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## 2 **Epstein–Barr Virus**

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### 6 **Advanced Article**

7 **(Advanced articles are aimed at advanced undergraduates, graduate students,**  
8 **postgraduates, and researchers reading outside their field of expertise.)**

### 9 **Abstract**

10 *Epstein–Barr virus* (EBV) is a ubiquitous gamma herpesvirus aetiologically linked to different lymphoid and  
11 epithelial malignancies, and a number of systemic autoimmune diseases. The virus has a unique ability to  
12 transform resting B lymphocytes *in vitro* by expressing a set of latent genes, subsets of which are present in  
13 EBV-associated tumours. EBV exploits the physiology of normal B-cell differentiation to persist within the memory  
14 B cell pool of the immunocompetent host with strong T-cell responses important for controlling EBV infection.  
15 Immunosuppressed transplant recipients and human immunodeficiency virus (HIV)-infected individuals are at  
16 increased risk of developing EBV-transformed B-cell proliferations which often present as monoclonal non-  
17 Hodgkin lymphomas. The major EBV-associated tumours (Burkitt lymphoma, Hodgkin lymphoma and  
18 nasopharyngeal carcinoma) show restricted forms of latent viral gene expression reflecting a more complex  
19 pathogenesis involving additional cofactors. A number of pharmacological and immunotherapeutic approaches  
20 are being developed to treat or prevent these EBV-associated tumours.

### 21 **Key concepts**

- 22 • *Epstein–Barr virus* (EBV) infection is implicated in the aetiology of several different lymphoid and  
23 epithelial malignancies, as well as a number of systemic autoimmune diseases.
- 24 • EBV exploits the physiology of normal B-cell differentiation to persist within the memory B cell pool of  
25 the immunocompetent host.
- 26 • EBV-encoded latent genes induce B-cell transformation *in vitro* by altering cellular gene transcription  
27 and constitutively activating key cell signalling pathways.
- 28 • Immunosuppressed transplant patients are at risk of developing EBV-transformed B-cell proliferations  
29 presenting as B-cell lymphomas.
- 30 • Other EBV-associated tumours display more restricted forms of latent gene expression, reflecting more  
31 complex pathogenesis involving additional cofactors.
- 32 • EBV sequence variation may reflect disease risk.
- 33 • Pharmacological and immunotherapeutic approaches are being developed to treat or prevent EBV-  
34 associated tumours.
- 35 • More direct vaccine approaches are being examined for the treatment and prevention of EBV-  
36 associated diseases.

37 **Keywords:** herpesvirus; B cells; latency; lymphoma; carcinoma; leiomyosarcoma; autoimmune diseases;  
38 vaccine; sequence variation; microRNAs

39

## 40 **Introduction: Discovery of *Epstein–Barr Virus***

41 Science often tells as gripping a tale as any detective story, and as such, the story of the discovery of *Epstein–Barr virus*  
42 (EBV), spanning 10 years on 4 continents, is worth recounting. The first investigator to pick up the trail in the 1950s was

1 Denis Burkitt. An Irish surgeon stationed at a mission hospital in rural Uganda, he described for the first time a paediatric  
2 malignancy that usually presented as a fast-growing tumour of the jaw. The afflicted children came from geographically  
3 distinct regions of Uganda. His curiosity piqued, Burkitt set out to visit hospitals in 10 African countries, in an attempt to  
4 correlate the incidence of the tumour with other environmental factors. He discovered that the tumour was found in an area  
5 overlapping the malaria belt (Burkitt, [1962](#)). Suspecting that a mosquito-borne virus might be causing the disease, Burkitt  
6 presented his findings in a seminar at the Middlesex Hospital in London in 1961. See also [Leukaemias and Lymphomas](#), and  
7 [Non-Hodgkin Lymphomas](#)

8 Attending that lecture was the next medical detective on the case, M Anthony Epstein, a pathologist. He knew that, while a  
9 large number of viruses were known to cause cancer in animals, none of these was mosquito-borne. Nonetheless, for the next  
10 3 years, he and his colleagues in London, Bert Achong and Yvonne Barr, examined biopsies of the tumour now called  
11 Burkitt lymphoma (BL), looking for evidence of a human tumour virus. A technical breakthrough came in 1964 with the  
12 establishment of several BL cell lines, from which herpesvirus-like particles were finally visualized by electron microscopy  
13 (Figure 1, Epstein *et al.*, [1964](#)). None of the three herpesviruses known at that time (*Herpes simplex virus 1* and 2 and  
14 *Varicella-Zoster virus*) was lymphotropic, nor were they associated with cancer or transmitted by mosquitoes. See also  
15 [Herpesviruses \(Human\)](#), and [Oncogenic Viruses](#)

16 <Figure 1 near here>

17

18 Nevertheless, now that the existence of a virus was confirmed, Werner and Gertrude Henle took up the investigation in  
19 Philadelphia, using immunological techniques to determine whether this African tumour-associated virus was in fact new.  
20 They found that the BL patients did indeed have antibodies that specifically recognized an antigen in the cultured tumour  
21 cells, and did not react with any of the three known herpesviruses in infected cells. Harald zur Hausen, a German scientist  
22 working with the Henles, also detected EBV deoxyribonucleic acid (DNA) in a BL cell line and in biopsies of  
23 nasopharyngeal carcinoma (NPC; zur Hausen *et al.*, [1970](#)). It appeared that the suspect, a new human herpesvirus now called  
24 EBV, had been identified.

25 At this point the Henles were surprised to discover that whereas all the African BL patients had anti-EBV antibodies, as did  
26 most of the normal population in the USA, no disease had been associated with the infection in the USA. Subsequently the  
27 Henles' research technician, who was among the relatively rare adults with no EBV antibodies, contracted infectious  
28 mononucleosis (IM). On her return to the laboratory, not only did she have EBV antibodies, but her B lymphocytes reacted  
29 with antibodies from BL patients, and when explanted to cell culture, grew continuously as an immortalized cell line,  
30 whereas before the infection they did not (Henle *et al.*, [1968](#)). This serendipitous discovery identified EBV as the causative  
31 agent for IM, later proven in a seroepidemiological study of university students and by molecular hybridization analyses  
32 designed to identify EBV in saliva. See also [Infectious Mononucleosis](#)

33 In the late 1960s, the ability of EBV to infect and immortalize human B lymphocytes in culture was discovered principally  
34 by John Pope working in Australia. This property singles EBV out as the most efficient transforming agent for human cells

1 in culture and still provides a useful tool for rescuing the human genome for genetic and functional studies. Since this time  
2 EBV has been implicated in the aetiology of an increasing number of tumours (B- and T-cell lymphomas, Hodgkin  
3 lymphoma (HL), a proportion of gastric adenocarcinomas, and although less commonly, a subset of breast cancers and a rare  
4 childhood cancer of the smooth muscle system called leiomyosarcoma). There are several associations of EBV with the  
5 pathogenesis of other diseases, such as oral hairy leukoplakia (OHL), chronic fatigue syndrome, and certain systemic  
6 autoimmune diseases including multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus (SLE) and systemic  
7 scleroderma (SSc), but for some of these diseases, the exact role of EBV infection in their pathogenesis has yet to be  
8 defined. See also [Electron Microscopy](#), [Epstein–Barr Virus and Cancer](#), [Nucleic Acids: Hybridisation, and Human](#)  
9 [Autoimmune Diseases](#).

