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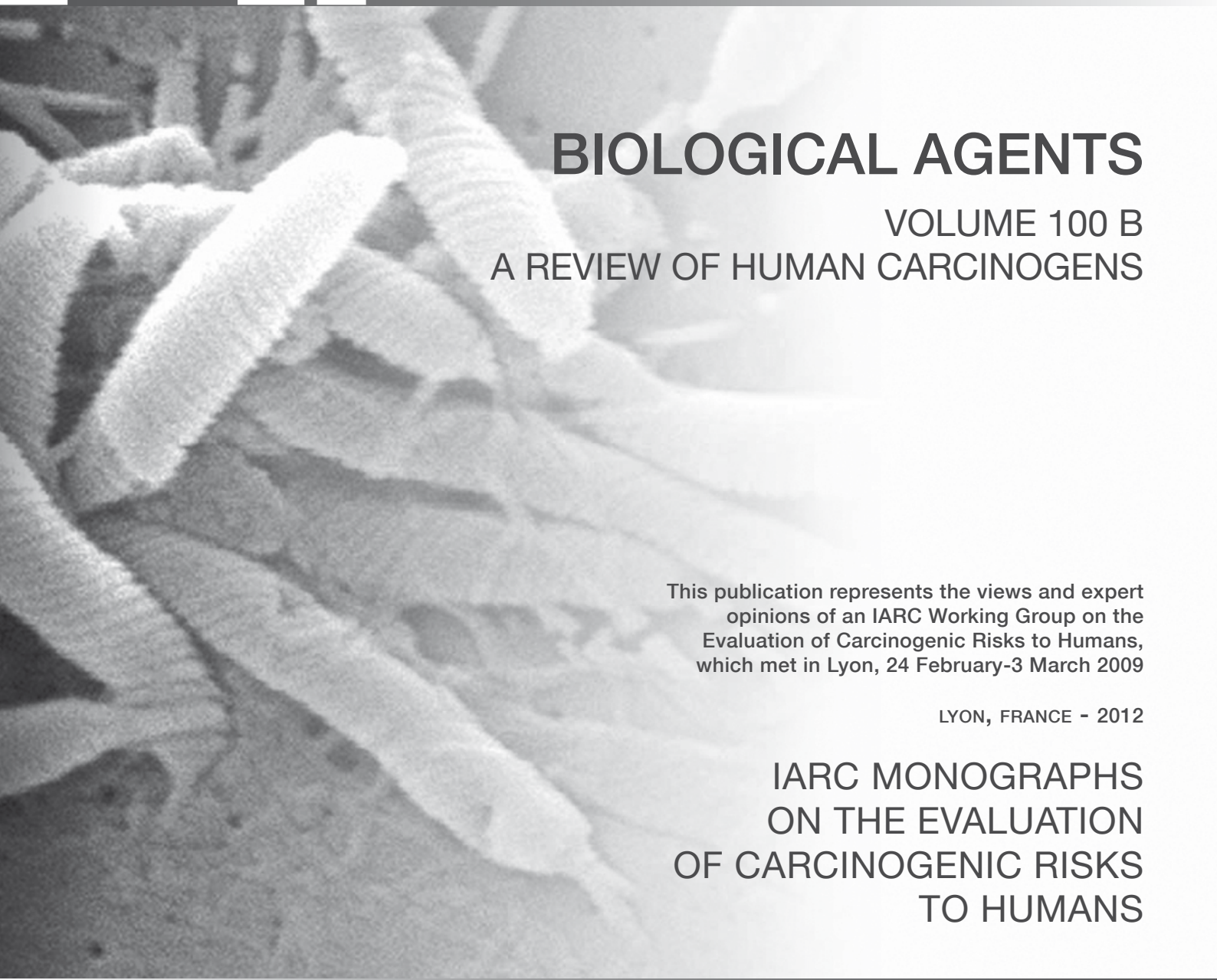

BIOLOGICAL AGENTS

VOLUME 100 B
A REVIEW OF HUMAN CARCINOGENS

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 24 February-3 March 2009

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TO HUMANS

International Agency for Research on Cancer
World Health Organization

EPSTEIN-BARR VIRUS

The Epstein-Barr virus was considered by a previous IARC Working Group in 1997 (IARC, 1997). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Taxonomy, structure, and biology

1.1.1 Taxonomy

The Epstein-Barr virus (EBV), the first isolated human tumour virus, was identified in 1964 by Epstein's group in a cell line derived from Burkitt lymphoma (Epstein *et al.*, 1964). EBV is a human herpesvirus, classified within the gammaherpesviruses subfamily, and is the prototype of the *Lymphocryptovirus* genus. In keeping with the systematic nomenclature adopted for all human herpesviruses, the formal designation of EBV is human herpesvirus 4 (HHV-4).

Two major EBV types have been detected in humans: EBV-1 and EBV-2 (also known as types A and B). EBV-1 and EBV-2 differ in the sequence of the genes that code for the EBV nuclear antigens (EBNA-2, EBNA-3A/3, EBNA-3B/4, and EBNA-3C/6) (Sample *et al.*, 1990). EBV-2 immortalizes B cells less efficiently than EBV-1 *in vitro*, and the viability of EBV-2-infected lymphoblastoid cell lines is less than that of EBV-1-infected lines (Rickinson *et al.*, 1987). The differences in the immortalizing efficiency of the EBV subtypes may relate to a divergence in the EBNA-2 sequences (Cohen *et al.*, 1989).

In addition to type-specific polymorphism, significant DNA-sequence heterogeneity has been found when comparing selected regions of the EBV genome isolated in certain geographic areas or even from the same area. These polymorphisms define different viral strains within both types (Aitken *et al.*, 1994).

1.1.2 Structure of the virion

Like other herpesviruses, EBV is a DNA virus with a toroid-shaped protein core that is wrapped with DNA, a nucleocapsid with 162 capsomers, a protein tegument between the nucleocapsid and the envelope, and an outer envelope with external virus-encoded glycoprotein spikes (Liebowitz & Kieff, 1993).

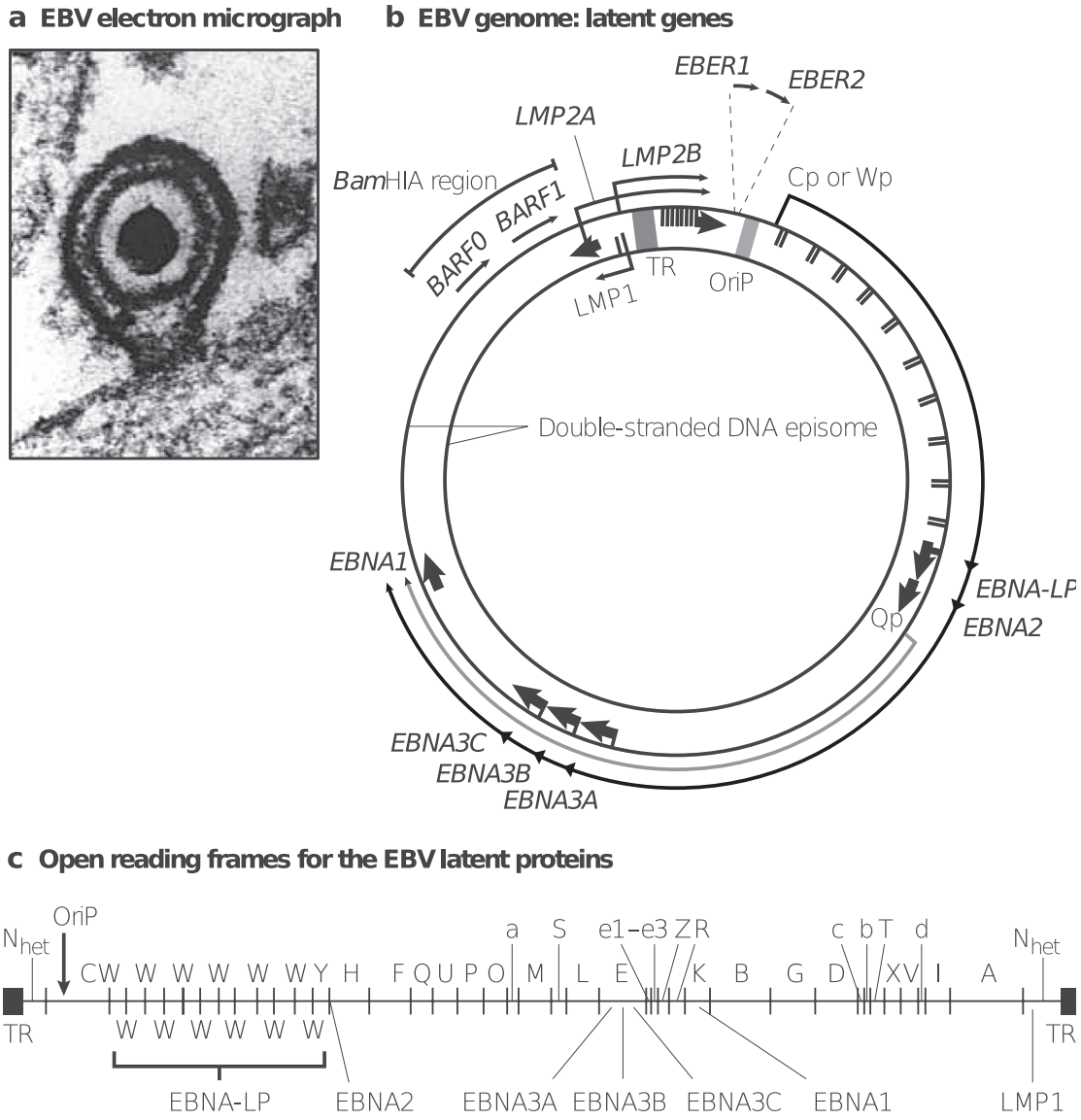
1.1.3 Structure of the viral genome

The EBV genome is a linear, double-stranded, ~172-kb DNA molecule that encodes > 85 genes (Fig. 1.1).

The nomenclature for EBV open-reading frames (ORFs) is based on the *Bam*HI-restriction fragment in which they are found. For example, the *BARF1* ORF is found in the *Bam*HI A fragment, and extends rightwards (Fig. 1.1).

The many EBV ORFs are divided into latent and lytic genes (further divided into immediate

Figure 1.1 The Epstein-Barr virus genome



a Electron micrograph of the Epstein-Barr virus (EBV) virion
b Diagram showing the location and transcription of the EBV latent genes on the double-stranded viral DNA episome
c Location of open reading frames of the EBV latent proteins on the Bam H1 restriction-endonuclease map of the prototype B95.8 genome
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Table 1.1 Examples of identified EBV gene products and their open reading frames

Open reading frame	Protein		Main proposed function
	Common name	Alternative nomenclature	
Latent genes			
BKRF1	EBNA-1[a]		Plasmid maintenance, DNA replication transcriptional regulation
BYRF1	EBNA-2[a]		*trans*-activation
BERF1	EBNA-3A[a]	EBNA-3	Transcriptional regulation
BERF2	EBNA-3B[a]	EBNA-4	Unknown
BERF3/4	EBNA-3C[a]	EBNA-6	Transcriptional regulation
BWRF1	EBNA-LP[a]	EBNA-5	*trans*-activation
BNLF1	LMP-1[a]		B-cell survival, anti-apoptosis
BNRF1	LMP-2A[a]/2B	TP1/2	Maintenance of latency
BARF0			Not shown to be translated, unknown function
EBER1/2			Non-translated, regulation of innate immunity
Lytic genes			
Immediate early genes			
BZLF1	ZEBRA		*trans*-activation, initiation of lytic cycle
BRLF1			*trans*-activation, initiation of lytic cycle
BI'LF4			*trans*-activation, initiation of lytic cycle
Early genes			
BMRF1			*trans*-activation
BALF2			DNA binding
BALF5			DNA polymerase
BORF2			Ribonucleotide reductase subunit
BARF1			Ribonucleotide reductase subunit
BXLF1			Thymidine kinase
BGLF5			Alkaline exonuclease
BSLF1			Primase
BBLF4			Helicase
BKRF3			Uracil DNA glycosylase
Late genes			
BLLF1	gp350/220		Major envelope glycoprotein
BXLF2	gp85 (gH)		Virus–host envelope fusion
BKRF2	gp25 (gL)		Virus–host envelope fusion
BZLF2	gp42		Virus–host envelope fusion, binds MHC class II
BALF4	gp110 (gB)		Unknown
BDLF3	gp100–150		Unknown
BILF2	gp55–78		Unknown
BCRF1			Viral interleukin-10
BHRF1[ab]			Viral *bcl-2* analogue

[a] Gene products involved in immortalization and/or other aspects of tumour cell phenotypes
[b] Expressed in latently infected cells as well
EBNA, EBV nuclear antigen; LP, leader protein; LMP, latent membrane protein; ZEBRA, Z EBV replication activation; gp, glycoprotein; MHC, major histocompatibility complex
Compiled from Liebowitz & Kieff (1993), Li *et al.* (1995), Nolan & Morgan (1995), Thompson & Kurzrock (2004)

early genes, early genes, and late genes). Most of these genes are translated into proteins whose main proposed functions are listed in Table 1.1. Several lytic genes encode for human homologues (Table 1.2). In addition, some latent genes are non-translated; this is the case for *EBV-encoded RNA* (*EBER)-1* and *-2* (Kieff, 1996; Kieff & Rickinson, 2001). EBV also encodes at least 17 micro-RNAs, arranged in two clusters: ten are located in the introns of the viral *BART* gene, and three adjacent to *BHRF1* (Cai *et al.*, 2006).

The viral genome also contains a series of 0.5-kb terminal direct repeats at either end and internal repeat sequences that serve to divide the genome into short and long unique sequence domains that have most of the coding capacity (Cheung & Kieff, 1982). These terminal repeats are good markers to determine if EBV-infected cells are from the same progenitor: when EBV infects a cell, the viral DNA circularizes and mainly persists as a circular episome with a characteristic number of terminal repeats that depends on the number of terminal repeats in the parental genome, with some variation introduced during viral replication. If the infection is permissive for latent infection but not replication, future generations will have EBV episomes with the same number of terminal repeats (Raab-Traub & Flynn, 1986).

1.1.4 Host range

Although herpesviruses are ubiquitous in nature, humans serve as the only natural host for EBV. Almost all higher primates have their own EBV-like virus. Antibodies to EBV have been detected in several primate species, probably due to the presence of cross-reactive antibodies against their own species-specific EBV homologues (Kieff *et al.*, 1979). Infection of newborn marmosets with EBV resulted in the establishment of a long-term permissive infection, indicating similarities in the responses of marmosets and humans to EBV (Cox *et al.*, 1996).

