch DJ, Rochford R, Kazura JW. 2007. Exposure to holoendemic malaria results in suppression of Epstein-Barr virus-specific T cell immunosurveillance in Kenyan children. *J. Infect. Dis.* 195:799–808
70. Juan R, Otim I, Nabalende H, Legason ID, Reynolds SJ, et al. 2017. Plasma magnesium is inversely associated with Epstein–Barr virus load in peripheral blood and Burkitt lymphoma in Uganda. *Cancer Epidemiol.* 52:70–74
71. McMahon SB. 2014. MYC and the control of apoptosis. *Cold Spring Harb. Perspect. Med.* 4:a014407
72. Farrell PJ, Allan GJ, Shanahan F, Vousden KH, Crook T. 1991. p53 is frequently mutated in Burkitt's lymphoma cell lines. *EMBO J.* 10:2879–87
73. Lindstrom MS, Klangby U, Wiman KG. 2001. p14ARF homozygous deletion or MDM2 overexpression in Burkitt lymphoma lines carrying wild type p53. *Oncogene* 20:2171–77
74. Hemann MT, Bric A, Teruya-Feldstein J, Herbst A, Nilsson JA, et al. 2005. Evasion of the p53 tumour surveillance network by tumour-derived *MYC* mutants. *Nature* 436:807–11
75. Fitzsimmons L, Boyce AJ, Wei W, Chang C, Croom-Carter D, et al. 2017. Coordinated repression of BIM and PUMA by Epstein–Barr virus latent genes maintains the survival of Burkitt lymphoma cells. *Cell Death Differ.* 25:241–54
76. Anderton E, Yee J, Smith P, Crook T, White RE, Allday MJ. 2008. Two Epstein–Barr virus (EBV) oncoproteins cooperate to repress expression of the proapoptotic tumour-suppressor *Bim*: clues to the pathogenesis of Burkitt's lymphoma. *Oncogene* 27:421–33
77. Paschos K, Smith P, Anderton E, Middeldorp JM, White RE, Allday MJ. 2009. Epstein–Barr virus latency in B cells leads to epigenetic repression and CpG methylation of the tumour suppressor gene *Bim*. *PLOS Pathog.* 5:e1000492

78. Kelly GL, Long HM, Stylianou J, Thomas WA, Leese A, et al. 2009. An Epstein–Barr virus anti-apoptotic protein constitutively expressed in transformed cells and implicated in Burkitt lymphomagenesis: the Wp/BHRF1 link. *PLOS Pathog.* 5:e1000341
79. Schmitz R, Young RM, Ceribelli M, Jhavar S, Xiao W, et al. 2012. Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. *Nature* 490:116–20
80. Abate F, Ambrosio MR, Mundo L, Laginestra MA, Fuligni F, et al. 2015. Distinct Viral and mutational spectrum of endemic Burkitt lymphoma. *PLOS Pathog.* 11:e1005158
81. Weiss LM, Movahed LA, Warnke RA, Sklar J. 1989. Detection of Epstein–Barr viral genomes in Reed–Sternberg cells of Hodgkin's disease. *N. Engl. J. Med.* 320:502–6
82. Steidl C, Shah SP, Woolcock BW, Rui L, Kawahara M, et al. 2011. MHC class II transactivator *CIITA* is a recurrent gene fusion partner in lymphoid cancers. *Nature* 471:377–81
83. Kushekhar K, van den Berg A, Nolte I, Hepkema B, Visser L, Diepstra A. 2014. Genetic associations in classical Hodgkin lymphoma: a systematic review and insights into susceptibility mechanisms. *Cancer Epidemiol. Biomark. Prev.* 23:2737–47
84. Hjalgrim H, Rostgaard K, Johnson PC, Lake A, Shield L, et al. 2010. HLA-A alleles and infectious mononucleosis suggest a critical role for cytotoxic T-cell response in EBV-related Hodgkin lymphoma. *PNAS* 107:6400–5