## 10 Pathogenesis and Epidemiology

### 11 Primary and persistent infection

12 The hallmark of herpesvirus infection is the ability to establish a quiescent, nonproductive infection that persists, often  
13 silently, for life. The cell type permitting this long-term latent infection varies, with *Herpes simplex virus* (HSV) persisting  
14 in neurons, *Cytomegalovirus* (CMV) in secretory glands, kidneys and other tissues, and EBV in B lymphocytes. It is  
15 generally believed that EBV replicates and is shed at epithelial sites in the oropharynx and salivary glands but that B  
16 lymphocytes are the main site of EBV persistence. This is supported by the B lymphotropism of EBV which is mediated by  
17 the binding of the major viral envelope glycoprotein gp350/220 to the CR2 (CD21) receptor on the surface of B cells. Since  
18 epithelial cells lack the CR2 receptor, the virus-encoded fusion proteins, gB and gH/gL are the key mediators of EBV entry  
19 into the epithelial compartment (Chen et al., 2016).

20 Detailed examination of EBV infection *in vivo* has shown that the virus persists in the IgD<sup>-</sup>CD27<sup>+</sup> memory B cell subset and  
21 that these cells have downregulated the expression of most, if not all, viral genes (Babcock *et al.*, 1998). This reservoir of  
22 infected cells is stably maintained thereafter, apparently subject to the same physiologic controls as the general mucosa-  
23 associated memory B cell pool. EBV persistence within this B-cell population brings with it the possibility of fortuitous  
24 antigen-driven recruitment of infected cells into germinal centres, leading to progeny that either reenter the circulating  
25 memory pool or differentiate to plasma cells that may migrate to mucosal sites (Figure 2). The different forms of latency that  
26 are manifest in virus-associated malignancies (discussed later) may represent latency programmes that have evolved to  
27 accommodate such changes in host cell physiology.

28 <Figure 2 near here>

29 The majority of the global population is infected subclinically during childhood. EBV-induced symptoms vary with the age  
30 of infection and whether the infection is primary or reactivated. The human diseases associated with EBV infection are  
31 summarized in Table 1. In developing countries, most infants are infected asymptotically within the first year of life,  
32 although they are protected for the first 3 months by maternal antibodies. In upper socioeconomic classes, infection usually  
33 occurs within the first three decades, which may manifest in adolescents and young adults as infectious mononucleosis (IM).

1 EBV elicits both humoral and cell-mediated immune responses in infected hosts. The levels of EBV-specific antibodies  
2 against latent and lytic antigens remain fairly stable in healthy EBV-positive individuals but are elevated in different EBV-  
3 associated diseases (see later discussion). As with other persistent viruses, cell-mediated immunity plays an important role in  
4 controlling EBV infection with high levels of virus-specific CD8<sup>+</sup> and CD4<sup>+</sup> T cells being detectable in the circulation. See  
5 also [Epstein–Barr Virus and Cancer](#)

6  
7 <Table 1 near here>

## 8 **B-cell (immunoblastic) lymphomas in the** 9 **immunosuppressed**

10 EBV-induced lymphoproliferative syndromes resulting from primary, or later from reactivated, infection arise in  
11 immunodeficiency states, because virus-induced B-cell proliferation is unchecked by EBV-specific T cells. Up to 10% of  
12 allograft recipients are affected, depending on the intensity of immunosuppressive therapy. Primary immunodeficiency  
13 states, whilst themselves rare, have a very high incidence of EBV-induced B-cell lymphomas; these include X-linked  
14 lymphoproliferative syndrome (Duncan syndrome), Wiscott–Aldrich syndrome, ataxia telangiectasia and combined  
15 immunodeficiency disease. These EBV-induced proliferations, initially polyclonal, express B-cell surface markers and do  
16 not have distinctive chromosomal abnormalities but can evolve into aggressive non-HLs. These lesions initially resemble the  
17 lymphoblastoid cell lines (LCLs) generated *in vitro* by infection of B lymphocytes with EBV (Young *et al.*, [1989](#)). Until  
18 recently the majority of these tumours presented in the context of human immunodeficiency virus (HIV) infection but with  
19 the introduction of highly active anti-retroviral therapy the incidence of these lesions has diminished. See also [AIDS:](#)  
20 [Clinical Manifestations](#), [Immunodeficiency](#), [Infections in the Secondary Immunocompromised Host](#), and [Immunodeficiency,](#)  
21 [Primary: Affecting the Adaptive Immune System](#)

## 22 **Burkitt lymphoma and Hodgkin lymphoma**

23 The first lymphoproliferative disease associated with EBV was BL, the most common childhood cancer in the endemic areas  
24 of sub-Saharan Africa, with an incidence of 10 per 100 000 during the first 15 years of life (Thorley-Lawson and Allday,  
25 [2008](#)). Although clonal EBV genomes are present in almost all African BL, they are mostly absent in the sporadic form,  
26 which occurs, rarely, in Europe and North America. For this reason and because of the role of the c-myc proto-oncogene  
27 (thought to be responsible for the neoplasm), EBV is now viewed as a nonessential cofactor. The definitive marker for BL,  
28 regardless of geographical origin and EBV positivity, is a set of specific chromosomal translocations. These result in the  
29 reactivation of the c-myc proto-oncogene at chromosome 8q24 by placing it adjacent to the immunoglobulin regulatory  
30 elements on chromosomes 14, 22 or 2 (Dalla-Favera *et al.*, [1982](#)). Higher EBV titres among children in the endemic area  
31 indicate increased risk for developing BL; however, EBV probably contributes as a cofactor by stimulating B-lymphocyte  
32 proliferation, increasing the likelihood of selection of cells bearing the c-myc translocation.

1 EBV infection is also associated with approximately 40% of HL cases where both viral DNA and latent proteins are  
2 localized to the malignant component of the tumour, the so-called Hodgkin and Reed-Sternberg (HRS) cells. The association  
3 of HL with EBV is age-related; paediatric and older adult cases are usually EBV-associated whereas HL in young adults is  
4 less frequently virus-positive. Although the incidence of HL is relatively low (1–3/100 000 per year), this tumour is not  
5 geographically restricted making its association with EBV significant in world health terms. See also [Chromosome](#)  
6 [Rearrangements](#), and [Oncogenes](#)

## 7 **EBV-associated T- and NK-cell lymphomas**

8 EBV-positive monoclonal lymphomas of either CD4<sup>+</sup> or CD8<sup>+</sup> T cell origin are more frequently found in Southeast Asian  
9 populations arising as a consequence of either virus-associated haemophagocytic syndrome (VAHS) or in the setting of  
10 chronic active EBV infection. A more aggressive extranodal tumour predominantly expressing the CD56 NK marker is also  
11 EBV-positive and manifests as an erosive lesion (lethal midline granuloma) of the nasal cavity.