1.1.5 Target cells

Like other gammaherpesviruses, EBV establishes latent infection in lymphocytes and can induce proliferation of the latently infected cells (Young & Rickinson, 2004). EBV infection of B cells is mediated through the interaction of the viral envelope glycoprotein gp350/220 with the cellular receptor for the C3d complement component CR2 (CD21) (Fingeroth *et al.*, 1984, 1988; Tanner *et al.*, 1987). After binding of the viral particle to the surface of the host cell and endocytosis, the viral envelope fuses with the host-cell membrane by a mechanism involving three other viral glycoproteins: gp85, gp25, and gp42 (Li *et al.*, 1995). It is worth noting that gp42 can bind to major histocompatibility complex (MHC) class II, and EBV uses this as a cofactor in the infection of B lymphocytes (Li *et al.*, 1997).

It has been shown nonetheless that EBV can also infect cells, albeit at low efficiency, via CD21-independent mechanisms. Indeed, cells that do not express CD21 (as some epithelial cells) can be infected by the virus, and furthermore a virus deficient in gp350/220 was shown to be still infectious (Imai *et al.*, 1998; Janz *et al.*, 2000).

Although EBV is considered to be a B-lymphotropic virus, it can also infect T lymphocytes or epithelial cells because it is found in some T-lymphoma cells and several important diseases of epithelial cells, including nasopharyngeal and gastric carcinomas, and oral hairy leukoplakia (Thompson & Kurzrock, 2004). Other CD21-independent pathways may be responsible for EBV infection of cells other than B lymphocytes (Imai *et al.*, 1998; Janz *et al.*, 2000).

Current evidence suggests that EBV infection in healthy chronic virus carriers is largely restricted to B cells, although in certain situations the virus can be detected in epithelial cells. The most likely role for epithelial cells is as a site for replication and amplification of EBV rather than as a site of persistent latent infection,

Table 1.2 Homology of EBV gene products with human proteins

Viral gene	Human homologue	Functional similarity established
BCRF1	Interleukin 10	Yes
BDLF2	Cyclin B1	No
BHRF1	BCL-2	Yes
BALF1	BCL-2	No
BARF1	C-FMS receptor	Yes
	ICAM-1 (CD54)	No

Amino acid homology between viral and human product varies from ~20% to >80%.
Adapted from Thompson & Kurzrock (2004)

however, this remains controversial (Kieff, 1996) (see Section 1.1.6 and Fig. 1.2).

1.1.6 Viral life cycle

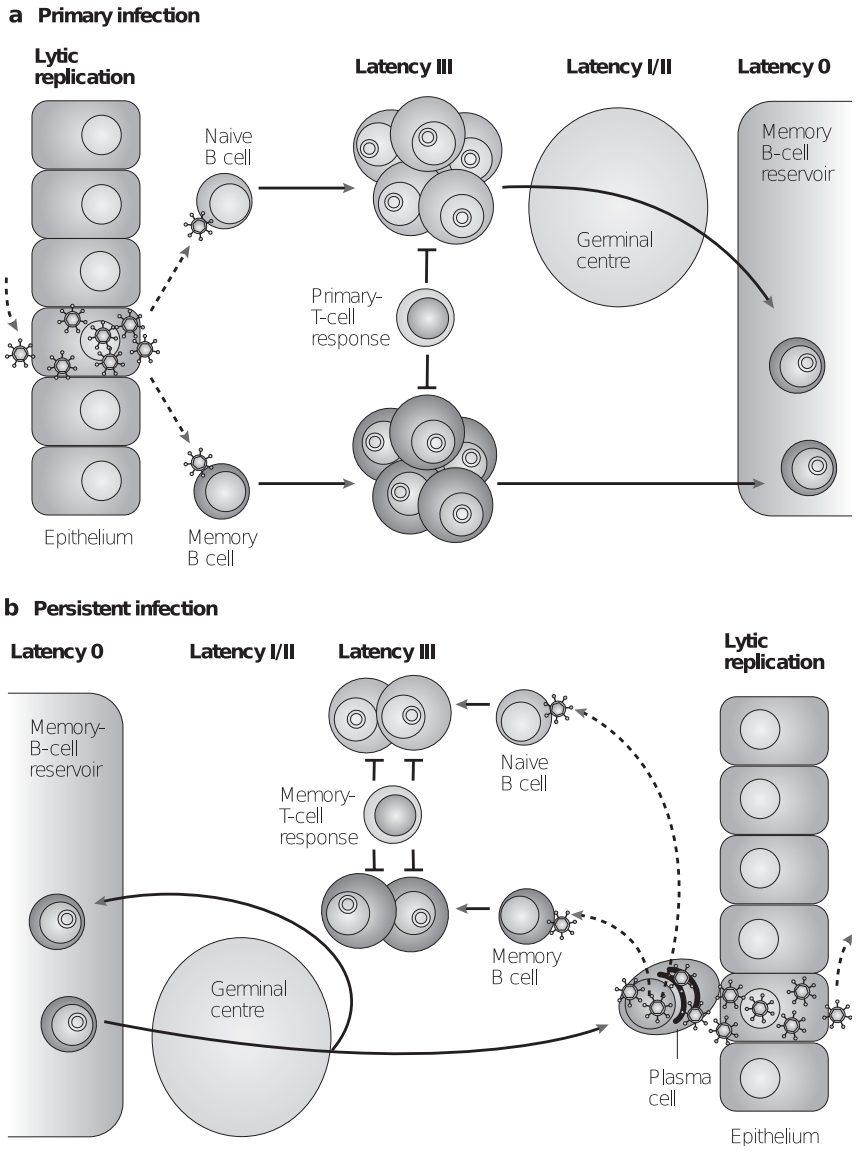
EBV, probably the most potent transforming human virus in culture, is nonetheless known to infect and persist for life in > 90% of human adults without causing disease.

Several reviews have described how EBV exploits the physiology of the normal B-cell differentiation, and uses different combinations of latent viral gene expression to progress from initial infection to long-term persistence within the memory B-cell pool of the immunocompetent host (Thorley-Lawson & Gross, 2004; Young & Rickinson, 2004; Thorley-Lawson & Allday, 2008).

Fig. 1.2 depicts the putative interactions between EBV and its host. EBV spreads via the saliva entering the epithelium of the Waldeyer tonsillar ring situated in the oropharynx where it probably initiates a lytic infection that leads to amplification of the virus. The virus then infects naïve B cells in the underlying lymphoid tissues, to become activated lymphoblasts using the growth transcription programme (latency III). Three of the growth-programme proteins (EBNA-3A, EBNA-3B, and EBNA-3C) negatively autoregulate the growth programme. This allows the cell to migrate into the follicle to initiate a germinal centre reaction, and to establish the default transcription programme (latency II). The default programme provides rescue or survival signals that allow the cell to exit the germinal centre as memory B cell. Then, the latency transcription programme (latency 0) in which all viral protein expression is turned off begins in the resting memory B cells. These cells are maintained by normal memory B cell homeostasis. When they occasionally divide, they express the EBNA-1-only programme (latency I). The memory B cells eventually return to the tonsil, where they occasionally undergo plasma-cell differentiation, which triggers viral replication. The resulting virus may be released into saliva for spreading to other hosts or may infect other B cells (Young & Rickinson, 2004; Thorley-Lawson & Allday, 2008).

Primary EBV infection elicits a strong cellular immune response which brings the infection under control, and newly infected cells are thought to be efficiently removed by the latent-antigen-specific T-cell response. The virus can persist for life in the host only in the resting memory B cells in which no viral proteins are expressed, and is therefore shielded from the immune system (Thorley-Lawson & Gross, 2004; Young & Rickinson, 2004; Thorley-Lawson & Allday, 2008).

Figure 1.2 Putative *in vivo* interactions between EBV and host cells



a Primary infection
b Persistent infection
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Table 1.3 The EBV transcription programmes in normal B cells

Transcription programme	Gene products expressed	Infected normal B-cell type[a]	Function
Growth (latency III)	EBNA-1, -2, -3A, -3B, -3C, -LP, LMP-1, LMP-2A and LMP-2B, EBERs	Naive	Activate B cell
Default (latency II)	EBNA-1, LMP-1 and LMP-2A, EBERs	Germinal centre	Differentiate activated B cell into memory
True latency (Latency 0)	EBERs	Peripheral memory	Allow lifetime persistence
EBNA-1 only (latency I)	EBNA-1, EBERs	Dividing peripheral memory	Allow virus in latency programme cell to divide
Lytic	All lytic genes	Plasma cell	Replicate the virus in plasma cell

[a] Except where indicated, the cell types are primarily restricted to the lymphoid tissue of the Waldeyer ring.
Adapted from Thorley-Lawson (2005)

1.1.7 Viral gene expression

(a) Viral gene expression in normal cells during the viral life cycle

Table 1.3 lists the different viral transcription programmes in normal B cells. The expression of EBV-encoded proteins differs depending on the type, differentiation, and activation status of the infected cell. The growth-stimulating programme is based on the expression of six nuclear and three membrane proteins. Six of these are essential for the activating and proliferation-driving effect of the virus. One virally encoded nuclear protein, EBNA-1 which is required for the maintenance of the viral episomes, is expressed to various degrees in these cells (Thorley-Lawson, 2005). In all forms of latency, EBV expresses two classes of non-coding small RNA (EBER) 1 and 2, which are highly structured RNAs of 167 and 172 nucleotides, respectively. The expression of EBER-1 and -2 is restricted to the cell nucleus where they are present at approximately 10^7 copies per cell (Sample & Sample, 2008). Also, EBV encodes at least 22 micro-RNAs which are expressed to various degrees in latency I, II, III (Cai *et al.*, 2006; Grundhoff *et al.*, 2006).

(b) Viral gene expression in EBV-associated malignancies

Specific latency EBV-transcription programmes have been demonstrated in many human tumours, including immunoblastic lymphoma in immunosuppressed patients, Burkitt lymphoma, Hodgkin disease, and nasopharyngeal carcinoma (Table 1.4). The origins of all of these tumours can be understood as arising from specific stages in the EBV life cycle, and appear to be associated with disturbances of the immune system as shown in Fig. 1.3 (Thorley-Lawson, 2005).

Latency I is generally associated with the EBV-related Burkitt lymphoma, latency II with Hodgkin disease, T-cell non-Hodgkin lymphoma, and nasopharyngeal and gastric carcinoma; latency III occurs mainly in immunocompromised individuals, in post-transplant lymphoproliferative disorders, and HIV-associated lymphoproliferative disorders (Liebowitz & Kieff, 1993; Sbih-Lammali *et al.*, 1996; Niedobitek *et al.*, 1997; Cesarman & Mesri, 1999; Kis *et al.*, 2006; Klein *et al.*, 2007).

Table 1.4 EBV latency pattern and associated malignancies

Latency Type	Viral products expressed	Associated malignancies
Latency 1	EBNA-1	Burkitt lymphoma
	EBERs	Gastric carcinoma[b]
	BARF0	
Latency II	EBNA-1	Hodgkin disease
	EBERs	Nasopharyngeal carcinoma
	LMP-1	Peripheral T/NK lymphoma
	LMP-2A	
	BARF0	
Latency III	All EBNAs[a]	AIDS-associated lymphomas
	EBERs	Post-transplant lymphoproliferative disorders
	LMP-1	
	LMP-2A	
	BARF0	

[a] EBNAs include EBNA-1, EBNA-2, EBNA-3A (EBNA-3), EBNA-3B (EBNA-4), EBNA-3C (EBNA-6), EBNA-LP (EBNA-5 or EBNA-4)
[b] Gastric carcinoma have been shown to express an intermediate Latency I/II pattern including expression of EBNA-1, EBERs, LMP-2A, BARF0 and some lytic infection proteins such as BARF-1, BNRF-1 (Luo *et al.*, 2005)
EBNA, EBV nuclear antigen; LMP, latent membrane protein; EBER, EBV-encoded RNA
Adapted from Thompson & Kurzrock (2004)

1.2 Epidemiology of infection

1.2.1 Prevalence, geographic distribution

In the 1970s, IARC demonstrated that more than 90% of adults worldwide are infected with EBV, based on the detection of antibodies to EBV (especially antibodies to viral capsid (VCA) and complement-fixing soluble (CF/S) antigens) (de Thé *et al.*, 1975). Many other epidemiological studies have shown since that EBV is highly prevalent throughout the world (IARC, 1997; Young, 2008), including in remote populations (Black *et al.*, 1970; Tischendorf *et al.*, 1970).