85. Hjalgrim H, Askling J, Rostgaard K, Hamilton-Dutoit S, Frisch M, et al. 2003. Characteristics of Hodgkin's lymphoma after infectious mononucleosis. *N. Engl. J. Med.* 349:1324–32
86. Sud A, Thomsen H, Law PJ, Forsti A, Filho M, et al. 2017. Genome-wide association study of classical Hodgkin lymphoma identifies key regulators of disease susceptibility. *Nat. Commun.* 8:1892
87. Jones JF, Shurin S, Abramowsky C, Tubbs RR, Sciotto CG, et al. 1988. T-cell lymphomas containing Epstein–Barr viral DNA in patients with chronic Epstein–Barr virus infections. *N. Engl. J. Med.* 318:733–41
88. Tse E, Kwong YL. 2017. The diagnosis and management of NK/T-cell lymphomas. *J. Hematol. Oncol.* 10:85
89. Lee S, Park HY, Kang SY, Kim SJ, Hwang J, et al. 2015. Genetic alterations of JAK/STAT cascade and histone modification in extranodal NK/T-cell lymphoma nasal type. *Oncotarget* 6:17764–76
90. Kimura H. 2018. EBV in T-/NK-cell tumorigenesis. *Adv. Exp. Med. Biol.* 1045:459–75
91. Kwong YL, Chan TSY, Tan D, Kim SJ, Poon LM, et al. 2017. PD1 blockade with pembrolizumab is highly effective in relapsed or refractory NK/T-cell lymphoma failing L-asparaginase. *Blood* 129:2437–42
92. Coleman CB, Wohlford EM, Smith NA, King CA, Ritchie JA, et al. 2015. Epstein–Barr virus type 2 latently infects T cells, inducing an atypical activation characterized by expression of lymphotactic cytokines. *J. Virol.* 89:2301–12
93. Coleman CB, Daud II, Ogolla SO, Ritchie JA, Smith NA, et al. 2017. Epstein–Barr Virus type 2 infects T cells in healthy Kenyan children. *J. Infect. Dis.* 216:670–77
94. Palser AL, Grayson NE, White RE, Corton C, Correia S, et al. 2015. Genome diversity of Epstein–Barr virus from multiple tumor types and normal infection. *J. Virol.* 89:5222–37
95. Ok CY, Papathomas TG, Medeiros LJ, Young KH. 2013. EBV-positive diffuse large B-cell lymphoma of the elderly. *Blood* 122:328–40
96. Lu TX, Liang JH, Miao Y, Fan L, Wang L, et al. 2015. Epstein–Barr virus positive diffuse large B-cell lymphoma predict poor outcome, regardless of the age. *Sci. Rep.* 5:12168
97. Montes-Moreno S, Odqvist L, Diaz-Perez JA, Lopez AB, de Villambrosia SG, et al. 2012. EBV-positive diffuse large B-cell lymphoma of the elderly is an aggressive post-germinal center B-cell neoplasm characterized by prominent nuclear factor-κB activation. *Mod. Pathol.* 25:968–82
98. Reddy A, Zhang J, Davis NS, Moffitt AB, Love CL, et al. 2017. Genetic and functional drivers of diffuse large B cell lymphoma. *Cell* 171:481–94.e15
99. Schuetz JM, Johnson NA, Morin RD, Scott DW, Tan K, et al. 2012. *BCL2* mutations in diffuse large B-cell lymphoma. *Leukemia* 26:1383–90
100. Xu PP, Zhong HJ, Huang YH, Gao XD, Zhao X, et al. 2017. B-cell function gene mutations in diffuse large B-cell lymphoma: a retrospective cohort study. *EBioMedicine* 16:106–14

101. White RE, Ramer PC, Naresh KN, Meixlsperger S, Pinaud L, et al. 2012. EBNA3B-deficient EBV promotes B cell lymphomagenesis in humanized mice and is found in human tumors. *J. Clin. Investig.* 122:1487–502
102. Tang M, Lautenberger JA, Gao X, Sezgin E, Hendrickson SL, et al. 2012. The principal genetic determinants for nasopharyngeal carcinoma in China involve the HLA class I antigen recognition groove. *PLOS Genet*. 8:e1003103