## 12 **Nasopharyngeal carcinoma and gastric** 13 **adenocarcinoma**

14 The epithelial malignancy NPC, like BL, is characterized by distinct patterns of geographical distribution. It is particularly  
15 common in areas of China and South-East Asia, reaching a peak incidence of 20–30 cases per 100 000 per year and also  
16 occurs with intermediate incidence among Alaskan Inuit people and in several pockets in North Africa. Incidence rates are  
17 particularly high in Cantonese males highlighting an important genetic predisposition as well as a role for environmental  
18 cofactors such as dietary components (e.g. salted fish). Arising in the posterior nasopharynx in the Waldeyer ring, the peak  
19 age for NPC incidence is 40–60 years, mainly in males. The association between EBV and NPC was first suspected from  
20 serological studies, as NPC patients carried extremely high titres of antibodies directed against the EBV early and viral  
21 capsid antigens (EA/VCA). These elevated titres may appear before the tumour develops, a discovery with enormous public  
22 health implications in the endemic area. Widespread EBV antibody screening in parts of Guangdong and Guangxi provinces  
23 in southern China, especially for IgA antibodies against VCA, has significantly increased the NPC early detection rate.  
24 Recent studies have demonstrated that the quantitative analysis of tumour-derived EBV DNA in the blood of patients with  
25 NPC using real-time polymerase chain reaction (PCR) is of both diagnostic and prognostic utility (Chan et al., 2002).

26 The clonal EBV genome is detected in all cells of undifferentiated NPC tissue specimens, regardless of geographic origin, as  
27 well as in premalignant lesions of the nasopharynx (Raab-Traub and Flynn, [1986](#)), but only a handful of EBV latency genes  
28 are expressed. NPC tumours are also characterized by a prominent lymphoid stroma and the interaction between these  
29 activated lymphocytes and adjacent carcinoma cells appears to be crucial for the continued growth of the malignant  
30 component. An aetiologically complex disease, NPC has been linked to several dietary, environmental and genetic cofactors.  
31 Several EBV-inducing tumour-promoting agents have been identified as environmental cofactors in the high-incidence  
32 areas. Detailed analysis of the genetic changes in NPC suggests that some of these (particularly deletions in chromosomes 3p  
33 and 9p) occur early before EBV infection (Lo and Huang, [2002](#)). Thus, EBV may be a tumour promoting agent in NPC and

1 one possibility is that the genetic changes induced by carcinogen exposure create a cellular environment in which EBV  
2 latency can be aberrantly established leading to the outgrowth of transformed cells. See also [Cancer](#), [Cancer Cytogenetics](#),  
3 [Cytogenetic Techniques](#), [Environmental Carcinogens and Mutagens](#), and [Tumour Suppressor Genes](#)  
4 EBV infection is also present in approximately 10% of typical gastric adenocarcinomas, accounting for up to 75 000 cases  
5 per year. These tumours resemble NPC in carrying monoclonal EBV genomes, having a restricted pattern of EBV gene  
6 expression and in the appearance of virus infection as a relatively late event in the carcinogenic process. The geographical  
7 variation in the association of EBV with gastric adenocarcinomas probably reflects ethnic and genetic differences. EBV-  
8 positive tumours have distinct phenotypic and clinical characteristics compared with EBV-negative tumours. See also  
9 [Epstein-Barr Virus and Cancer](#)

## 10 **Rarer EBV-associated tumours**

### 11 **Breast cancer:**

12 Albeit controversial, EBV infection has also been linked to breast cancer incidence, with approximately 30-40% of breast  
13 cancers exhibiting EBV latent gene product expression (Labrecque et al., 1995; Ballard, 2015). By contrast, other studies  
14 failed to detect EBV gene expression in breast cancer tissues (Fuentes-Pananá et al., 2016). To this day, the nature of the  
15 relationship between EBV infection and breast cancer is poorly understood and remains controversial, although evidence  
16 points to an association of EBV infection with more aggressive forms of breast cancer (Mazouni et al., 2011). Moreover, it is  
17 believed there is a correlation between EBV-positivity in breast cancer with mutations in the APOBEC family of cytosine  
18 deaminases – enzymes which ordinarily serve to protect against viral infections (Hu et al., 2016).

19

### 20 **Leiomyosarcoma:**

21 Leiomyosarcomas are extremely rare, differentiated smooth muscle tumours that are associated with EBV infection,  
22 primarily in immunocompromised children, such as transplant recipients, children with congenital immunodeficiencies, and  
23 HIV patients (Lee et al., 1995; Tetzlaff et al., 2011). The presence of the EBV-encoded RNAs (EBERs) in the smooth  
24 muscle tissue suggests that EBV is capable of infecting smooth muscle cells in addition to its normal tissue tropism in B  
25 cells and epithelial cells, findings which are supported by the evidence that the EBV receptor, CR2 (CD21), is also present  
26 on these cells (McClain et al., 1995).

27

### 28 **Oral hairy leukoplakia**

29 Oral hairy leukoplakia (OHL), a benign lesion of the tongue, may be the only EBV-associated disease whose pathology is a  
30 direct result of full-blown viral replication (Greenspan *et al.*, 1985). OHL occurs in up to 25% of patients with acquired  
31 immunodeficiency syndrome (AIDS) and in other immunosuppressed individuals, and can contain multiple strains of EBV.  
32 EBV episomes are not detected, and there are masses of EBV particles in the lesions. The lesions respond well to the  
33 antiviral drug acyclovir.

# 1 EBV-associated autoimmune disorders

2 A number of genetic and environmental factors are involved in the aetiology of systemic autoimmune diseases, including  
3 dysregulation of EBV. The restricted pattern of gene expression observed in latent EBV infection in memory B-cells  
4 enables the virus to evade the host immune defence, which may also lead to initiation of autoimmune diseases or exacerbate  
5 disease progression. Common features of EBV-associated autoimmune disorders such as Multiple Sclerosis (MS),  
6 Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) include elevated titres of EBV antibodies, reduced  
7 anti-EBV T cell responses, and increased viral load.

8 The mechanisms underlying the causal role of EBV in promoting disease onset or progression remains poorly understood.  
9 For example, in MS, it is generally believed that causation is multifactorial, with EBV infection or reactivation representing  
10 one of many causal factors, alongside genetic defects in the immune response to EBV infected B cells, vitamin D deficiency  
11 and molecular mimicry between viral peptides and host epitopes, leading to an autoimmune attack on host cells and their  
12 components (Tao et al., 2017).