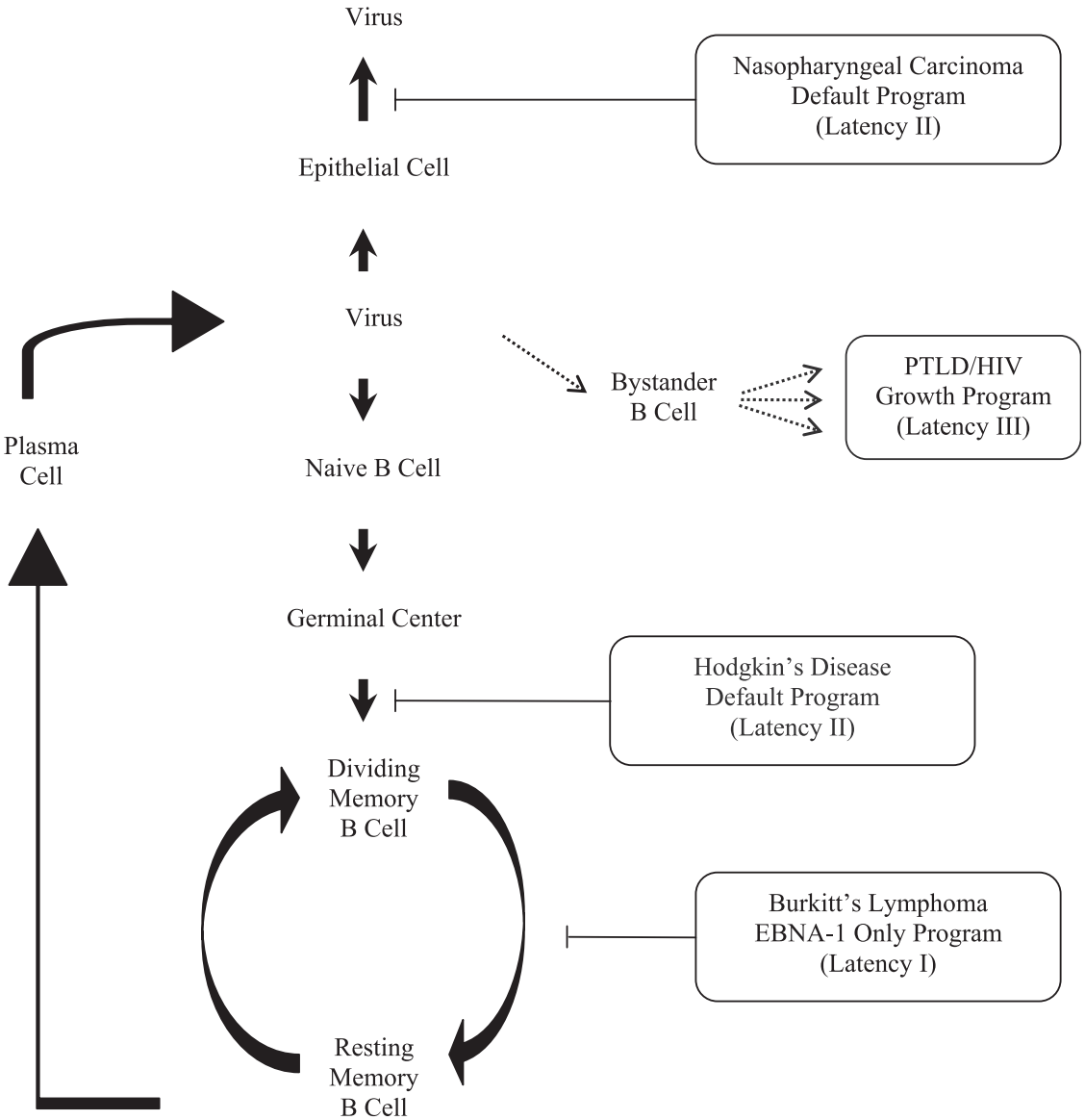
The age at primary infection varies substantially worldwide, and exposure to EBV is likely to be due to socioeconomic factors (Evans, 1971) such as overcrowded living conditions with poor sanitation (de Thé *et al.*, 1975). For example, while more than 80% of children in Uganda are estimated to be seropositive for EBV by age one (Kafuko *et al.*, 1972), this estimate is only approximately 45% in the rural United States of America (Sumaya *et al.*, 1975; Hsu & Glaser, 2000).

Although primary EBV infection during early childhood is usually subclinical or has symptoms that are similar to other respiratory illnesses, a delay in acquiring a primary EBV infection at an older age in childhood or adolescence, which usually occurs in more developed countries (Rickinson & Kieff, 1996), can manifest itself as infectious mononucleosis (occurring in approximately 25–75% of EBV-infected persons) (Evans, 1971; Sawyer *et al.*, 1971; Niederman & Evans, 1997; Hsu & Glaser, 2000).

In a study in the Hong Kong Special Administrative Region (Chan *et al.*, 2001), sequential measurements for markers of EBV infection from serum samples of a group of infants demonstrated a sudden seroconversion at the age of 8 months, which may imply a protective role for persistent maternal antibodies, and also partially explain why primary EBV infection in early childhood, unlike during adolescence, is usually asymptomatic (Chan *et al.*, 2001).

Two major types of EBV – EBV-1 and EBV-2 – have been identified and differ in geographic distribution. The role of specific EBV types in

Figure 1.3 Putative check points in the EBV life cycle that give rise to tumours



Events occurring normally in healthy carriers are denoted in thick arrows. EBV normally infects naive B cells in the Waldeyer ring, and can differentiate into memory cells and out of the cell cycle, and are not pathogenic. *PTLD/HIV.* If a cell other than the naive B cell in the Waldeyer ring is infected, it will express the growth programme and continue to proliferate because it cannot differentiate out of the cell cycle (thin dashed arrows) - a very rare event - highlighting how carefully controlled EBV infection is. Normally, these bystander B-cell blasts would be destroyed by CTLs, but if the CTL response is suppressed, then they grow into PTLD or AIDS-associated lymphomas. Note: a bystander-type cell could also arise if a latently infected germinal center or memory cell fortuitously switched on the growth programme. *Hodgkin disease* occurs from the default programme. *Burkitt lymphoma* evolves from a germinal-centre cell that is entering the memory compartment but is stuck proliferating. Consequently, the cell expresses EBNA-1 only. *Nasopharyngeal carcinoma* is assumed to occur from a latently infected epithelial cell blocked from terminal differentiation and viral replication.

the etiology of different cancers is unknown. Immunocompromised patients more commonly harbour both subtypes of EBV (Borisch *et al.*, 1992; Thompson & Kurzrock, 2004). EBV-2 may be more common in Africa (Gratama & Ernberg, 1995), and in homosexual men (van Baarle *et al.*, 2000; Higgins *et al.*, 2007). It has been hypothesized that the attenuated transforming ability of EBV-2 along with an immunosuppressive condition (HIV or malaria) may be necessary for EBV-2 to be capable of maintaining infection of B lymphocytes, and to cause transformation (Buisson *et al.*, 1994; Thompson & Kurzrock, 2004). However, other studies showing that HIV-infected haemophiliacs have lower rates of EBV-2 infection than HIV-infected homosexuals have challenged this hypothesis, and suggest that the acquisition of EBV-1 versus EBV-2 would rather be due to the opportunity for exposure (van Baarle *et al.*, 2000; Thompson & Kurzrock, 2004).

The fact that EBV is ubiquitous, and consequently causes widespread and largely asymptomatic infection, suggests that the specific geographic distribution of EBV-associated malignancies, such as endemic Burkitt lymphoma and nasopharyngeal carcinoma, is probably not due to differences in EBV infection but rather due to the activation of viral replication by additional cofactors (Young, 2008).

1.2.2 Transmission and risk factors for infection

EBV infection usually occurs in individuals of a young age, with low socioeconomic status or development, from a larger than average family, and with poor hygienic standards. By their third decade of life, 80–100% of these individuals become carriers of the infection (IARC, 1997).

The oral route is the primary route of transmission of the virus; however, transmission by transfusion has been documented. In developing countries, infection is acquired in the first few years of life. Crowding and/or the practice of pre-chewing food for infants may be contributing factors. In the developed world, infection is often delayed to adolescence, when transmission is more likely because of intimate oral exposure (Hjalgrim *et al.*, 2007a). About 50% of primary EBV infections during young adulthood result in clinical infectious mononucleosis (CDC, 2006).

Infectious mononucleosis is usually acquired from a transfer of saliva, and in young adults, this is more likely to occur after the onset of sexual activity. However, only limited data are available to support this hypothesis (Macsween & Crawford, 2003). In a cohort study of sexually active young women, the development of detectable antibodies against EBV after primary infection increased with increasing number of sexual partners, and was greatest when a new sexual partner was encountered in the 2 years before seroconversion. In addition, transient EBV DNA loads were detected in cervical cytology samples in some of the women (Woodman *et al.*, 2005). [The Working Group noted, however, that it is difficult to distinguish in this study whether transmission occurs through saliva or genital contact.]

1.2.3 Persistency, latency, and natural history of infection

Following primary infection via transmission of cell-free virus and/or of productively infected cells in saliva, EBV will enter into the circulating B-cell pool, and then remain in most cases undetected for life in a latent state (Young & Rickinson, 2004; Thorley Lawson, 2005). EBV can also infect the mucosal epithelial cells in which intermittent viral productive replication occurs (Frangou *et al.*, 2005). The B-cell compartment, more precisely resting memory B cells, appears to be the true reservoir of the latent virus in healthy carriers. Resting memory B cells express a very restricted pattern of latent viral gene expression (see Table 1.3 and Miyashita *et al.*, 1995); this is

how these infected cells can persist in the face of efficient cytotoxic T lymphocyte (CTL) surveillance (Masucci & Ernberg, 1994). Nonetheless, cells that express the full repertoire of growth-transformation-associated antigens are likely be generated sporadically in asymptomatic virus carriers, because memory CTLs that are reactive against most EBNAs are maintained at high levels for life (see Fig. 1.2, and further details in Section 1.1, this *Monograph*; IARC, 1997).

Primary EBV infections occurring in adolescence or early in adult life are manifested as infectious mononucleosis, which is an acute form of primary infection occurring asymptomatically in early childhood.

EBV-associated malignancies are suspected to result from viral reactivation that is most likely due to interaction with additional cofactors (Young, 2008).

1.2.4 Biological markers of the different status of EBV infection

(a) Antibody responses to EBV

The detection of antibodies to EBV in biological fluids has been until recently the major means of diagnosis for EBV infection. Distinct patterns of antibody response have been identified during primary infection, latent infection of healthy carriers, viral reactivation, and in various EBV-associated diseases. Serological parameters include the detection of IgG, IgM, and occasionally IgA, directed against EBNAs, early antigens (EAs, divided into two components, EA-D (encoded by *BMRF-1*) and EA-R (a human BCL-2 homologue encoded by *BHRF-1*)), and VCAs (for a review see IARC, 1997).

(i) Infectious mononucleosis

Most information available on primary antibody response has been provided by studies on infectious mononucleosis. Table 1.5 shows the variation over time of serological parameters both at and after the onset of infectious mononucleosis. At the onset of clinical symptoms of the disease, substantial titres of IgM antibodies to VCA are detected, with rising titres of IgG to EA, and to VCA. IgA antibodies to these antigens may also appear. Whereas anti-VCA IgM titres disappear over the next few months, anti-VCA IgG titres rise to a peak that may fall slightly, and anti-EA IgG titres become either undetectable or stabilize at very low levels. Neutralizing antibodies to the major envelop glycoprotein gp350 are detected during the acute phase of infectious mononucleosis but only at very low titres, and increase to stable levels thereafter (IARC, 1997).

The serology of infectious mononucleosis for the anti-EBNA response presents an interesting pattern. For the anti-EBNA response, during the acute phase of infectious mononucleosis, patients show an IgG response to EBNA-2 (and also probably to EBNA-3A, -3B, and -3C), whereas an IgG response to EBNA-1 is not usually detected until convalescence. The production of antibodies to EBNA-1 and EBNA-2 in the course of infectious mononucleosis follows an ordered progression. Anti-EBNA-2 is the first to be detected, reaches peak titres, and then declines to a lower persistent level, and can remain undetected in about 1/3 of the cases. Anti-EBNA-1 is detected long after anti-EBNA-2, and then persists indefinitely once it has reached its concentration plateau. Therefore, within the first year following infectious mononucleosis, the ratio of anti-EBNA-1: anti-EBNA-2 is well below 1, but becomes well above 1 over time. The switch from dominant anti-EBNA-2 to dominant anti-EBNA-1 titres occurs in individual cases over a long span of time (Table 1.5; Henle *et al.*, 1987; IARC, 1997).

(ii) Healthy EBV-carriers

IgG antibodies to VCA, to neutralizing anti-gp350, and to EBNA-1 are consistently detected in the serum of healthy carriers. The titre of these antibodies is usually stable over time but can markedly differ among individuals (Henle & Henle, 1976). Antibodies to EA are only detected

Table 1.5 Serological parameters at various times after the onset of infectious mononucleosis

	Months after onset					Healthy controls (n=38)
	0 (n=74)	2–3 (n=44)	4–12 (n=65)	13–24 (n=83)	25–48 (n=35)	
IgM anti-VCA						
% positive	100.0	73.1	0.0	0.0	0.0	0.0
Range of titres	80–640	10–80	<10	<10	<10	<10
Anti-EA						
% positive	81.8	88.5	87.8	60.9	39.4	30.0
Range of titres	10–320	10–160	10–160	10–80	10–80	10–40
Anti-D						
% positive	81.8	57.7	10.2	13.0	0.0	0.0
Range of titres	10–320	10–160	10–160	10–40	<10	<10
Anti-R						
% positive	–[a]	46.2	83.7	47.9	39.4	30.0
Range of titres	–	10–160	10–160	10–80	10–80	10–40
Anti-EBNA-1						
% positive	0.0	4.5	73.8	97.6	97.1	100.0
Geometric mean titre	<2	<2	5.3	21.2	24.8	48.2
Anti-EBNA-2						
% positive	0.0	93.2	87.9	60.2	71.4	71.1
Geometric mean titre	<2	7.3	11.6	4.1	4.3	3.9
Ratio anti-EBNA-1/anti-EBNA-2						
≤1.0	–	97.7	76.9	22.9	17.1	5.3
>1.0	–	2.3	23.1	77.1	82.9	94.7

[a] Anti-R can be measured only when exceeding anti-D in titre.
Adapted from Henle *et al.* (1987)

in a proportion of healthy carriers. Although persisting for life, anti-EBNA-1 and anti-VCA do not appear to have much of a protective role (Moss *et al.*, 1992).

Virus shed can be frequently detected from throat washes of asymptomatic carriers. The levels of shedding are thought to be quite stable over many months, although with different rate depending on the individuals. A direct relationship appears to exist between the level of virus shedding in the throat and the level of virus-infected B cells in the blood. However, no obvious relationship was shown between the levels of EBV virus shedding from the throat and either anti-VCA or anti-EA titres in the serum of healthy carriers (Yao *et al.*, 1985).

(iii) EBV-associated malignant diseases

The major features of the humoral response to EBV in different EBV-associated malignancies have been reported (Khanna *et al.*, 1995) but no specific pattern could be defined as useful prognostic markers for these diseases.

Although anti-VCA IgA serology was proposed as an effective and sensitive prognostic and diagnostic marker for nasopharyngeal carcinoma, more recent studies have shown high false positive rates for this antibody (Low *et al.*, 2000). Furthermore, the follow-up of individuals with high titres of IgA to VCA demonstrated that a significant portion of those seroconverted back to normal, and did not develop nasopharyngeal carcinoma (Lo *et al.*, 2004).

(b) Detection of EBV in tissues and serum

(i) Healthy carriers

In healthy carriers, EBV is mostly present in a latent form as episomal DNA in resting memory B cells. The frequency of EBV-carrying cells in peripheral blood ranges from 1 in $2x10^5$–10^7 whole mononuclear cells or 1 in $2x10^4$–10^6 B cells, and is quite stable in an individual over time. It was estimated that less than 1 in 40 EBV-infected cells can replicate the virus in the peripheral blood of healthy carriers (Miyashita *et al.*, 1995; Decker *et al.*, 1996). Therefore, only very highly sensitive polymerase chain reaction (PCR) assays can detect EBV DNA in peripheral blood cells.