103. Imai S, Nishikawa J, Takada K. 1998. Cell-to-cell contact as an efficient mode of Epstein–Barr virus infection of diverse human epithelial cells. *J. Virol.* 72:4371–78
104. Shannon-Lowe CD, Neuhierl B, Baldwin G, Rickinson AB, Delecluse HJ. 2006. Resting B cells as a transfer vehicle for Epstein–Barr virus infection of epithelial cells. *PNAS* 103:7065–70
105. 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
106. Temple RM, Zhu J, Budgeon L, Christensen ND, Meyers C, Sample CE. 2014. Efficient replication of Epstein–Barr virus in stratified epithelium in vitro. *PNAS* 111:16544–49
107. Tsang CM, Yip YL, Lo KW, Deng W, To KF, et al. 2012. Cyclin D1 overexpression supports stable EBV infection in nasopharyngeal epithelial cells. *PNAS* 109:E3473–82
108. Raab-Traub N, Flynn K. 1986. The structure of the termini of the Epstein–Barr virus as a marker of clonal cellular proliferation. *Cell* 47:883–89
109. Li YY, Chung GT, Lui VW, To KF, Ma BB, et al. 2017. Exome and genome sequencing of nasopharynx cancer identifies NF-κB pathway activating mutations. *Nat. Commun.* 8:14121
110. Zheng H, Dai W, Cheung AK, Ko JM, Kan R, et al. 2016. Whole-exome sequencing identifies multiple loss-of-function mutations of NF-κB pathway regulators in nasopharyngeal carcinoma. *PNAS* 113:11283–88
111. Qiu J, Smith P, Leahy L, Thorley-Lawson DA. 2015. The Epstein–Barr virus encoded BART miRNAs potentiate tumor growth in vivo. *PLOS Pathog*. 11:e1004561
112. Kang D, Skalsky RL, Cullen BR. 2015. EBV BART microRNAs target multiple pro-apoptotic cellular genes to promote epithelial cell survival. *PLOS Pathog*. 11:e1004979
113. Cancer Genome Atlas Res. Netw. 2014. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 513:202–9
114. Shinozaki-Ushiku A, Kunita A, Fukayama M. 2015. Update on Epstein–Barr virus and gastric cancer (review). *Int. J. Oncol.* 46:1421–34
115. Strong MJ, Xu G, Coco J, Baribault C, Vinay DS, et al. 2013. Differences in gastric carcinoma microenvironment stratify according to EBV infection intensity: implications for possible immune adjuvant therapy. *PLOS Pathog*. 9:e1003341
116. Kaneda A, Matsusaka K, Aburatani H, Fukayama M. 2012. Epstein–Barr virus infection as an epigenetic driver of tumorigenesis. *Cancer Res*. 72:3445–50
117. Birdwell CE, Queen KJ, Kilgore PC, Rollyson P, Trutschl M, et al. 2014. Genome-wide DNA methylation as an epigenetic consequence of Epstein–Barr virus infection of immortalized keratinocytes. *J. Virol.* 88:11442–58
118. Saha A, Jha HC, Upadhyay SK, Robertson ES. 2015. Epigenetic silencing of tumor suppressor genes during in vitro Epstein–Barr virus infection. *PNAS* 112:E5199–207
119. Hernandez-Vargas H, Gruffat H, Cros MP, Diederichs A, Sirand C, et al. 2017. Viral driven epigenetic events alter the expression of cancer-related genes in Epstein-Barr-virus naturally infected Burkitt lymphoma cell lines. *Sci. Rep.* 7:5852
120. Ushiku T, Chong JM, Uozaki H, Hino R, Chang MS, et al. 2007. p73 gene promoter methylation in Epstein–Barr virus-associated gastric carcinoma. *Int. J. Cancer* 120:60–66