## 13 Classification and Structure

14 Sharing nearly indistinguishable morphology, herpesvirus virions are all enveloped, with an amorphous protein tegument  
15 region encasing a 162-capsomer icosahedral capsid. All have double-stranded linear DNA genomes, and, based on their  
16 biological properties, are classified into three subfamilies. EBV and *Human herpesvirus 8* (HHV-8) are the only members of  
17 the *Gammaherpesvirinae* to infect humans routinely. Most likely the causative agent of AIDS-related Kaposi's sarcoma,  
18 HHV-8 has an endothelial cell tropism, and shares EBV's B-cell tropism. See also [Herpesviruses \(Human\)](#)

19 The 184-kb EBV genome is characterized by distinctive sequence reiterations. At both termini are 2–5 copies of a 500-bp  
20 direct repeat unit (the terminal repeat, TR), through which the linear genome circularizes to form the episome, the molecular  
21 basis for latent infection (Figure 3). The genome structure and organization of EBV are most homologous with the other Old  
22 World primate members of its genus *Lymphocryptovirus* (LCV), distinguished by their lymphotropism and propensity to  
23 oncogenicity. A transforming, EBV-related virus has also been isolated from spontaneous B-cell lymphomas of common  
24 marmosets and is thus the first EBV-like virus to be identified in New World primates.

## 25 Virology

26 The EBV genome assumes different forms, depending on the state of infection. During latency, it exists as a chromatin-  
27 associated episome with a varying number of TR units for different clonal isolates. Using host-cell DNA polymerase, each  
28 EBV episome is replicated during the cell cycle, maintaining a constant copy number from one cell generation to the next.  
29 Very few of EBV's 100-odd genes are expressed during latency, all of them localizing to either the nucleus or membrane of  
30 the infected cell. EBNA1 is the only virally encoded gene required for persistence of the episome (Yates *et al.*, 1985), and is  
31 the only EBV protein expressed in all EBV-associated malignancies. A protein of low abundance, EBNA1 maintains EBV

1 latency by binding as a homodimer in a highly sequence-specific manner to a series of repeat units comprising the EBV  
2 origin of plasmid replication, ori-P. A unique structural feature of EBNA1 is the presence of an internal Gly Gly Ala repeat  
3 unit of varying length which serves to prevent recognition of this protein by cytotoxic T lymphocytes (CTLs; Levitskaya *et*  
4 *al.*, 1997). This may contribute to the ability of EBV to persist in memory B cells (Figure 3). See also [Immune Response:](#)  
5 [Evasion and Subversion by Pathogens](#), and [T Lymphocytes: Cytotoxic](#)

6 <Figure 3 near here>

## 7 **Sequence variation**

8 The first complete EBV sequence to be published was the B95.8 laboratory strain in 1984 (Baer *et al.*, 1984), which at the  
9 time was the largest DNA sequence to have been determined and made up 10% of the EMBL data library. This strain of  
10 EBV was isolated from the only EBV cell line available at the time that produced sufficient levels of virus to enable cloning  
11 of the viral restriction fragments; however over the years it has become apparent that there are multiple strains of the virus  
12 worldwide. Broadly speaking, they can be categorised into Type 1 and Type 2 – a classification system based on the gene  
13 sequence of EBV nuclear antigens 2 and 3 (EBNA2 and EBNA3; Tzellos and Farrell, 2012). Type 2 is common in parts of  
14 Africa, whilst Type 1 EBV is the most prevalent strain globally and is more efficient at transforming B cells into LCLs *in*  
15 *vitro*. It has long been hypothesised that the genetic variation in EBV strains across different geographical regions may  
16 account for the substantial geographic differences in EBV-associated disease incidence (Jenkins and Farrell, 1996). In  
17 support of this hypothesis is the recent findings from Tsai *et al.*, (2017), which mapped out the different biological properties  
18 of particular EBV strains to explain their association with different EBV-associated cancers. For example, the YCCEL1  
19 EBV strain isolated from a gastric carcinoma and the M81 strain isolated from a nasopharyngeal carcinoma both infected  
20 primary epithelial cells, gastric carcinoma cells and gastric spheroids more efficiently than the well-characterised Akata or  
21 B95.8 laboratory strains (Tsai *et al.*, 2017). Furthermore, interestingly there is a high frequency of co-infection with both  
22 Type 1 and Type 2 virus strains in MS patients, suggesting a synergistic or increased risk association in individuals  
23 harbouring more than one virus strain, presumably having undergone more than one primary infection event (Santón *et al.*,  
24 2011). In addition to this broad distinction between EBV Types 1 and 2, there is also minor heterogeneity within each virus  
25 type particularly within the latent membrane protein 1 (LMP1) gene. Although strong biological evidence is lacking, it  
26 remains possible that variation in EBV genes within different virus strains can contribute to the risk of developing virus-  
27 associated tumours.

## 28 **Viral Gene Expression during EBV Latency**

29 Only a small fraction of the genes encoded by EBV is expressed during latency because transcription in latently infected  
30 cells is tightly regulated and restricted to a small portion of the genome. Four latency types are distinguished by the number  
31 of EBV latency proteins made. They range from the EBNA1-only type I latency found in the majority of BL tumours to type  
32 III latency in EBV-transformed LCLs, in which all of the known latent genes are expressed. Latency type 0 is characterised  
33 by the lack of all viral gene expression observed in non-dividing B cells. The gene expression and viral promoter usage



1 characteristic of type III latency is accompanied by a lymphoblastoid pattern of cellular gene expression, which includes  
2 high levels of the B-cell activation antigens and cellular adhesion molecules. The first proteins expressed after primary EBV  
3 infection of B lymphocytes *in vitro*, which results in type III latency, are EBNA2 and EBNA-LP (leader protein). Within  
4 48 h the other EBNA genes, including EBNA1 and a pair of nonpolyadenylated ribonucleic acid (RNA) polymerase III  
5 transcripts, the Epstein–Barr-encoded RNAs (EBERs), are expressed. The transcripts encoding all of the EBNAs are highly  
6 spliced, and can arise from either of the two promoters, Wp and Cp (Figure 2a). Concomitant with EBNA expression,  
7 latently infected cells also express two membrane proteins: (i) LMP1, EBV's principal oncoprotein and (ii) LMP2A and  
8 LMP2B which regulate virus latency. The promoters for the LMP proteins are EBNA-2 responsive and are probably  
9 coordinately regulated. Thus the full range of latency genes plus the EBERs are expressed in type III latency as observed in  
10 LCLs and EBV-induced lymphoproliferations *in vivo*.

11 At the other extreme is type I latency which is observed in fresh BL biopsies, with expression of EBNA1 and the EBERs  
12 only. In this situation EBNA1 expression is driven from a different promoter (Qp) compared to that used in type III latency  
13 (Cp/Wp). This differential promoter usage is regulated by epigenetic modification of various EBV promoters. Recently, a  
14 novel form of EBV latency has been found in some BL biopsies where deletion of EBNA2 has occurred and EBNA  
15 expression is driven from the Wp promoter (Kelly *et al.*, 2002). Type II latency is observed in NPC, and HL is restricted to  
16 expression of EBNA1 along with LMP1 and LMP2A/B. However, LMP1 expression at the protein level is only observed in  
17 approximately 40% of NPC biopsies and these tumours also display high levels of BART transcripts and the BamHIA-  
18 encoded protein 1 (BARF1).