(ii) EBV-associated malignancies

- EBV DNA in tumour tissues

The EBV genome can be detected in tumour cells by PCR or in-situ hybridization assays using the BamH1 internal fragment of the viral genome as a probe. However, a major technical breakthrough has been the use, especially by in-situ hybridization, of probes specific for the small nuclear EBV-encoded RNAs, EBER-1 and EBER-2, which are highly expressed in all forms of EBV infection (Wu *et al.*, 1990). The high sensitivity of this method allowed the determination of the incidence of EBV infection in the very scarce neoplastic Hodgkin and Reed-Sternberg cells (HRC) in biopsies from Hodgkin disease patient (IARC, 1997).

Detection of EBV genomic DNA by PCR using EBV genes (e.g. *EBNA-1*, *EBNA-2*, and *LMP-1*) as targets in tissue obtained from nasopharyngeal biopsy and fine-needle aspirate samples have also been shown as being reliable and accurate methods for the diagnosis of nasopharyngeal carcinoma (Yap *et al.*, 2007). In addition, nasopharyngeal carcinoma patients have a very high load of EBV DNA as collected in non-invasive nasopharyngeal brushing. EBNA1 and BARF1 mRNAs are detected at even higher levels in such samples, whereas no EBV mRNA is detected from nasopharyngeal brushing samples of healthy donors (Stevens *et al.*, 2006).

- Cell-free EBV DNA in serum

Cell-free EBV DNA has been detected in the plasma and serum of patients with several EBV-associated malignant diseases: Hodgkin disease, post-transplant lymphoproliferative diseases, NK/T-cells lymphoma, Burkitt lymphoma, nasopharyngeal carcinoma, and EBER-positive gastric carcinoma; and, this detection correlated with the EBV status in tumours. In contrast, cell-free EBV DNA was not detected in any of the healthy control subjects (Lei *et al.*, 2000, 2002; Lo *et al.*, 2001; Musacchio *et al.*, 2006). Plasma EBV DNA, as measured by real-time quantitative PCR, has been proposed as a sensitive and specific tumour marker for diagnosis, disease monitoring, and prediction of outcome for several of the EBV-associated diseases (Lo *et al.*, 2001; Lei *et al.*, 2002; Shao *et al.*, 2004).

Low-level EBV DNA positivity in serum has been reported to occur relatively frequently after stem-cell transplantation, and may subside without specific treatment. However, high EBV DNA levels (i.e. > 50 000 copies/mL) are strong predictors for the development of post-transplantation lymphoproliferative disease (Aalto *et al.*, 2007).

The lack of detectable viral DNA in the serum of healthy carriers indicates that although most of these individuals are expected to be carrying EBV DNA in their lymphocytes, EBV DNA is not usually found in serum in the absence of active EBV disease. It is likely, however, that the viral DNA in serum is present in cases of EBV reactivation as well as in cases of primary infection, and tests for viral DNA can not discriminate between the two cases unless they are used in conjunction with serology. EBV reactivation is particularly relevant in immunocompromised patients (Chan *et al.*, 2001).

2. Cancer in Humans

In the previous *IARC Monograph*, EBV infection was associated with several cancer types: Burkitt lymphoma, immunosuppression-related non-Hodgkin lymphoma, extranodal NK/T-cell lymphoma (nasal type – Swerdlow *et al.*, 2008; previously known as angiocentric T-cell lymphoma – IARC, 1997); Hodgkin lymphoma, and nasopharyngeal carcinoma (IARC, 1997). The following text comprises updated relevant data from case–control and cohort studies for several cancer types in relation to infection with EBV.

2.1 Virus-associated B-cell lymphoma

2.1.1 Burkitt lymphoma

There are three subtypes of Burkitt lymphoma: endemic Burkitt lymphoma, sporadic Burkitt lymphoma, and immunodeficiency-associated Burkitt lymphoma. Endemic Burkitt lymphoma is defined as affecting children in equatorial Africa and New Guinea, sporadic Burkitt lymphoma affects children and young adults throughout the world, and immunodeficiency-associated Burkitt lymphoma is primarily associated with HIV infection. The majority of endemic Burkitt lymphoma, sporadic Burkitt lymphoma, and immunodeficiency-associated Burkitt lymphoma form three distinct clinical entities. It has been reported that EBV is detected in the tumour tissue of almost 100% of the cases of endemic Burkitt lymphoma, this proportion is less in cases of sporadic and immunodeficiency-associated Burkitt lymphoma (Carbone *et al.*, 2008).

With regard to endemic Burkitt lymphoma, two new studies (Carpenter *et al.*, 2008; Mutalima *et al.*, 2008) from Uganda and Malawi (with 325 and 148 cases, respectively) add to the evidence from five case–control studies (including 431 cases in total) (Henle *et al.*, 1969, 1971b; Klein *et al.*, 1970; Hirshaut *et al.*, 1973; Nkrumah *et al.*, 1976), and one cohort study (with 16 cases) (Geser *et al.*, 1982) outlined in the previous *IARC Monograph* (IARC, 1997). Both studies (Carpenter *et al.*, 2008; Mutalima *et al.*, 2008) demonstrate there is a relationship between an increase in the titre of antibodies against EBV-VCA and an increase in risk for endemic Burkitt lymphoma (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.1.pdf). There are no new data available relating to sporadic Burkitt lymphoma to add to the 90 cases in the four studies previously reported (Hirshaut *et al.*, 1973; Ablashi *et al.*, 1974; Gotleib-Stematsky *et al.*, 1976; Çavdar *et al.*, 1994).

(a) Cofactors for endemic Burkitt lymphoma

A thorough review of potential cofactors for endemic Burkitt lymphoma (e.g. malaria, sickle-cell trait, *Euphorbia tirucalli*, and other medicinal plants) is outlined in the previous *IARC Monograph* (IARC, 1997), and, with the exception of malaria, no new data were available to the Working Group. Previously, the relationship between malaria and endemic Burkitt lymphoma was based mostly on ecological data – for example, geographic correlations between the prevalence of malaria and the reported incidence of endemic Burkitt lymphoma, and apparent declines in the incidence following widespread malaria eradication programmes. One intervention study (Geser *et al.*, 1989) in the United Republic of Tanzania confirmed the relationship between malaria prevalence and the incidence of Burkitt lymphoma. More recently, two case–control studies have demonstrated an increasing risk of endemic Burkitt lymphoma in relation to an increase in the titre of antibodies against malaria, and also suggested that EBV and malaria act synergistically in the etiology of the disease (Carpenter *et al.*, 2008; Mutalima *et al.*, 2008). In addition, the use of insecticides or bed nets in the home was associated with substantially

lower risks of endemic Burkitt lymphoma. There is evidence that malaria reduces T-cell-mediated immunosurveillance of EBV-infected cells, and is linked to an increased viral load of EBV (Moormann *et al.*, 2005, 2007, 2009).

2.1.2 Hodgkin lymphoma

EBV is more commonly associated with classic Hodgkin lymphoma, especially the mixed-cellular subtypes. The non-classical nodular lymphocyte-predominant Hodgkin lymphoma cases are very rarely associated with EBV (Khalidi *et al.*, 1997). Developing countries have an increased incidence of EBV-positive cases, which may be attributed to the existence of underlying immunosuppression (Jarrett *et al.*, 1991; Murray & Young, 2005). A bimodal age distribution has been recognized for EBV-positive Hodgkin lymphoma patients; children (< 15 years) and older-age groups tend to have much higher rates than young adults (Flavell & Murray, 2000). In western populations, the EBV genome has been detected in the tumour tissue of 40–50% of Hodgkin lymphoma cases (Weiss, 2000). The subclassification of Hodgkin lymphoma cases as EBV-positive or EBV-negative provides the potential to identify etiological subgroups (Alexander *et al.*, 2000). EBV has been identified as a cause of infectious mononucleosis, a potential risk factor for Hodgkin lymphoma, which results from hyperproliferation of EBV-containing B cells, and a reactive T-cell response (Henle *et al.*, 1968).

Previously, epidemiological data on the association between Hodgkin lymphoma and EBV were derived from several sources:

- Investigations of the relationship between infectious mononucleosis and Hodgkin lymphoma from six case–control studies (odds ratios (ORs) ranging from 1.0–8.2) (Table 18, IARC, 1997; Henderson *et al.*, 1979; Gutensohn & Cole, 1981; Gutensohn, 1982; Evans & Gutensohn, 1984; Bernard *et al.*, 1987; Serraino *et al.*, 1991) and six cohort studies (ORs ranging from 2.0–5.0) (Table 22, IARC, 1997; Miller & Beebe, 1973; Connelly & Christine, 1974; Rosdahl *et al.*, 1974; Carter *et al.*, 1977; Muñoz *et al.*, 1978; Kvåle *et al.*, 1979). A 2–4-fold increased risk for Hodgkin lymphoma within the first 3 years following infectious mononucleosis was also demonstrated (Rosdahl *et al.*, 1974; Muñoz *et al.*, 1978)
- 41 case–control studies of Hodgkin lymphoma in which there was evidence of antibodies against EBV-VCA (22 studies; ORs, 0.8–79; Table 19, IARC, 1997), EA (11 studies; ORs, 1.2–infinity; Table 20, IARC, 1997), and EBNA (eight studies; five with equivocal results, and three with ORs ranging from 1.7–infinite; Table 21, IARC, 1997)
- Two large cohort studies, both reporting statistically significant excess risks associated with antibodies against EBV (Mueller *et al.*, 1989; Lehtinen *et al.*, 1993)

One further cohort study (Hjalgrim *et al.*, 2000) of patients with infectious mononucleosis (reporting 46 cases of Hodgkin lymphoma; OR, 2.6; 95%CI: 1.9–3.4), together with seven case–control studies (Gallagher *et al.*, 1999; Alexander *et al.*, 2000, 2003; Glaser *et al.*, 2005; Berrington de González *et al.*, 2006; Musacchio *et al.*, 2006; Dinand *et al.*, 2007; Hjalgrim *et al.*, 2007b) addressed the association between EBV and Hodgkin lymphoma (see Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.2.pdf and Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.3.pdf). In two studies, one in the United Kingdom (Gallagher *et al.*, 1999) and one in Brazil (Musacchio *et al.*, 2006), examining the association between EBV DNA (*Bam*HI-W) in serum and risk for Hodgkin lymphoma, cases of both EBV-positive and EBV-negative Hodgkin lymphoma had a much higher proportion with detectable EBV DNA

in serum/plasma than that of healthy controls. In a case–control study involving 145 Hodgkin lymphoma cases and 25 follicular hyperplasia controls in India, EBV DNA was detected in the lymph nodes of 140 (96.6%) Hodgkin lymphoma cases and but not in controls (0%) (Dinand *et al.*, 2007). One case–control study from South Africa did not find a positive association between levels EBV antibody in serum and Hodgkin lymphoma (Berrington de González *et al.*, 2006). In case–control studies on the association between a history of infectious mononucleosis and risk for Hodgkin lymphoma, a significant association was observed in EBV-positive Hodgkin lymphoma cases, particulary in those with young age at onset in the United Kingdom (Alexander *et al.*, 2000, 2003), and Denmark and Sweden (Hjalgrim *et al.*, 2007b). However, no significant association between a history of infectious mononucleosis and Hodgkin lymphoma was found in the USA (Glaser *et al.*, 2005). A case–case analysis in a population-based case–control study compared 95 EBV-positive and 303 EBV-negative Hodgkin lymphoma cases (Chang *et al.*, 2004). EBV antibody titres were significantly higher in the EBV-positive cases, including anti-VCA IgG and IgA, EA and an EBNA-1:EBNA-2 ratio ≤ 1. With mutual adjustment, the odds ratios for elevated VCA IgG were 3.6 (95%CI: 1.4–8.7), and for low EBNA-1:EBNA-2 ratio, 3.2 (95%CI: 1.1–9.0).

2.1.3 *Lymphomas in immunosuppressed individuals*

(a) Post-transplant lymphoproliferative disorders

Since the original reports of post-transplant lymphoproliferative disorders in 1969 (McKhann, 1969; Penn *et al.*, 1969), a higher incidence of lymphoproliferative disorders in transplant recipients of both a solid organ and bone marrow has been observed (Carbone *et al.*, 2008). According to the WHO classification (Swerdlow *et al.*, 2008), post-transplant lymphoproliferative disorders may be classified into: a) early lesions, generally represented by EBV-driven polyclonal lymphoproliferations; and, b) true monoclonal diseases, including polymorphic post-transplant lymphoproliferative disorders and monomorphic post-transplant lymphoproliferative disorders.

In addition to data presented previously (IARC, 1997), a case–control study of EBV DNA in plasma samples of four cases of post-transplant EBV-associated nasal NK/T-cell lymphoma, two cases of post-transplant lymphoproliferative disorders, and 35 healthy controls in the Hong Kong Special Administrative Region (Lei *et al.*, 2000) were considered – all six cases (100%) and no control (0%) had EBV DNA (*Bam*HI-W) levels in plasma (see Table 2.4 available http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.4.pdf).

(b) HIV-associated lymphoproliferative disorders

HIV-associated lymphoproliferative disorders are a heterogeneous group of diseases that occur in the presence of HIV-associated immunosuppression. These aggressive disorders include both central nervous system and systemic lymphomas. Primary effusion lymphoma (reviewed in this volume, see *Monograph* on Kaposi Sarcoma Herpes Virus (KSHV)) also occurs and often involves EBV in addition to KSHV. The categories of HIV-associated non-Hodgkin lymphoma (HIV-NHL) confirmed in the latest WHO Classification of Tumours (Raphael *et al.*, 2001, 2008) are grouped as follows (Carbone *et al.*, 2008):

- Lymphomas also occurring in immunocompetent patients. Most of these HIV-NHLs belong to three high-grade B-cell lymphomas: Burkitt lymphoma, diffuse large B-cell lymphoma with centroblastic features, and diffuse large B-cell lymphoma with immunoblastic features. According to the site of involvement, the present spectrum of HIV-NHL includes extranodal/nodal lymphomas, and

primary central nervous system lymphomas (Carbone *et al.*, 2008);
- Unusual lymphomas occurring more specifically in HIV-positive patients – these lymphomas include two rare entities, namely, primary effusion lymphoma (Cesarman *et al.*, 1995), and plasmablastic lymphoma of the oral cavity (Delecluse *et al.*, 1997; Carbone *et al.*, 1999);
- Lymphomas also occurring in other immunodeficient states (Carbone *et al.*, 2008).