121. Ribeiro J, Malta M, Galaghar A, Silva F, Afonso LP, et al. 2017. P53 deregulation in Epstein–Barr virus-associated gastric cancer. *Cancer Lett*. 404:37–43
122. Fox JG, Wang TC. 2007. Inflammation, atrophy, and gastric cancer. *J. Clin. Investig.* 117:60–69
123. Lin Z, Swan K, Zhang X, Cao S, Brett Z, et al. 2016. Secreted oral epithelial cell membrane vesicles induce Epstein–Barr virus reactivation in latently infected B cells. *J. Virol.* 90:3469–79
124. Tsai MH, Raykova A, Klinke O, Bernhardt K, Gartner K, et al. 2013. Spontaneous lytic replication and epitheliotropism define an Epstein–Barr virus strain found in carcinomas. *Cell Rep*. 5:458–70

125. Dheekollu J, Malecka K, Wiedmer A, Delecluse HJ, Chiang AK, et al. 2017. Carcinoma-risk variant of EBNA1 deregulates Epstein–Barr Virus episomal latency. *Oncotarget* 8:7248–64
126. Feng FT, Cui Q, Liu WS, Guo YM, Feng QS, et al. 2015. A single nucleotide polymorphism in the Epstein–Barr virus genome is strongly associated with a high risk of nasopharyngeal carcinoma. *Chin. J. Cancer* 34:563–72
127. Smith PR, de Jesus O, Turner D, Hollyoake M, Karstegl CE, et al. 2000. Structure and coding content of CST (BART) family RNAs of Epstein–Barr virus. *J. Virol.* 74:3082–92
128. Al-Mozaini M, Bodelon G, Karstegl CE, Jin B, Al-Ahdal M, Farrell PJ. 2009. Epstein–Barr virus BART gene expression. *J. Gen. Virol.* 90:307–16
129. Suspene R, Aynaud MM, Koch S, Pasdeloup D, Labetoulle M, et al. 2011. Genetic editing of herpes simplex virus 1 and Epstein–Barr herpesvirus genomes by human APOBEC3 cytidine deaminases in culture and in vivo. *J. Virol.* 85:7594–602
130. Kalchschmidt JS, Bashford-Rogers R, Paschos K, Gillman AC, Styles CT, et al. 2016. Epstein–Barr virus nuclear protein EBNA3C directly induces expression of AID and somatic mutations in B cells. *J. Exp. Med.* 213:921–28
131. Apcher S, Daskalogianni C, Manoury B, Fahraeus R. 2010. Epstein Barr virus–encoded EBNA1 interference with MHC class I antigen presentation reveals a close correlation between mRNA translation initiation and antigen presentation. *PLOS Pathog.* 6:e1001151
132. Green MR, Rodig S, Juszczynski P, Ouyang J, Sinha P, et al. 2012. Constitutive AP-1 activity and EBV infection induce PD-L1 in Hodgkin lymphomas and posttransplant lymphoproliferative disorders: implications for targeted therapy. *Clin. Cancer Res.* 18:1611–18
133. Derks S, Liao X, Chiaravalli AM, Xu X, Camargo MC, et al. 2016. Abundant PD-L1 expression in Epstein–Barr virus-infected gastric cancers. *Oncotarget* 7:32925–32
134. Song H, Park H, Kim J, Park G, Kim YS, et al. 2011. IDO metabolite produced by EBV-transformed B cells inhibits surface expression of NKG2D in NK cells via the c-Jun N-terminal kinase (JNK) pathway. *Immunol. Lett.* 136:187–93
135. Liu P, Xie BL, Cai SH, He YW, Zhang G, et al. 2009. Expression of indoleamine 2,3-dioxygenase in nasopharyngeal carcinoma impairs the cytolytic function of peripheral blood lymphocytes. *BMC Cancer* 9:416
136. Wein F, Weniger MA, Hoing B, Arnolds J, Huttmann A, et al. 2017. Complex immune evasion strategies in classical Hodgkin lymphoma. *Cancer Immunol. Res.* 5:1122–32