## 19 **Function of Key EBV Latent Genes**

20 Several of the latent gene products exert profound effects on cellular gene expression, perturbing the network of cellular  
21 factors controlling cell growth. EBNA2 is a transcription factor, activating an array of viral and cellular genes. It does not  
22 directly bind DNA but is targeted to specific DNA sequences by the cellular protein RBP-Jκ (recombination signal  
23 sequence-binding protein-J kappa), and is associated with other cellular proteins within the transcription complex (Ling *et*  
24 *al.*, 1993). Interestingly EBNA2 is functionally homologous to the *Drosophila* Notch-1 factor. See also [Drosophila](#)  
25 [Patterning: Delta–Notch Interactions](#)

26 Apart from its effect in the replication and maintenance of the episomal EBV genome, EBNA1 may also contribute directly  
27 to oncogenesis via the modulation of various cell signalling pathways. Thus, EBNA1 has been shown to promote survival in  
28 BL cells and, via interaction with a cellular ubiquitin-specific protease, USP7 or HAUSP, promotes disruption of PML  
29 (promyelocytic leukaemia protein) nuclear bodies thereby affecting the efficiency of DNA repair.

30 LMP1 is the major transforming protein of EBV behaving as a classical oncogene in rodent fibroblast transformation assays  
31 and being essential for EBV-induced B-cell transformation *in vitro* (Wang *et al.*, 1985). LMP1 functions as a constitutively  
32 activated member of the tumour necrosis factor receptor (TNFR) superfamily activating a number of signalling pathways in  
33 a ligand-independent manner via engagement with TNFR-associated factors (TRAFs; Kilger *et al.*, 1998; Mosialos *et al.*,

1 [1995](#)). The LMP1 protein activates a number of downstream signalling pathways (e.g. NF- $\kappa$ B (necrosis factor  $\kappa$ B), ERK-  
2 MAPK (extracellular signal-regulated kinase/mitogen-activated protein kinase), phosphatidylinositol 3-kinase, activin A  
3 and/or TGF $\beta$ ) that contribute to the many growth-promoting and phenotypic consequences of LMP1 expression including  
4 the induction of various antiapoptotic and cytokine genes, influencing cell motility (Morris et al., 2009; 2016). Activation of  
5 these signalling pathways is essential for *in vitro* immortalization of B cells by EBV, and is likely to contribute to the  
6 initiation of EBV-associated malignancies *in vivo*.

7 The gene encoding LMP2 yields two distinct proteins, LMP2A and LMP2B. LMP2A can drive the proliferation and survival  
8 of B cells in the absence of signalling through the B-cell receptor (BCR). It is also able to prevent the inappropriate  
9 activation of the EBV lytic cycle. A modulatory role for LMP2B in regulating LMP2A function has been suggested  
10 (Longnecker, [2000](#)). The precise contribution of LMP2A/B to the pathogenesis of NPC remains unknown.

11 Transcripts from the BamHIA region of the EBV genome are highly expressed in NPC and EBV-positive gastric carcinoma.  
12 These encode a set of miRNAs (micro RNAs; BARTs) which are evolutionarily conserved and have been found to play a  
13 role in modulating LMP1 expression (Pfeffer *et al.*, [2004](#)). EBV also encodes 25 viral precursor miRNAs within its genome  
14 that are expressed during both lytic and latent infection. The virus also induces the expression of some cellular oncogenic  
15 miRNAs, including miR-155, miR-146a and miR-21, amongst others, that facilitate persistence of latently infected cells  
16 (Skalsky, 2017). BARF1 is also expressed from this region of the EBV genome and encodes a secreted protein with  
17 oncogenic activity (Seto *et al.*, [2008](#)).

## 18 **Viral Gene Expression during EBV Lytic** 19 **Replication**

20 Although, the biological signals triggering productive infection are unknown, it is widely acknowledged that viral reactivation  
21 often precedes disease onset, and there is some evidence in elite athlete cohorts to suggest that high intensity exercise and the  
22 subsequent stress on the immune system and T cell exhaustion this causes may trigger EBV lytic replication (Gleeson et al.,  
23 2002; 2017).

24 *In vitro*, viral replication can be chemically induced in latently infected B cells by phorbol esters, sodium butyrate or  
25 treatment with anti-immunoglobulin-G (anti-IgG). Superinfection or transfection with the BZLF1 (BamHIZ-encoded protein  
26 1) activation gene will also induce a fraction of the cells into productive replication (Countryman and Miller, [1985](#)), but  
27 there is no permissive experimental cell system for EBV growth or virus quantitation.

28 Unlike EBV's extensively spliced gene transcripts expressed during latency, the lytic cycle gene transcripts are very  
29 compact, with relatively small introns. In productive infection, the 'immediate early' class of genes is the first to be  
30 expressed, so designated because their transcription is not dependent on *de novo* protein synthesis. Lytic induction triggers a  
31 cascade of gene expression of both early viral genes and cellular genes, comprising the viral replicative cycle. Over-  
32 expression of the BZLF1 protein (also known as Z, Zta, EB1 and Zebra) in lymphoid cells is alone sufficient to disrupt

1 latency by transactivating an array of viral promoters containing Z-responsive elements. In epithelial cells, the BRLF-1 gene  
2 product by itself can readily trigger the lytic cycle. [Recent evidence suggests that LMP1 can promote efficient lytic](#)  
3 [reactivation in EBV-infected epithelial cells by augmenting expression of the immediate early proteins BZLF1 and BRLF1,](#)  
4 [and that LMP1 expression itself is induced by epithelial cell differentiation via the transcription factors KLF4 and BLIMP1](#)  
5 [\(Nawandar et al., 2017\).](#)

6 The 'early' genes, whose expression is triggered by the products of the 'immediate early' genes, are all involved with viral  
7 DNA replication. These early antigens include a viral DNA polymerase, processivity factor, helicase, primase,  
8 ribonucleotide reductase, thymidine kinase and other DNA-binding proteins that aid in replication. Because they are unique  
9 to viral DNA replication, several of them are useful targets for antiviral drugs. Lytic replication of unit-length linear  
10 genomes is initiated at two copies of ori-lyt (Figure 2b), which like the SV40 papovavirus origin, contains promoter and  
11 enhancer regions. Following viral DNA synthesis, the early gene products are thought to activate the 'late' or structural  
12 genes. To complete the cycle, nucleocapsids are assembled and enveloped virus particles are released by cell lysis. See also  
13 [DNA Plant and Animal Virus Replication](#), and [Eukaryotic DNA Polymerases](#)