One nested case–control study of non-Hodgkin lymphoma among HIV-infected people identified an association between anti-VCA antibodies and risk of disease, although no division by histological subtype of lymphoma was possible (Newton *et al.*, 2006; see Table 2.5 available http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.5.pdf).

2.1.4 EBV-positive diffuse large B-cell lymphoma of the elderly

This is defined as an EBV-containing diffuse large B-cell lymphoma occurring in patients over 50 years of age without any prior lymphoma or other known immunodeficiency. These EBV-positive lymphomas account for 8–10% of diffuse large B-cell lymphomas in Asian countries, but there are little data from western countries (Oyama *et al.*, 2007; Swerdlow *et al.*, 2008).

2.2 Virus-associated T-cell and NK-cell lymphomas

EBV is an established cause of extranodal NK/T-cell lymphoma (nasal type); previously called angiocentric T-cell lymphoma (IARC, 1997; Chan *et al.*, 2001b; Swerdlow *et al.*, 2008). A higher incidence of extranodal NK/T-cell lymphoma (nasal type) has been described in central and south America, and in several east Asian countries (Suzuki *et al.*, 2008). Several recent large case series confirm the presence of EBV in tumour cells in nearly 100% of cases (Barrionuevo *et al.*, 2007; He *et al.*, 2007). In a case–control study of anti-EBV VCA, EA, and EBNA in serum samples of 100 cases of peripheral NK/T-cell proliferative disease/lymphoma, and 100 age- and sex-matched controls in Thailand (Mitarnun *et al.*, 2002), elevated serum levels of anti-EBV VCA IgG and EA IgG were associated with an increased risk of the disease (see Table 2.4 on-line). In another case–control study of seven cases of peripheral NK/T-cell proliferative disease, 38 cases of peripheral NK/T-cell lymphoma, and 45 age- and sex-matched healthy controls in Thailand (Suwiwat *et al.*, 2007), 37 (82.2%) cases and no control had detectable EBV DNA levels (*Bam*HI-W) in their plasma .

Other T-cell lymphoproliferative disorders that have been reported to be associated with EBV include a subset of peripheral T-cell lymphomas (Dupuis *et al.*, 2006a, b; Tan *et al.*, 2006), enteropathy-type T-cell lymphomas (de Bruin *et al.*, 1995; Quintanilla-Martínez *et al.*, 1998; Isaacson *et al.*, 2001), γδ T-cell lymphomas (hepatosplenic and non-hepatosplenic) (Arnulf *et al.*, 1998; Ohshima *et al.*, 2000), T-cell lymphoproliferative disorders after chronic EBV infection (Quintanilla-Martinez *et al.*, 2000), EBV-associated cutaneous T-cell lymphoproliferative disorders (especially in Asia) (Chan *et al.*, 2001a; Kim *et al.*, 2006), and aggressive NK-cell leukaemias/lymphomas (Chan *et al.*, 2001b).

Angioimmunoblastic T-cell lymphoma is a distinct entity of peripheral T-cell lymphoma (Dupuis *et al.*, 2006b). Angioimmunoblastic T-cell lymphomas are also nearly always associated with EBV; however, the cellular origins remain unknown. The virus is present in B cells, rather than in the neoplastic T cells, suggesting an indirect role, hypothetically through antigenic stimulation (Knecht *et al.*,1990; Dupuis *et al.*, 2006b; Dunleavy *et al.*, 2007).

2.2.1 Other non-Hodgkin lymphoma

Two cohort studies including a total of 115 cases of non-Hodgkin lymphoma occurring in apparently immunocompetent individuals reported no excess risk in relation to anti-VCA antibody titres (Mueller *et al.*, 1991; Lehtinen *et al.*, 1993; IARC, 1997). Since then, four case–control studies have investigated the serological evidence of infection with EBV: two reported no associations (Hardell *et al.*, 2001a; Berrington de González *et al.*, 2006), one reported a borderline increased association (Hardell *et al.*, 2001b), and the other showed a significant association between abnormal reactive EBV antibody patterns and non-Hodgkin lymphoma (OR, 1.4; 95%CI: 1.2–1.7; based on 1085 cases; de Sanjosé *et al.*, 2007).

2.3 Cancers of the nasopharynx, stomach, and lymphoepithelium

2.3.1 Cancer of the nasopharynx

Cancer of the nasopharynx is rare in most populations around the world but common in South-East Asia (Ferlay *et al.*, 2010). According to the WHO Classification of Tumours, cancers of the nasopharynx are classified into three types: keratinizing squamous cell carcinoma (Class I), non-keratinizing carcinoma (Class II), and basaloid squamous cell carcinoma (Class III) (Chan *et al.*, 2005). Most cancers of the nasopharynx diagnosed in the high-risk areas belong to Class II. In the previous *IARC Monograph*, an increased risk of cancer of the nasopharynx was demonstrated in five case–control studies in which all 671 cases had evidence of infection with EBV (de Thé *et al.*, 1978b; Lanier *et al.*, 1980a; Pearson *et al.*, 1983b; Chen *et al.*, 1987; Zheng *et al.*, 1994a), and one cohort study in which all seven cases had evidence of infection with EBV (Chan *et al.*, 1991). Since then, two cohort (Chien *et al.*, 2001; Ji *et al.*, 2007) and eight case–control studies (Mutirangura *et al.*, 1998; Lo *et al.*, 1999; Chen *et al.*, 2001; Lin *et al.*, 2001, 2004; Fan *et al.*, 2004; Leung *et al.*, 2004; Tiwawech *et al.*, 2008) on the association between EBV and cancer of the nasopharynx have been reported.

The prospective cohort studies on the association between EBV biomarkers and the development of nasopharyngeal carcinoma are shown in Table 2.6 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.6.pdf) and include 22 and 131 cases (Chien *et al.*, 2001; Ji *et al.*, 2007, respectively). In one study (Chien *et al.*, 2001), the relative risks of developing nasopharyngeal carcinoma were 22.0 (95%CI: 7.3–66.9) for anti-EBV VCA IgA-seropositivity, and 3.5 (95%CI: 1.4–8.7) for anti-EBV DNase-seropositivity. Compared with those who were seronegative for both anti-EBV markers as the referent group, the adjusted relative risk was 32.8 (95%CI: 7.3–147.2) for those who were seropositive for both anti-EBV markers (Chien *et al.*, 2001). In the other study (Ji *et al.*, 2007), seropositivity of anti-EBV VCA IgA was associated with an increased risk of nasopharyngeal carcinoma during follow-up with a crude relative risk of [9.4; 95%CI: 6.8–13.5]. For seven case–control studies (see Table 2.7 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.7.pdf), the odds ratios for nasopharyngeal carcinoma in relation to the evidence of infection with EBV ranged from 20.6 to infinity (Mutirangura *et al.*, 1998; Lo *et al.*, 1999; Lin *et al.*, 2001, 2004; Chen *et al.*, 2001; Fan *et al.*, 2004; Leung *et al.*, 2004). For only one study were the reported odds ratios below 3 (Tiwawech *et al.*, 2008).

Since the previous *IARC Monograph*, no new cofactors for cancer of the nasopharynx have been identified.

2.3.2 Lymphoepithelioma-like carcinomas

Rare carcinomas with a histological similarity to nasopharyngeal carcinoma (in that both have lymphoid stroma) are called lymphoepithelial-like carcinomas (Tsang & Chan, 2005). These can occur in multiple organ sites with epithelial lining, and have been reported most frequently in the salivary glands, and in the stomach. In the previous *IARC Monograph* (Table 30, IARC, 1997), 19 case series reported on the association of EBV with cancers of the stomach. These included a total of 102 lymphoepithelial cancers of which 90 had evidence of infection with EBV. In addition, five case series of lymphoepithelial cancers of the salivary gland indicated that 25/27 reported cases had evidence of infection with EBV (Table 28, IARC, 1997). More recently, in a case–control study of lymphoepithelial cancer of the salivary gland, 16/16 cases had evidence of EBV DNA in tumour tissue compared to 0/12 salivary gland tumours of other histology (Wang *et al.*, 2004; see Table 2.8 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.8.pdf). More recently, multiple case series including 209 cases of parotid gland lymphoepithelial carcinoma reported that EBV DNA was present in the tumour cells of 208 cases (Leung *et al.*, 1995; Kim *et al.*, 1999; Squillaci *et al.*, 2000; Białas *et al.*, 2002; Saku *et al.*, 2003; Wu *et al.*, 2004; Jen *et al.*, 2005; Hsu *et al.*, 2006; Saqui-Salces *et al.*, 2006). Therefore, the evidence of an association between EBV infection and lymphoepithelioma-like carcinomas has become substantially stronger since the previous *IARC Monograph* (IARC, 1997).

2.3.3 Cancer of the stomach

In 19 case series of cancer of the stomach reported in 1997 (Table 30, IARC, 1997), 115/1322 (9%) of cases had evidence of EBV DNA in tumour tissue. None of these studies provided information on possible infection with *H. pylori*. Since then, three case–control studies have been published in which two include a total of 69/174 cases with evidence of EBV DNA in tumour tissue (Shinkura *et al.*, 2000; Lo *et al.*, 2001; see Table 2.9 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.9.pdf), while the other, a nested case–control study, considered only serological responses against EBV in relation to all cancers of the stomach (Koshiol *et al.*, 2007; see Table 2.10 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.10.pdf). Recent findings indicate that people with EBV-positive tumours tend to have higher antibody titres against EBV (Shinkura *et al.*, 2000) or higher EBV viral loads (Lo *et al.*, 2001) than people with EBV-negative tumours or controls without cancer of the stomach. A recent review of over 30000 cancers of the stomach identified evidence of EBV DNA in 8% of the patients (Sousa *et al.*, 2008). It is important to note that EBV DNA is present within tumour cells and not in the surrounding epithelium, and that virus monoclonality has been demonstrated in tumour cells only (Sousa *et al.*, 2008). [The Working Group noted that the interaction of *H.pylori* and EBV in the etiology of cancer of the stomach needs further clarification.]

2.4 Other cancers

Several other studies investigating the evidence of infection with EBV in relation to cancers of the oral cavity, breast, cervix, testis, prostate, and multiple myeloma and leukaemia show limited or no evidence of an association.

See Table 2.11 available online at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.11.pdf), Table 2.12 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.12.pdf, and Table 2.13 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.13.pdf.

3. Cancer in Experimental Animals

In this volume, the Working Group decided not to include a separate section on "Cancer in Experimental Animals" in the *Monographs* on viruses but rather to include description of such studies under Section 4 (below). The reasoning for this decision is explained in the General Remarks.

4. Other Relevant Data

The mechanistic evidence for EBV-associated oncogenesis was thoroughly reviewed in the previous *IARC Monograph* (IARC, 1997), and is based on the following:

- The ability of EBV to immortalize human B lymphocytes *in vitro* (Nilsson *et al.*, 1971)
- Other effects of EBV infection of human cells *in vitro* affecting their phenotype – migration and invasion (Pegtel *et al.*, 2005; Dawson *et al.*, 2008)
- Convincing links of these phenotypic effects on cell proliferation, apoptosis, and cell migration to single EBV proteins or combinations thereof, primarily by the expression of or "knock down" of single proteins (Klein & Ernberg, 2007)
- Induction of EBV-positive lymphoproliferative diseases or lymphomas by infection of animals (New World monkeys) with EBV, or transplantation of infected human B lymphocytes to immunosuppressed mice (SCID or nude; Mosier *et al.*, 1988; Young *et al.*, 1989)

Although more circumstantial, *in vivo* evidence is also strong.

The EBV genome and the constant expression of viral proteins detected in a wide spectrum of human malignancies strongly support a role for EBV in carcinogenesis. EBV-associated tumours include diffuse large B-cell lymphomas that occur in immunocompromised persons, such as transplant recipients, certain congenitally immunocompromised individuals, and HIV-infected persons. EBV is also involved in the pathogenesis of Burkitt lymphoma, Hodgkin lymphoma, some T/NK-cell lymphomas, cancer of the nasopharynx, and some cancers of the stomach (Ambinder & Cesarman, 2007; Table 4.1).

In several of the high-risk groups for EBV-associated cancers, the EBV-genome load found in the peripheral blood lymphocytes or plasma precedes the development of malignancy (Lin *et al.*, 2004).

Further convincing evidence comes from the successful prevention or regression of EBV-carrying lymphoid tumours in humans by the adoptive transfer of EBV-specific cytotoxic T cells (Heslop & Rooney, 1997; Gustafsson *et al.*, 2000; Bollard *et al.*, 2007; Merlo *et al.*, 2008).

4.1. Transforming capacity of EBV

In vitro, EBV has the unique ability to transform resting B cells into permanent, latently infected lymphoblastoid cell lines, a system that has provided an invaluable, albeit incomplete, model of the lymphomagenic potential of the virus (Young & Rickinson, 2004). EBV can infect human B lymphocytes from any human donor as long as they express the CD21 consensus receptor for the attachment of EBV particles, and entry into the host cell (see Section 1.1). The B lymphocytes of several differentiation stages can be infected. The most efficient infection – and immortalization – has been seen in mature virgin B lymphocytes, which are IgM- and IgD-positive The virus establishes a 'latent' blastogenic "growth programme" (latency III) expressing nine latency-associated proteins and several non-translated genes, the two EBERs, micro-RNAs and BARF-transcripts. The infection results in the immortalization of the target

Table 4.1 EBV-associated tumours

Tumour type	Approx % EBV positivity
Lymphoid tissues	
Burkitt lymphoma	
Endemic (Subequatorial Africa)	>95[a]
Sporadic (Other countries)	20–80[b]
AIDS	30–50
AIDS-related DLBCL	
Immunoblastic	70–100
Non-immunoblastic	10–30
CNS lymphomas	>95
Plasmablastic lymphoma	60–75[c]
Primary effusion lymphoma	70–90
Post-transplant lymphoproliferative disorders	>90
Primary effusion lymphoma	70–90
Hodgkin lymphoma	20–90[d]
EBV-positive diffuse large B cell lymphoma of the elderly	100
Extranodal T/NK cell lymphoma, nasal type	100
Epithelial tissues	
Nasopharyngeal carcinoma	100
Lymphoepithelioma-like carcinoma	>80[e]
Gastric carcinoma	5–10
Other tissues	
Leiomyosarcoma in immunodeficient individuals	100

[a] The small fraction of EBV-negative cases in endemic regions may represent cases of sporadic Burkitt lymphoma.
[b] The proportion of positive cases varies widely with geographic distribution, ranging from 20% in some US and European studies to close to 80% in India, Egypt, and Northeastern Brazil.
[c] EBV positivity in plasmablastic lymphoma of the oral cavity in AIDS patients is close to 100%.
[d] The proportion of positive cases varies widely with geographic distribution, age, histological type and immunocompetence status. It is more frequent in low-resource areas, in very young or older patients, in the mixed cellularity subtypes, and approaches 100% in patients with AIDS.
[e] Stomach, parotid and liver
Compiled by the Working Group mainly from two reviews (Ambinder & Cesarman, 2007; Cesarman & Chadburn, 2007)

cells with a high efficiency, resulting in proliferation with a 30–40-hour extended cell cycle (Einhorn & Ernberg, 1978).