137. Ressing ME, van Gent M, Gram AM, Hooykaas MJ, Piersma SJ, Wiertz EJ. 2015. Immune evasion by Epstein–Barr virus. *Curr. Top. Microbiol. Immunol.* 391:355–81
138. Sokal EM, Hoppenbrouwers K, Vandermeulen C, Moutschen M, Leonard P, et al. 2007. Recombinant gp350 vaccine for infectious mononucleosis: a phase 2, randomized, double-blind, placebo-controlled trial to evaluate the safety, immunogenicity, and efficacy of an Epstein–Barr virus vaccine in healthy young adults. *J. Infect. Dis.* 196:1749–53
139. Kanekiyo M, Bu W, Joyce MG, Meng G, Whittle JR, et al. 2015. Rational design of an Epstein–Barr virus vaccine targeting the receptor-binding site. *Cell* 162:1090–100
140. Snijder J, Ortego MS, Weidle C, Stuart AB, Gray MD, et al. 2018. An antibody targeting the fusion machinery neutralizes dual-tropic infection and defines a site of vulnerability on Epstein–Barr virus. *Immunity* 48:799–811.e9
141. Coghill AE, Bu W, Nguyen H, Hsu WL, Yu KJ, et al. 2016. High levels of antibody that neutralize B-cell infection of Epstein–Barr virus and that bind EBV gp350 are associated with a lower risk of nasopharyngeal carcinoma. *Clin. Cancer Res.* 22:3451–57
142. Gottschalk S, Rooney CM. 2015. Adoptive T-cell immunotherapy. *Curr. Top. Microbiol. Immunol.* 391:427–54
143. Bollard CM, Tripic T, Cruz CR, Dotti G, Gottschalk S, et al. 2018. Tumor-specific T-cells engineered to overcome tumor immune evasion induce clinical responses in patients with relapsed Hodgkin lymphoma. *J. Clin. Oncol.* 36:1128–39
144. Tzannou I, Papadopoulou A, Naik S, Leung K, Martinez CA, et al. 2017. Off-the-shelf virus-specific T cells to treat BK virus, human herpesvirus 6, cytomegalovirus, Epstein–Barr virus, and adenovirus infections after allogeneic hematopoietic stem-cell transplantation. *J. Clin. Oncol.* 35:3547–57

145. Lo YM, Chan LY, Chan AT, Leung SF, Lo KW, et al. 1999. Quantitative and temporal correlation between circulating cell-free Epstein–Barr virus DNA and tumor recurrence in nasopharyngeal carcinoma. *Cancer Res.* 59:5452–55
146. Kwong YL, Pang AW, Leung AY, Chim CS, Tse E. 2014. Quantification of circulating Epstein–Barr virus DNA in NK/T-cell lymphoma treated with the SMILE protocol: diagnostic and prognostic significance. *Leukemia* 28:865–70
147. Welch JJG, Schwartz CL, Higman M, Chen L, Buxton A, et al. 2017. Epstein–Barr virus DNA in serum as an early prognostic marker in children and adolescents with Hodgkin lymphoma. *Blood Adv.* 1:681–84
148. Chan KCA, Woo JKS, King A, Zee BCY, Lam WKJ, et al. 2017. Analysis of plasma Epstein–Barr virus DNA to screen for nasopharyngeal cancer. *N. Engl. J. Med.* 377:513–22
149. Coghill AE, Hsu WL, Pfeiffer RM, Juwana H, Yu KJ, et al. 2014. Epstein–Barr virus serology as a potential screening marker for nasopharyngeal carcinoma among high-risk individuals from multiplex families in Taiwan. *Cancer Epidemiol. Biomark. Prev.* 23:1213–19
150. Liu Z, Ji MF, Huang QH, Fang F, Liu Q, et al. 2013. Two Epstein–Barr virus-related serologic antibody tests in nasopharyngeal carcinoma screening: results from the initial phase of a cluster randomized controlled trial in Southern China. *Am. J. Epidemiol.* 177:242–50