## 14 Treatment and Control

15 Therapeutic approaches are mostly hypothetical compared to the effectiveness of antiviral therapy for several other human  
16 herpesviruses. A number of drugs inhibit EBV replication in cell culture, including the pyrophosphate analogues  
17 phosphonoformic acid (PFA) and phosphonoacetate (PAA), and the nucleoside analogues ara-A, ganciclovir, acyclovir,  
18 FIAC (1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-5-iodocytosine) and HPMPC ((S)-1-(3-hydroxy-2-  
19 phosphonylmethoxypropyl)cytosine), all of which inhibit the virus-encoded DNA polymerase. Acyclovir suppresses viral  
20 replication *in vivo*, but it has been largely ineffective as a treatment for IM in clinical trials, possibly because most of the  
21 symptoms of IM are caused by secondary immunopathological responses. Success in treatment of IM will probably hinge on  
22 earlier diagnosis and use of higher drug dosage, which can be achieved with an acyclovir prodrug. Most EBV-infected  
23 tumour cells are latently infected, and would therefore be unaffected by any antiviral agents that inhibit viral replication and  
24 virus production. The only EBV disease which clearly responds to antiviral therapy is OHL, perhaps because it is an acute  
25 disease based almost entirely on active viral replication and not latent infection (Israel and Kenney, 2003). See also [Antiviral](#)  
26 [Drugs](#)

27 Demethylating agents such as 5'-azacytidine are able to derepress lytic, as well as potentially immunogenic, latent genes and  
28 are currently in early stage clinical trials in patients with NPC, HL and AIDS-associated lymphoma (Chan *et al.*, 2004).  
29 Hydroxyurea is a chemotherapeutic agent that can induce loss of EBV episomes and has shown some clinical efficacy in  
30 patients with EBV-positive AIDS-related central nervous system (CNS) lymphoma. Other approaches are based on the more  
31 specific targeting of individual EBV proteins using either single chain antibodies, siRNA (small interference RNA) or  
32 dominant negative molecules. The use of adoptive EBV-specific T cell therapy for the treatment of existing EBV-induced B-  
33 cell lymphomas and in the prophylactic setting has been extremely successful (Rooney *et al.*, 1995). This approach is also

1 showing signs of clinical efficacy in the treatment of NPC and HL but in this setting the use of T cells enriched for  
2 reactivities to subdominant targets (e.g. LMP2A and EBNA1) is important.

### 3 **EBV vaccine update**

4 More direct vaccine approaches to treat patients with EBV-associated tumours or to prevent disease development are being  
5 examined including: (i) whole EBV latent antigens delivered by a virus vector or in autologous dendritic cells; (ii) peptide or  
6 polytope vaccination and (iii) prophylactic vaccination against the MA (gp350/220) to generate a protective neutralizing  
7 antibody response, which in phase 2 clinical trials reduced the rate of IM but not of virus infection (Sokal *et al.*, [2007](#);  
8 [Cohen, 2015](#)). One promising alternative approach for an EBV vaccine is the production of virus-like particles in which  
9 several latent gene products are functionally lost, including EBNA2, 3A, 3B, 3C, LMP1 and BZLF1, and which also lacks  
10 the TR packaging element required for packaging viral DNA (Ruiss *et al.*, 2011). The resulting virus-like particles do not  
11 possess detectable EBV DNA, yet vaccination of mice with these particles still induced EBV-neutralising antibodies and  
12 cellular immune responses. Since EBV is associated with nearly 200,000 new malignant diagnoses per annum worldwide,  
13 there is a strong case to continue pursuing an EBV vaccine to prevent these and other EBV-associated diseases, with great  
14 potential for economic and societal benefit.

## 15 **Future Perspectives**

16 EBV was discovered over 40 years ago and its DNA was fully sequenced in 1984. Much work has contributed to the  
17 unequivocal identification of EBV as oncogenic in humans and it remains the most efficient agent for transforming human  
18 cells *in vitro*. In recent years, the nature of the interaction of EBV with the immune host has become clearer illustrating the  
19 complex mechanisms that the virus exploits to persist in the memory B cell pool. The development of more efficient *in vitro*  
20 systems for studying EBV infection and replication in different cell types will help to unravel the complex interplay between  
21 the virus and the cell. The use of EBV recombinants continues to shed light on the role of latent genes in the transformation  
22 process and on the requirements for the efficient production of progeny virus. Understanding the host cell:virus interaction  
23 will be dependent on the generation of appropriate *in vitro* models particularly systems which allow a more detailed  
24 understanding of the tumour microenvironment and the role of the local cytokine milieu. There are many interesting aspects  
25 of EBV that remain to be understood including the role of virus-encoded miRNAs and the possible contribution of lytic  
26 cycle antigens to virus persistence and oncogenesis. The challenge will be to exploit these new mechanistic insights both to  
27 gain a better understanding of EBV infection *in vivo* and to develop novel therapies for treating virus-associated disease.

### 28 **End Notes**

29 Based in part on the previous version of this Encyclopedia of Life Sciences (ELS) article, *Epstein–Barr Virus* by  
30 Lawrence S Young, Nancy S Sung, Joseph S Pagano.

### 31 **Glossary**

32 **APOBEC**

1 “Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like” – A family  
2 of cytidine deaminase enzymes involved in generating protein diversity via mRNA  
3 editing.

4 **BARF1**

5 A protein encoded by the BamHIA region of the EBV genome.

6 **BART**

7 Abundant RNA transcripts encoded by the BamHIA region of the EBV genome that  
8 produce miRNAs.

9 **BZLF1**

10 A protein encoded from the BamHI Z region of the EBV genome which is an  
11 immediate early protein responsible for the switch between latent and lytic infection.

12 **Cell transformation**

13 A change to a state of unrestricted growth in culture, resembling the tumourigenic  
14 condition.

15 **Clonal tumour**

16 Malignancy in which all cells share a single common genetic ancestor.

17 **EBNA**

18 *Epstein–Bar virus*-encoded nuclear antigen.