In vivo, mucosal epithelial cells can also be infected (see Section 1.1). By contrast, the infection of epithelial cells *in vitro* does not activate the full growth-transforming programme of the virus, and rarely – if ever – achieves full lytic replication (Young & Rickinson, 2004).

4.2 Biochemical and biological properties of EBV gene products

This section provides an overview of the properties of the EBV-encoded latency-associated gene products, and their mechanism of action relevant to transformation and tumorigenesis.

4.2.1 The latent EBV nuclear gene products (EBNAs)

The main known functions of the six nuclear proteins, EBNA-1, -2, -3A, -3B, -3C and -LP are summarized in Table 4.2.

(a) EBNA-1

EBNA-1, encoded by the ORF BKRF1, is a protein of highly variable size (60–100 kDa) due to a glycine–alanine repetitive sequence (Hennessy *et al.*, 1983). With the possible exception of latently infected resting B cells, EBNA-1 is expressed in most EBV-carrying cells, irrespective of the cell phenotype, level of differentiation or, in the case of lymphocytes, activation status (Section 1.1.7; Tables 1.3 and 1.4). EBV-positive B cells that only express EBNA-1 are poorly recognized by CTLs. The glycine–alanine repeat of EBNA-1 inhibits its processing through the ubiquitin–proteasome system and the subsequent MHC-class 1 association of the derived peptides, a prerequisite for recognition by CD8-positive CTLs (Levitskaya *et al.*, 1995). This results in a dramatically extended half-life of EBNA-1 to more than 2 weeks, and may contribute to its likely presence in resting B cells without *de novo* synthesis.

EBNA-1 is a DNA-binding protein that can bind to three different specific palindromic target sites on the viral DNA, each of which occurs multiple times in the viral genome, and is involved in the control of episomal maintenance, DNA replication, and viral gene expression in latency. Twenty binding sites are located in the family of repeats (FR) element, four in the dyad symmetry (DS) element, both these elements being localized to the origin of replication (OriP), and finally, two EBNA-1-binding sites are situated downstream of the Q promoter (Ambinder *et al.*, 1990; see also the map of the EBV genome depicted in Fig. 1.1). The dyad symmetry element controls S-phase-associated viral DNA replication. EBNA-1 regulates viral promoters via its multiple binding sites. The family of repeats element acts as an enhancer for the C promoter, directing transcription for all six EBNAs, and the Q-promoter elements are negative regulators of Q-promoter-driven EBNA-1 transcription through a negative autoregulatory feedback loop.

EBNA-1 acts as a transcriptional regulator of viral programmes, and maintains the viral genomes in the host cell. It is therefore necessary for cell transformation. EBNA-1 might also directly contribute to the tumorigenic process as it is expressed in all tumour types (Sample & Sample, 2008). It has been shown to exert an anti-apoptotic effect. Blocking its function with dominant negative mutants induces apoptosis in Burkitt lymphoma cell lines (Kirchmaier & Sugden, 1997).

EBNA-1 induces the specific recombinases RAG-1 and -2, which could contribute to genomic instability or even specific translocations (Tsimbouri *et al.*, 2002). EBNA-1 also induces genomic instability involving increases in the levels of reactive oxygen species (Gruhne *et al.*, 2009).

Recently, it was shown that EBNA-1 can also physically bind to cellular promoters, but whether

Table 4.2 Overview of the EBNA proteins: Functions and Interactions

Name (alternative nomenclature)	Functions	Interaction with cellular proteins	Expression in B cells	Evidence for role in B-cell immortalization
EBNA-1	- Viral episome maintenance - Viral DNA replication - Regulation of viral promoters	Karyopherins 2 α and β; TAP/p32; USP7 (HAUSP); RPA	Latency I, II, and III	Yes
EBNA-2	Activation of viral and cellular promoters	PU.1; hSNF5; Spi-B; CBF1/RBP-J kappa; p300/CBP; DP103; p100; TFIIE; TFIIH; TFIIB; TAF40; Myb; TBP	Latency III	Yes
EBNA-3A (EBNA-3)	Repression of the CBF1/RBP-J kappa dependent transcription	CBF1/RBP-J kappa; RBP-2N; CtBP; epsilon-subunit of TCP-1; XAP-2 ; F538 (UK/UPRT); AhR	Latency III	Yes
EBNA-3B (EBNA-4)		CBF1/RBP-J kappa; RBP-2N	Latency III	No
EBNA-LP (EBNA-5)	Co-activation of EBNA-2-dependent transcription	Hsp27; Hsp70 (Hsp72); Hsc70 (Hsp73); HAX-1; HA95; alpha & beta tubulins; prolyl-4-hydroxylase alpha-1 subunit; p14ARF; Fte-1/S3a	Latency III	Yes
EBNA-3C (EBNA-6)	Repression of the CBF1/RBP-J kappa dependent transcription	CBF1/RBP-J kappa; RBP-2N; DP103; ProT- alpha; SMN; NM23-H1; pRB	Latency III	Yes

Adapted from Klein & Ernberg (2007)

or not this binding plays a role in regulating the transcription of these genes *in vivo* remains to be demonstrated (Dresang *et al.*, 2009).

(b) EBNA-2

EBNA-2 is a phosphoprotein of about 82 kDa, and is among the earliest viral protein expressed in newly infected B cells. EBNA-2 is a potent transactivator of many cellular and viral genes but does not bind directly to DNA. It influences the responding promoters through its interaction with CBF1/RBP-Jk, PU1, and other cellular proteins. The EBNA-2 protein complexes formed induce chromatin remodelling. Elements for EBNA-2 responsiveness have been found in the EBV-Cp, LMP-1, LPMP-2, and CD23 promoters (Klein & Ernberg, 2007).

EBNA-2 is essential for the transformation of B cells into immunoblasts, and for the derivation of lymphoblastoid cell lines. EBNA-2-defective viral substrains cannot immortalize B cells. EBNA-2 is the EBV-encoded oncoprotein that differs most extensively between EBV types 1 and 2. EBV type 1 is a more efficient transformer of primary B lymphocytes than is type 2 (Rickinson *et al.*, 1987). Recruitment of EBNA-2 to DNA is essential for the transforming activity of EBV, and CBF1/RBP-Jk is its most extensively studied partner. CBF1/RBP-Jk functions as a downstream target of the Notch cell-surface receptor. Notch genes encode cell-surface receptors that regulate the developmental processes in a wide variety of organisms. The cleaved product of Notch is targeted to the nucleus where it binds to CBF1/RBP-Jk, and can activate transcription

(Strobl *et al.*, 1997). EBNA-2 is regarded as a constitutively active homologue of Notch. However, Notch can only partially substitute for EBNA-2 in B-cell transformation experiments, probably owing to its inability to upregulate the transcription of LMP1 or c-myc. Thus, the functional homology is partial (Höfelmayr *et al.*, 2001).

EBNA-2 induces a variety of activation markers and other cellular proteins in B cells, including CD23, CD21, c-fgr, and c-myc. It is required for the expression of EBV-encoded LMP-1 and LMP-2A in immunoblastic cells (Wang *et al.*, 1990a, b; Kaiser *et al.*, 1999; Klein & Ernberg, 2007). The essential role of EBNA-2 in the immortalization of B cells is therefore due to its role in the transactivation of viral promoters (Cp, LMP-1 and -2) and of cellular genes associated with B-cell activation and growth, among them c-myc. C-myc activation in lymphocytes, in turn, induces protein synthesis (e.g. D-type cyclins and cyclin E) but also the downregulation of the inhibitors p21 and p27. The induction of c-myc is regarded as a major link between EBV infection and cell-cycle control (Kaiser *et al.*, 1999). EBNA-2 is required to maintain the EBV-driven proliferation of B cells. EBNA-2 can be replaced by the constitutive expression of exogenous c-myc. The switch from the EBNA-2-driven to the c-myc-driven state is accompanied by a phenotypic change of the lymphoblastoid cell line-like cell to a more Burkitt lymphoma-like cell, resembling dividing germinal centre B cells (Polack *et al.*, 1996).

(c) EBNA-LP

EBNA-LP (also known as EBNA-5) is a nuclear phosphoprotein. Together with EBNA-2, EBNA-LP is the earliest viral protein expressed in freshly infected B cells. Co-expression of EBNA-LP with EBNA-2 enhances EBNA-2-mediated transcriptional activation (Klein & Ernberg, 2007). The two proteins can induce the entry of resting B cells into the G1-phase (Sinclair *et al.*, 1994). EBNA-LP is tightly associated with the nuclear matrix, and often accumulates in the nuclear promyelocytic leukaemia bodies. EBNA-LP is also necessary for immortalization (Pokrovskaja *et al.*, 2001). EBNA-LP was shown *in vitro* to exert an inhibitory effect on the p53–Rb axis by targeting the p53 regulator p14 ARF. The latter can bind MDM2, suppress its ability to mediate in the degradation of p53, and thereby increase the expression level of p53. It was suggested that EBNA-LP participates in the elimination of the p14 ARF–MDM2–p53 complexes and contributes to the downregulation of p14 ARF and p53 protein levels in EBV-infected B cells (Kashuba *et al.*, 2003).

(d) EBNA-3

The EBNA-3 family – EBNA-3A (ORF: BLRF3 + BERF1), EBNA-3B (or EBNA-4, ORF: BERF2a + BERF2b), and EBNA-3C (or EBNA-6, ORF: BERF3 + BERF4) – comprises three large nuclear phosphoproteins in sizes ranging from 140–180 kDa. All three proteins are stable proteins that accumulate in intranuclear clumps, sparing the nucleolus (Klein & Ernberg, 2007).

All EBNA-3 proteins share a limited homology in a region near the *N* terminus, and this conserved domain mediates the binding to CBF1/RBP-Jk. This is how they all act as repressors of EBNA-2-mediated transactivation of the CBF1/RBP-Jk-dependent Cp, LMP-2A, and LMP-1 promoters. EBNA-3C also physically associates with histone deacetylase HDAC1, and can repress transcription through the Notch signalling pathway (Radkov *et al.*, 1999).

EBNA-3C (but not EBNA-3A or -3B) can also activate the transcription of both cellular and viral genes (e.g. *CD21*, *CD23*, and *LMP-1*). This activation is clearly distinct from the interaction of EBNA-3C with CBF1/RBP-Jk, and requires an intact Spi binding site as well as a fully functional EBNA-2 protein (Zhao & Sample, 2000). EBNA-3C disrupts cell-cycle checkpoints at several levels. One is by recruiting the SCF^{skp2}

ubiquitin ligase complex, which mediates the ubiquitination and degradation of pRB (Knight *et al.*, 2005).

EBNA-3A and EBNA-3C, but not EBNA-3B, are necessary for *in vitro* immortalization (Tomkinson *et al.*, 1993).

4.2.2 The latent membrane proteins

EBV expresses three latent membrane proteins (LMPs) during latency II and III in immunoblasts as well as in derived tumours and cell lines: LMP-1, LMP-2A, and LMP-2B. All three proteins are also detected in epithelial tumours of the nasopharynx, and during the early stages of oral hairy leukoplakia (Webster-Cyriaque & Raab-Traub, 1998) (see Section 1.1; Table 1.4). LMP-2A transcripts can also be expressed in resting virus-carrying B lymphocytes in healthy individuals – the reservoir of persistently latent EBV (Chen *et al.*, 1995).

LMP-2A together with LMP-1 are necessary for continued lymphoma cell survival via TRAF2 regulation of NF-κB (Guasparri *et al.*, 2008).

The three LMP proteins are highly multifunctional and interact with several cellular signalling pathways (Table 4.3). They are expressed at the cell surface membrane as well as in intracellular membranes of the Golgi and endoplasmic reticulum (Hennessy *et al.*, 1984; Lynch *et al.*, 2002).

(a) LMP-1

LMP-1 is a 356-amino-acid protein which consists in a short *N*-terminal cytoplasmic domain, six membrane-spanning domains, and a *C*-terminal cytoplasmic domain 200 amino-acid long (Liebowitz *et al.*, 1986).

LMP-1 is essential although not mandatory for the transformation of B lymphocytes into lymphoblastoid cell lines, and EBV mutants lacking LMP-1 fail to efficiently immortalize B cells (Dirmeier *et al.*, 2003).

LMP-1 can induce lymphomas and epithelial tumours in transgenic mice, acting as tumour promoter after chemical initiation (Curran *et al.*, 2001).

LMP-1 as an integral membrane protein acts like a constitutively activated receptor. It almost completely mimics the CD40-mediated signalling, and is thus functionally homologous to the TNF-receptor (TNFR)-family of proteins in B lymphocytes and epithelial cells. Indeed, it constitutively activates major signalling systems such as NF-κB (canonical and non-canonical), JNK-kinase, and JAK/STAT-pathways. Protection from apoptosis is one of its major downstream effects (Lam & Sugden, 2003a, b).

LMP-1 has been shown to interact with several proteins of the TNFR-signalling pathway through its *C*-terminal activation region (CTAR) 1 and 2 (Table 4.3; Lam & Sugden, 2003b). These interactions result in the NF-κB-dependent upregulation of several genes. LMP-1 can block apoptosis due to the upregulation of several anti-apoptotic proteins, including A20 and Bcl-2, and the block of p53-mediated apoptosis by the latter (Henderson *et al.*, 1991; Hatzivassiliou & Mosialos, 2002). It may also alter the ratio of caspase-8, an initiator caspase, and its competitor FLIP (FLICE inhibitor protein) (Tepper & Seldin, 1999).

LMP-1 also activates JNK-kinase (Eliopoulos *et al.*, 1999; Kieser *et al.*, 1999). It can also induce telomerase activity (Terrin *et al.*, 2008).