19 **Episome**

20 A circular extrachromosomal DNA element which can associate with the  
21 chromosomal DNA of the host.

22 **Latency**

23 The phase in the viral life cycle in which the virus persists in the host cell without  
24 making virions.

25 **LMP**

26 EBV-encoded latent membrane protein.

27 **Lytic infection**

28 The phase in the viral life cycle in which virions are produced and released,  
29 destroying the host cell.

30 **miRNA**

31 Small noncoding RNA molecules that posttranscriptionally control gene expression.

32 **Terminal repeats**

33 Repetitive units of DNA sequence situated at the ends of a linear viral genome.

34

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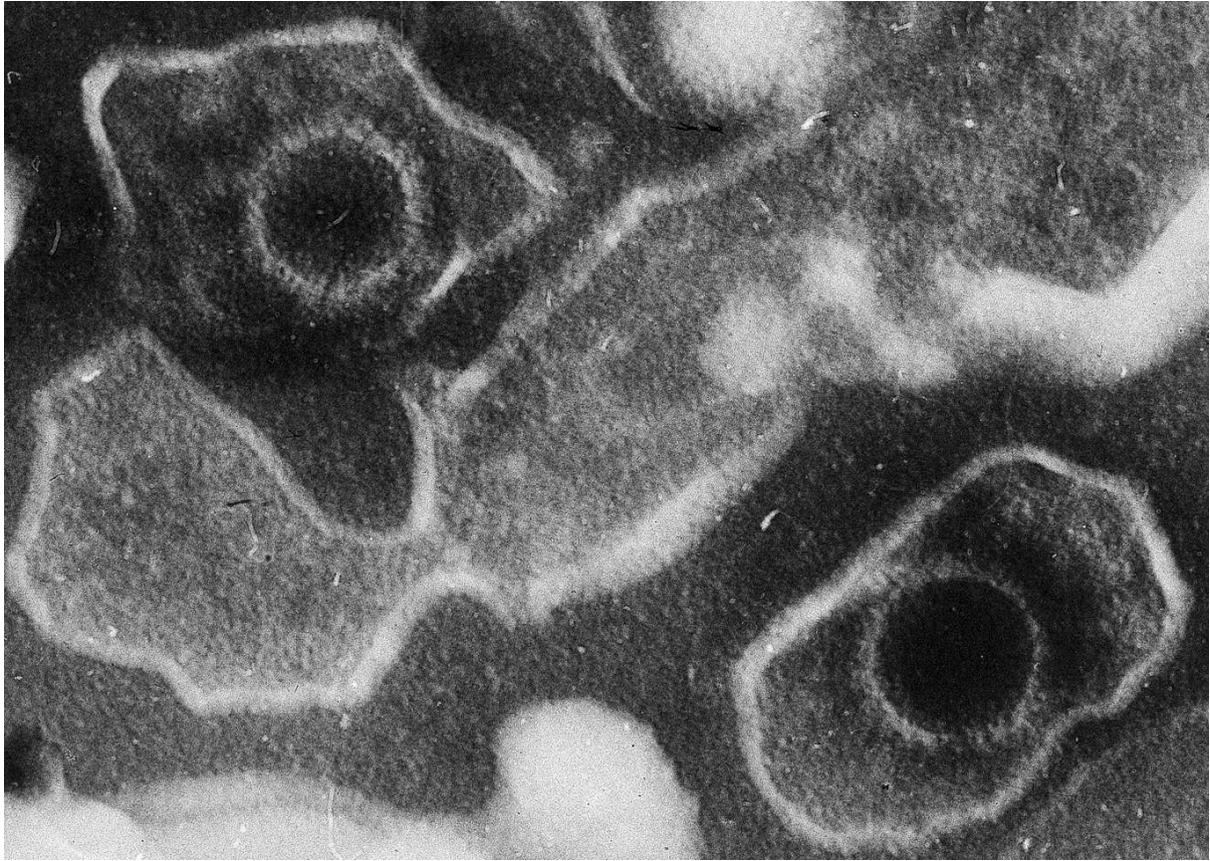
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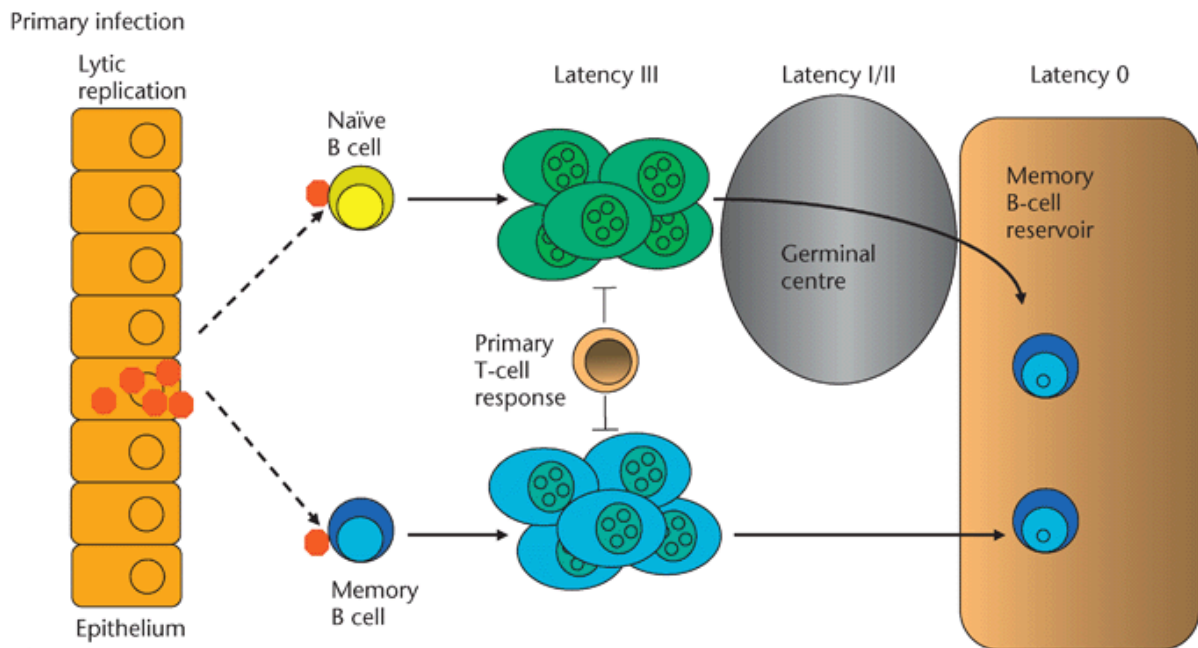
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7 **Figure 1.** Electron micrograph of the *Epstein–Barr virus* virion.

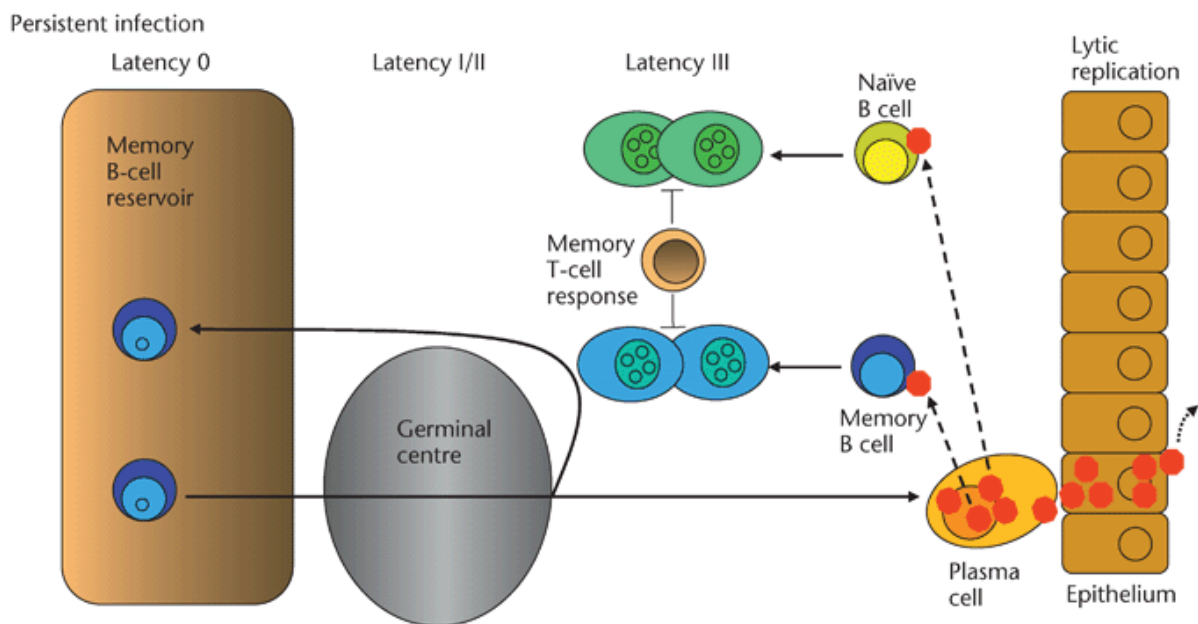
**Table 1** – Diseases associated with *Epstein-Barr virus* (EBV) infection