Through its interference with several major signalling pathways in B cells and epithelial cells, LMP-1 mediates deregulation of several hundred cellular proteins. LMP-1 induces the expression of adhesion molecules such as ICAM-1 and LFA, and also MHC Class I and II (Hatzivassiliou & Mosialos, 2002).

Moreover, LMP-1 expressed in epithelial cell lines *in vitro* inhibits DNA repair and induces micronuclei formation, chromosomal aberrations, and consequent genomic instability (Liu *et al.*, 2004).

Table 4.3 Overview of the EBV latent membrane proteins: functions and interactions

Name	Functions	Major protein interactions	Expression in B cells	Evidence for role in oncogenesis
LMP-1	Mimics CD 40 Activation of NFkB, JNK kinase, JAK/STAT, MAP kinase, Akt Cell survival Induction of adhesion and immune regulatory membrane proteins	RAF 1,2,3, TRADD BRAM 1 LMP-2A	Latency II-III	***Yes*** - Anti-apoptotic - Survival of lymphoma cells - Transformation of primary rat embryo fibroblasts - Tumours in transgenic animals
LMP-2A	Interacts with phosphotyrosine kinases including Src-family and PI3-kinase Blocks lytic cycle Block BCR activation	Src, Lyn, Lck ZAP-70, Syk, AIP4/Nedd4	Latency I-III	***Yes*** - Survival of B-cells and lymphoma cells - Cell migration and invasion
LMP-2B	Modulates function of LMP-2A	LMP-2A	Latency III	***No***

Adapted from Klein & Ernberg (2007)

(b) LMP-2A and LMP-2B

LMP-2A contains 12 trans-membrane domains, and two intracellular tails: a 27-amino-acid *C*-terminal tail, important for protein aggregation, and a 119-amino-acid *N*-terminal tail that confers the capacity of LMP-2A to activate signal cascade. LMP-2A has been reported to aggregate into 'cap-like' structures at the plasma membrane and specifically associate with lipid rafts, sites enriched for signalling molecules (Dykstra *et al.*, 2001; Higuchi *et al.*, 2001).

LMP-2A signalling mimics signalling through the B-cell receptor with which it shares structural and functional similarities. The *N*-terminal tail of LMP-2A contains eight phosphotyrosine motifs that interact with SH2-domain-containing proteins such as the immunoglobulin-receptor (IgR)-induced kinases Lyn. In addition, LMP-2A also possesses an Immunoglobulin Transactivation Motif (ITAM) with complete homology to the corresponding IgR-ITAM-motif of its gamma-chain that binds the Syk kinase in its activated phosphorylated state (Klein & Ernberg, 2007).

When expressed as a B-lineage-specific transgene in mice, it can both drive B-cell development, and promote the survival of mature B-cells in the absence of surface immunoglobulin expression (Merchant *et al.*, 2000). EBV mutants with a deleted *LMP-2A* gene fail to allow germinal centre B cells to survive; it is thus essential for growth transformation of these B cells (Mancao & Hammerschmidt, 2007).

If LMP-2A mimics B-cell receptor signalling, there is evidence however that expression of LMP-2A in B lymphocytes also attenuates normal activation through B-cell receptors. It was shown that LMP-2A blocks both B-cell receptor signalling and antigen-processing function in lymphoblastoid cell lines (Dykstra *et al.*, 2001). It inhibits apoptosis pathways that are normally activated by B-cell receptor activation in Ramos and Akata cells, and prevents EBV reactivation in these cells. Thus, LMP-2A has an important role in maintaining viral latency.

LMP-2A has also been shown to activate PI3 kinase and the downstream phosphorylation of Akt in epithelial cells and B cells. This may modulate cell growth and apoptosis (Swart *et al.*, 2000; Moody *et al.*, 2005). It was shown to induce cell mobility and invasion in epithelial cells (Pegtel *et al.*, 2005).

LMP-2A can also associate with Nedd4-ubiquitin ligases via its PPPPY-motif located at its AA-terminus. It is conceivable that the binding of LMP-2A to the Nedd4 family of proteins can result in fast destruction of LMP-2A itself and LMP-2A-associated kinases, by guiding the complex to the ubiquitin–proteasome system (Winberg *et al.*, 2000).

A major role of LMP-2A in relation to latent EBV infection may stem from its ability to inhibit the activation of lytic EBV replication in infected B cells by cell-surface-mediated signal transduction (Miller *et al.*, 1994). This may prevent lytic replication in latently infected B cells as they circulate in the blood, bone marrow or lymphatic tissues, where they might encounter antigens or other ligands capable of engaging B-cell receptors and activating the viral cycle.

LMP-2B is a splice variant of LMP-2A which lacks the *N*-terminal tail with its kinase-interacting domains. It is thought to interact with LMP-2A, and thereby modulates its functions (Rovedo & Longnecker, 2007).

4.2.3 The non-coding RNAs

(a) The EBV-encoded RNAs (EBERs)

The EBERs are two non-coding, non-polyadenylated RNAs, EBER-1 (166 nucleotide long) and EBER-2 (172 nucleotides long), which are always expressed in very high abundance (10^5–10^6 copies/cell) in latently EBV-infected cells irrespective of cell phenotype. Structural predictions suggest that they can form a compact structure with five major hairpin structures. They act as regulators of signalling and transcription factors, resulting in the production of interferons and cytokines (Samanta *et al.*, 2008). The EBERs were shown to induce the anti-inflammatory cytokine IL10 as an autocrine growth factor in Burkitt lymphoma cells. This effect is produced via retinoic-acid-inducible gene I (*RIG-I*, a sensor of innate immunity)-mediated activation of IRF-3. In cell lines derived from nasopharyngeal carcinoma, the EBERs induce insulin-like growth factor 1 (IGF-1), which also acts as an autocrine growth factor. This is corroborated *in vivo* because nasopharyngeal carcinoma biopsies consistently express IGF-1 (Wu *et al.*, 2007; Samanta *et al.*, 2008).

EBERs may also contribute to B-cell transformation; this was shown for EBER-2 RNA via its efficient induction of IL6 (Wu *et al.*, 2007).

(b) The EBV micro-RNAs

Micro-RNAs are small non-coding RNAs, generally 20–24 nucleotides in length, that can transcriptionally downregulate the expression of mRNAs, bearing complementary sequences. EBV encodes at least 22 micro-RNAs which are expressed to various degrees in all forms of latency, and in tumour tissues (Pfeffer *et al.*, 2004; Cai *et al.*, 2006; Grundhoff *et al.*, 2006). All EBV tumours that have been studied express at least some of the EBV-encoded micro-RNAs. They have been shown to target several interesting cellular genes, and thus they may very well turn out to play a central role in the tumorigenesis of EBV. Target genes identified so far include PUMA of the p53 pathway, and the chemokine CXCL11 (Choy *et al.*, 2008; Xia *et al.*, 2008).

4.3 In vivo and in vitro evidence for a role of EBV in human malignancies

4.3.1 EBV-associated B-cell lymphomas

There are three histologically and clinically distinct types of EBV-associated B-cell lymphomas that show different patterns of latent gene expression and seem, from the immunoglobulin gene sequencing, to derive from cells at different position in the B-cell differentiation pathway (Fig. 1.3).

(a) *Lymphomas in immunosuppressed individuals*

T-cell-immunocompromised patients — organ transplant recipients, congenitally immunocompromised individual, particularly the X-linked lymphoproliferative syndrome (XLP) and AIDS patients — are at a high risk of developing B-cell lymphomas.

Most post-transplant lymphoproliferative diseases occur as polyclonal or monoclonal lesions within the first year of allografting, when immunosuppression is most severe. Almost all of these early onset tumours are EBV-positive, and express the full latency III programme, which identifies them as virus-transformed B cells that grow out in the absence of effective T-cell surveillance. Some of the lymphomas that are seen in highly immunocompromised AIDS patients, particularly central nervous system lesions, show essentially the same phenotype. (Young & Rickinson, 2004). The EBV proteins expressed include the highly immunogenic members of the EBNA-3 triad (EBNA-3A, -3B, and -3C), which is why passive immunotherapy with *in vitro*-expanded EBV-antigen-specific CD8-positive CTLs can bring about dramatic regression, even of widely disseminated tumours (Rooney *et al.*, 1995; Khanna *et al.*, 2001).

(b) *Burkitt lymphoma*

EBV is associated with almost all of the paediatric Burkitt lymphomas in high endemicity areas, but only with a fraction of sporadic or AIDS-associated Burkitt lymphomas (Kelly & Rickinson, 2007).

In equatorial African endemic areas, high EBV VCA antibody titres are regularly detected in children as early as 4 years before tumour development, which indicates an early infection and a high viral load (Geser *et al.*, 1982). In these countries, malaria is holoendemic and this infection appears to be a strong risk factor of Burkitt lymphoma (reviewed in Rochford *et al.*, 2005).

EBV gene expression in Burkitt lymphoma is strictly latent and very constrained. Most cells express only EBNA-1 and the EBERs (latency I programme). The BARTs have also been detected in Burkitt lymphoma samples by PCR. In addition, some genes traditionally thought to be confined to expression during the lytic cycle can also be expressed in Burkitt lymphoma cells, by alternative splicing of transcripts driven from latent promoters. The expression of the bcl-2 homologue *BHRF-1* by this mechanism might be particularly significant (Kelly *et al.*, 2006).

All Burkitt lymphomas, irrespective of form or EBV status, carry *c-myc* translocations to one of the immunoglobulin loci, the heavy chain locus on chromosome 14 or the light chain loci on chromosomes 2 or 22. Theses translocations are the hallmark of all Burkitt lymphomas. This may reflect the timing of the initiation of the lymphoma in relation to B-cell differentiation and at the time of the immunoglobulin gene rearrangement. As a result, the *c-myc* gene is in these cells under the control of a highly active immunoglobulin gene promoter leading to constitutive expression of *c-myc* (Klein, 1983).

Apart from the possible role of expanding the lifespan of the EBV-carrying B cells before lymphomagenesis, thus increasing the likelihood of secondary genetic events (such as the c-myc translocation), EBV can also play a direct role in lymphoma initiation. First, the expression of dominant-negative EBNA-1 mutants in Burkitt lymphoma cells *in vitro* induces apoptosis, which points to the requirement of EBNA-1 for the continued survival of EBV-positive Burkitt lymphoma cells (Kirchmaier & Sugden, 1997). In addition, genomic instability induced by EBNA-1 could be another possible mechanism (Tsimbouri *et al.*, 2002; Gruhne *et al.*, 2009).

It has also been proposed that EBV, by virtue of its anti-apoptotic *BHRF1* gene, provides protection against apoptosis induced by deregulated c-*myc* expression (Kelly *et al.*, 2006). Another

possibility is apoptotic protection by the EBERs (Takada, 2001).

(c) Hodgkin lymphoma

Hodgkin lymphoma is characterized by an expansion of Reed-Sternberg cells, which are now postulated to be of B-cell lineage. Several lines of evidence link EBV to Hodgkin lymphoma:

- A 4-fold increase in risk in individuals with a past history of infectious mononucleosis;
- Increased antibody titres to EBV viral capsid antigen; and,
- The detection of monoclonal EBV genomes in the HRCs.

Almost half of the Hodgkin lymphoma cases in Western countries carry EBV-positive HRCs that express the latency II pattern with EBNA-1, LMP-1, LMP-2A, LMP-2B, and the EBERs being expressed (reviewed in Thompson & Kurzrock, 2004).

4.3.2 EBV-associated epithelial cancers

(a) Cancer of the nasopharynx

EBV is consistently detected in patients with cancer of the nasopharynx, with a stronger association with non-keratinizing carcinoma than with keratinizing carcinoma (Maeda *et al.*, 2009). Regardless of whether the patient with nasopharyngeal carcinoma lives in an area of endemic or sporadic incidence (see Section 1.2), all tumour cells contain EBV DNA as multiple clonal episomes as shown by terminal repeats analysis. The clonality of EBV DNA suggests that nasopharyngeal carcinoma occurs from the clonal expansion of a single EBV-infected cell, and that EBV infection is an early, possibly initiating, event in the development of nasopharyngeal carcinoma (Raab-Traub & Flynn, 1986). This is further supported by studies showing that preneoplastic and preinvasive lesions of the nasopharynx are also infected by EBV, and express the same latency programme (Pathmanathan *et al.*, 1995).

Nasopharyngeal carcinoma cells express an EBV-latency II pattern (see Section 1.1) including the expression of EBNA-1, LMP-1, LMP-2A, LMP-2B, the EBERS, and micro-RNAs.

Several genes relevant for the tumorigenic phenotype of the nasopharyngeal carcinoma cell are induced by LMP-1 (Thornburg & Raab-Traub, 2007).

However, only about two-thirds of nasopharyngeal tumours express LMP-1 *in vivo* as measured by Western blot or in-situ staining. Clinical and follow-up data from 74 cases of nasopharyngeal carcinoma showed that LMP-1-positive nasopharyngeal carcinoma grew faster and more expansively than LMP-1-negative tumours, in a short two-year follow-up (Hu *et al.*, 1995). In the LMP-1 non-expressing tumours, the promoter region of the LMP-1 gene is hypermethylated (Hu *et al.*, 1991).

In a few early precancerous lesions *in situ* that could be studied, LMP-1 is always expressed (Pathmanathan *et al.*, 1995). It might thus have an important role in the early process, but its functions can later be replaced by cellular genes.

LMP-2A induces migration and invasion of epithelial cells including nasopharyngeal-carcinoma-derived cell lines, which could affect the *in vivo* phenotype of the tumour (Allen *et al.*, 2005).

Both EBERs and some of the EBV micro-RNAs are expressed in nasopharyngeal carcinoma, but their respective role in tumorigenesis has not yet been addressed (Cosmopoulos *et al.*, 2009).

(b) Cancers of the stomach

EBV is detected in 5–10% of gastric carcinomas worldwide (see Section 1.2). It has been suggested that EBV-positive gastric carcinoma belongs to a separate clinico-histopathological entity, distinguishable from most gastric carcinomas as this occurs at younger age, with a distinct histopathology (ranging from

adenocarcinoma with lymphoid infiltration to lymphoepithelioma-like), and with a more proximal location (Fukayama *et al.*, 2008). In these tumours, the EBV genome is present in (almost) all cells and is monoclonal, suggesting that the infection takes place at tumour precursor cell state. The latent pattern of EBV in gastric carcinoma corresponds to an intermediate latency I / II programme, with EBNA-1, EBERs, BARF-0, LMP-2A, and micro-RNAS. In addition, some lytic infection genes such as *BARF-1* and *BHRF-1* have also been detected in these tumours. All tumour cells express the EBERs as shown by PCR and by in-situ hybridization, while expression of LMP-2A and the lytic genes is variable (Luo *et al.*, 2005).