<b>Tissues affected and diseases</b>	<b>Role of EBV</b>
<i>Lymphoid</i>	
Burkitt Lymphoma	Cofactor, nonessential; clonal association <sup>a</sup>
B-cell immunoblastic lymphomas	Causal
T-cell lymphomas	Clonal association <sup>a</sup>
Hodgkin disease	Clonal association <sup>a</sup> , Reed-Sternberg cells infected
Chronic interstitial lymphocytic pneumonitis	Causal
<i>Epithelial</i>	
Nasopharyngeal carcinoma	Essential cofactor
Parotid carcinoma	Clonal association <sup>a</sup>
Gastric carcinoma	Clonal association <sup>a</sup>
Oral hairy leukoplakia	Causal
<i>Lymphoid and epithelial</i>	
Infectious mononucleosis	Causal
<i>Other</i>	
Leiomyosarcoma	Clonal association <sup>a</sup>

<sup>a</sup>Clonal association refers to episomes which reflect clonality of tumour cells in which they are found, with differences in the number of terminal repeats (TRs; **Figure 3**) characterising different ‘clones’.



(a)

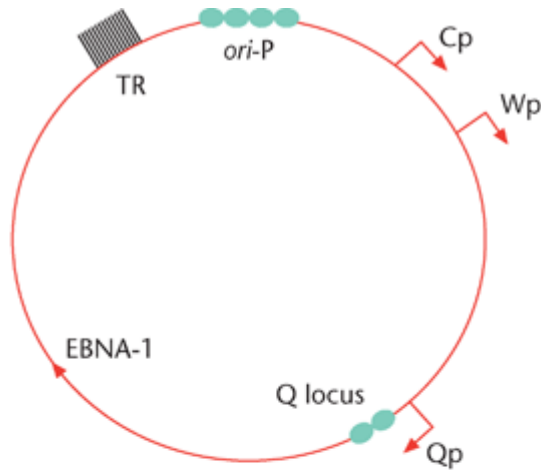


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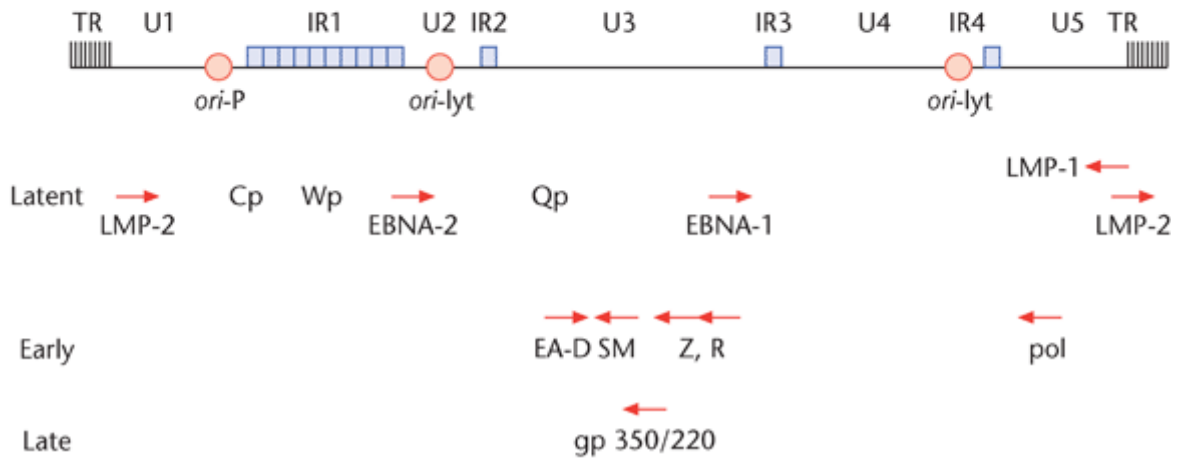
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2

3 **Figure 2.** EBV primary infection and persistence. Figure showing putative *in vivo*  
 4 interactions between EBV and host cells. (a) Primary infection. EBV replicates in epithelial  
 5 cells and spreads to lymphoid tissues as a latent growth-transforming (latency III) infection of  
 6 B cells. Many infected B cells are removed by emerging EBV-specific T-cell response. Some  
 7 infected cells escape by downregulating EBV latent genes to establish a stable pool of resting  
 8 virus-positive memory B cells. (b) Persistent infection. EBV-infected memory B cells  
 9 become subject to the physiological controls governing memory B-cell migration and  
 10 differentiation. Occasional recruitment into germinal centre (GC) reactions resulting in

1 activation of different EBV latency programmes and reentry into the memory cell reservoir or  
 2 plasma cell differentiation with activation of virus lytic cycle. Infectious virions then initiate  
 3 foci of EBV replication in epithelial cells and also new growth-transforming infection of  
 4 naïve and/or memory B cells. For more detailed explanation see Young and Rickinson [2004](#)  
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 7 permission from Elsevier.



(a)



8 (b)

9 **Figure 3.** *Epstein–Barr virus* (EBV) genome forms. (a) The episome, in which the EBV  
 10 genome circularizes via the terminal repeats (TR), is the hallmark of EBV latency. EBNA1 is  
 11 the principal EBV latency protein. EBNA promoters are shown (Cp, Wp, Qp), as well as the  
 12 EBNA1 open reading frame (triangle). Shaded circles represent the two areas of the EBV

1 genome where EBNA1 dimers bind. Two binding sites are found in the Q locus, whereas 24  
2 binding sites are found within the plasmid origin of replication (*ori-P*). EBNA-1 activates *ori-*  
3 *P* and autoregulates *Qp*. (b) The linear EBV genome is diagnostic of productive infection.  
4 The positions of terminal (TR) as well as internal direct (IR) repeat units are shown. Regions  
5 of unique sequence are designated U. Latent and lytic (*ori-lyt*) origins of DNA replication are  
6 shown as open circles. Arrows pointing rightward or leftward indicate the location and  
7 direction of transcription of the coding sequences of the EBV genes mentioned in the text.  
8