In gastric carcinoma cells in culture, EBV expresses a latency pattern that is similar to gastric carcinoma *in vivo*, including the viral micro-RNAs. In these cells, EBV uses LMP-2A to activate the NF-κB-surviving pathway which confers some resistance to apoptosis induced by serum deprivation (Hino *et al.*, 2008). In parallel with the results *in vitro*, the NF-κB-surviving pathway has been shown to be highly activated in nearly all EBV-associated gastric carcinomas in the advanced stage, and the frequency is significantly higher than that in EBV-negative gastric carcinomas (Luo *et al.*, 2005). Various viral proteins (e.g. HTLV-1 Tax, HPV-16E6, HBx) are known to upregulate surviving protein expression in human neoplasms; this may be a common denominator in the mechanisms of human viral oncogenesis (Hino *et al.*, 2008).

(c) Other carcinomas

Carcinomas showing morphological features that are similar to undifferentiated nasopharyngeal carcinomas or EBV-related gastric carcinomas, so-called lymphoepithelial carcinomas, can occur at other sites. Lymphoepithelial carcinomas of the salivary glands, of the lungs, and possibly of the thymus are frequently associated with EBV infection (IARC, 1997), but there is no mechanistic data demonstrating a specific role of EBV in these tumours.

4.4 Interaction between EBV and other agents; mechanisms involved in EBV reactivation

EBV coexists for a lifetime in a latent state in most human hosts without overt serious consequences. This strongly suggests that cofactors able to reactivate EBV viral replication may potentially be required for EBV-associated carcinogenesis.

EBV can be reactivated from its latent state by several means, and its reactivation could potentially lead to the development of EBV-related pathology.

(a) Foreign antigen

In healthy carriers, EBV remains silent and expresses only EBERs in infected resting memory B cells. Viral replication can only occur in dividing cells; this is the case when memory B cells divide for cell maintenance or when they differentiate into plasma cells following activation by the presence of a foreign antigen (Laichalk & Thorley-Lawson, 2005). This means that any additional infection may potentially reactivate EBV in these cells.

(b) Immunodeficiency

EBV infection is strictly kept under very tight control by cell-mediated immunity in immunocompetent individuals (see Fig. 1.2). Immunodeficiency (iatrogenic as in transplant recipients, congenital, or HIV-related) allows the spread of uncontrolled reactivated EBV from infected memory B cells, which can give rise to various lymphoproliferative disorders (Fig. 1.3 and Section 4.3.1).

(c) Malaria

Infection with both EBV and *Plasmodium falsiparum* are recognized to be required for the genesis of endemic Burkitt lymphoma. Children living in areas endemic for malaria have an elevated EBV load, and have diminished EBV-specific T-cell immunosurveillance between the ages of 5–9 years, which coincides with the peak age incidence of the diseases (Moormann *et al.*, 2005, 2007). In addition, acute malaria infection leads to increased levels of circulating EBV that are cleared following anti-malaria treatment (Rasti *et al.*, 2005; Donati *et al.*, 2006). A direct molecular mechanism of interaction has been demonstrated between P. *falsiparum* and EBV. CIDR1α, a cystein-rich domain of the P. *falsiparum* membrane protein 1 was shown to act as a polyclonal B-cell activator, and to induce the EBV lytic cycle (Chêne *et al.*, 2007).

(d) Food

In the southern region of the People's Republic of China, where nasopharyngeal carcinoma is a very common malignancy, the ingestion of salted fish especially during weaning has been shown to be an important risk factor for the condition (Yuan *et al.*, 2000). Other preserved food preparations such as the spiced mixture "harissa" in Tunisia have also been identified as potential risk factors for nasopharyngeal carcinoma (Jeannel *et al.*, 1990). Using Raji cells, an *in vitro* study demonstrated a strong EBV reactivation activity in aqueous extracts of some Cantonese salted fish from China, and harissa, and to a lesser extent qaddid (dry mutton preserved in olive oil) from Tunisia (Shao *et al.*, 1988).

(e) Inflammation

TGFβ-1, a multifunctional cytokine, induces EBV reactivation in EBV-infected gastric carcinoma cell lines *in vitro* as shown by the induction of EBV-early immediate *BZLF-1* RNA, and its protein product ZEBRA (Fukuda *et al.*, 2001). [The Working Group noted that TGFβ is highly expressed during inflammation suggesting that chronic inflammation may potentially reactivate latent EBV infection *in vivo*; this hypothesis still needs to be demonstrated.]

(f) Chemical agents and drugs

Stimuli that can activate the latency-to-lytic switch in cultured cell lines, include phorbol esters, which are protein kinase C agonists; sodium butyrate and trichostatin A, which are histone deacetylase inhibitors; 5-aza-2-deoxycytidine, which is a DNA methyltransferase inhibitor; and anti-immunoglobuline G, which activates the B-cell antigen receptor. While operating by different modes of action, these agents all lead to the expression of the EBV lytic activator genes *BZLF-1* and *BRLF-1*, which encode ZEBRA and Rta (Countryman *et al.*, 2009).

4.5 Transgenic models for EBV-associated cancers

Transgenic mice models expressing EBNA-1 and LMP-1 under various tissue-specific promoters are available. They show dysregulation of the haematopoetic and epithelial compartments, depending on which tissue the transgene is directed to. Transplantable tumours occur with an elevated frequency in LMP-1-transgenic mice (Kulwichit *et al.*, 1998; Curran *et al.*, 2001; Shair *et al.*, 2007). EBNA-1-transgenic mice have also been shown to develop tumours (Wilson *et al.*, 1996).

A humanized mouse model in which the functional human immune system (including T, B, and natural killer lymphocytes) is reconstituted, can simulate key aspects of EBV infection. Inoculation of EBV in these mice causes B-cell lymphoproliferative disorder, with histopathological findings and latent EBV gene expression that are similar to that in immunocompromised patients (Yajima *et al.*, 2008).

4.6 Synthesis

Mechanistic data that strongly support an oncogenic role of EBV in human cancer can be summarized as follows:

- EBV immortalizes normal B cells in culture.
- One or several EBV gene products are expressed in all EBV-associated cancers.
- At the molecular level, these EBV-encoded gene products associated with latent viral infection induce cell proliferation, block apoptosis, induce genomic instability or modulate cell migration. These events occur before or during tumour initiation. Several of these gene products are also involved in mechanisms contributing to continued tumour maintenance, cell growth, and progression.

Mechanistic data strongly support an oncogenic role of EBV in diffuse large B-cell lymphomas in immunocompromised individuals (post-transplant patients, XLP, AIDS). In these tumours, EBV adopts the growth-proliferative programme seen in EBV-infected human B cells, which is solely driven by the virus.

Mechanistic data strongly support an oncogenic role of EBV in Burkitt lymphoma, where EBV promotes the survival of B cells that have undergone the pro-apototic *myc*-translocation.

Mechanistic data strongly support an oncogenic role of EBV in Hodgkin lymphoma and nasopharyngeal carcinoma, where LMP-1 can act as a transforming viral protein.

There is positive mechanistic data for a role of the virus in EBV-positive gastric carcinoma.

Regarding the role of EBV in EBV-positive T/NK-cell lymphomas, *in vitro* model systems still require optimization, and as a result, only weak mechanistic evidence is available to support a role of EBV in these types of cancer.

At the time of writing, no mechanistic studies have been published that directly investigate a role of EBV in lymphoepitheliomas of the salivary gland.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of EBV. EBV causes Burkitt lymphoma, immunosuppression-related non-Hodgkin lymphoma, extranodal NK/T-cell lymphoma (nasal type), Hodgkin lymphoma, and cancer of the nasopharynx. Also, a positive association has been observed between exposure to EBV and lymphoepithelioma-like carcinoma.

In the case of gastric carcinoma, there is insufficient epidemiological evidence for the involvement of EBV. However, the fact that the EBV genome is present in the tumour cell in a monoclonal form, and that transforming EBV proteins are expressed in the tumour cell provides a mechanistic explanation of how EBV might cause a proportion of gastric cancer.

EBV is *carcinogenic to humans (Group 1).*

References

Aalto SM, Juvonen E, Tarkkanen J *et al.* (2007). Epstein-Barr viral load and disease prediction in a large cohort of allogeneic stem cell transplant recipients. *Clin Infect Dis*, 45: 1305–1309. doi:10.1086/522531 PMID:17968825

Ablashi DV, De-Thé GB, Easton JM *et al.* (1974). Antibodies to Epstein-Barr virus (EBV) antigens in sera of American Burkitt lymphoma patients. *Biomedicine*, 21: 288–293. PMID:4139981

Aitken C, Sengupta SK, Aedes C *et al.* (1994). Heterogeneity within the Epstein-Barr virus nuclear antigen 2 gene in different strains of Epstein-Barr virus. *J Gen Virol*, 75: 95–100. doi:10.1099/0022-1317-75-1-95 PMID:8113744

Alexander FE, Jarrett RF, Lawrence D *et al.* (2000). Risk factors for Hodgkin's disease by Epstein-Barr virus (EBV) status: prior infection by EBV and other agents. *Br J Cancer*, 82: 1117–1121. PMID:10737396

Alexander FE, Lawrence DJ, Freeland J *et al.* (2003). An epidemiologic study of index and family infectious mononucleosis and adult Hodgkin's disease (HD): evidence for a specific association with EBV+ve HD in

young adults. *Int J Cancer*, 107: 298–302. doi:10.1002/ijc.11156 PMID:12949811

Allen MD, Young LS, Dawson CW (2005). The Epstein-Barr virus-encoded LMP2A and LMP2B proteins promote epithelial cell spreading and motility. *J Virol*, 79: 1789–1802. doi:10.1128/JVI.79.3.1789-1802.2005 PMID:15650203

Ambinder RF, Cesarman E (2007). *Clinical and pathological aspects of EBV and KSHV Infection*. In: *Human Herpesviruses*. Arvin A, Campadelli-Fiume G, Mocarski E *et al.*, editors. Cambridge: Cambridge University Press, pp. 885–903.

Ambinder RF, Shah WA, Rawlins DR *et al.* (1990). Definition of the sequence requirements for binding of the EBNA-1 protein to its palindromic target sites in Epstein-Barr virus DNA. *J Virol*, 64: 2369–2379. PMID:2157891

Arnulf B, Copie-Bergman C, Delfau-Larue MH *et al.* (1998). Nonhepatosplenic gammadelta T-cell lymphoma: a subset of cytotoxic lymphomas with mucosal or skin localization. *Blood*, 91: 1723–1731. PMID:9473239

Barrionuevo C, Zaharia M, Martinez MT *et al.* (2007). Extranodal NK/T-cell lymphoma, nasal type: study of clinicopathologic and prognosis factors in a series of 78 cases from Peru. *Appl Immunohistochem Mol Morphol*, 15: 38–44. doi:10.1097/01.pai.0000205062.27174.56 PMID:17536305

Bernard SM, Cartwright RA, Darwin CM *et al.* (1987). Hodgkin's disease: case control epidemiological study in Yorkshire. *Br J Cancer*, 55: 85–90. doi:10.1038/bjc.1987.18 PMID:3814482

Berrington de González A, Urban MI, Sitas F *et al.* (2006). Antibodies against six human herpesviruses in relation to seven cancers in black South Africans: a case control study. *Infect Agent Cancer*, 1: 2 doi:10.1186/1750-9378-1-2 PMID:17150131

Białas M, Sińczak A, Choińska-Stefańska A, Zygulska A (2002). EBV-positive lymphoepithelial carcinoma of salivary gland in a woman of a non-endemic area–a case report. *Pol J Pathol*, 53: 235–238. PMID:12597342

Black FL, Woodall JP, Evans AS *et al.* (1970). Prevalence of antibody against viruses in the Tiriyo, an isolated Amazon tribe. *Am J Epidemiol*, 91: 430–438. PMID:4316199

Bollard CM, Gottschalk S, Leen AM *et al.* (2007). Complete responses of relapsed lymphoma following genetic modification of tumor-antigen presenting cells and T-lymphocyte transfer. *Blood*, 110: 2838–2845. doi:10.1182/blood-2007-05-091280 PMID:17609424

Borisch B, Finke J, Hennig I *et al.* (1992). Distribution and localization of Epstein-Barr virus subtypes A and B in AIDS-related lymphomas and lymphatic tissue of HIV-positive patients. *J Pathol*, 168: 229–236. doi:10.1002/path.1711680212 PMID:1334141

Buisson M, Morand P, Genoulaz O *et al.* (1994). Changes in the dominant Epstein-Barr virus type during human immunodeficiency virus infection. *J Gen Virol*, 75: 431–437. doi:10.1099/0022-1317-75-2-431 PMID:8113767

Cai X, Schäfer A, Lu S *et al.* (2006). Epstein-Barr virus microRNAs are evolutionarily conserved and differentially expressed. *PLoS Pathog*, 2: e23 doi:10.1371/journal.ppat.0020023 PMID:16557291

Carbone A, Gaidano G, Gloghini A *et al.* (1999). AIDS-related plasmablastic lymphomas of the oral cavity and jaws: a diagnostic dilemma. *Ann Otol Rhinol Laryngol*, 108: 95–99. PMID:9930548

Carbone A, Gloghini A, Dotti G (2008). EBV-associated lymphoproliferative disorders: classification and treatment. *Oncologist*, 13: 577–585. doi:10.1634/theoncologist.2008-0036 PMID:18515742

Carpenter LM, Newton R, Casabonne D *et al.* (2008). Antibodies against malaria and Epstein-Barr virus in childhood Burkitt lymphoma: a case-control study in Uganda. *Int J Cancer*, 122: 1319–1323. doi:10.1002/ijc.23254 PMID:18000823

Carter CD, Brown TM Jr, Herbert JT, Heath CW Jr (1977). Cancer incidence following infectious mononucleosis. *Am J Epidemiol*, 105: 30–36. PMID:831462

Çavdar AO, Yavuz G, Babacan E *et al.* (1994). Burkitt's lymphoma in Turkish children: clinical, viral [EBV] and molecular studies. *Leuk Lymphoma*, 14: 323–330. doi:10.3109/10428199409049685 PMID:7950922

CDC (2006). *Epstein-Barr Virus and Infectious Mononucleosis*. Atlanta, GA: Centers for Disease Control and Prevention. Available at: http://www.cdc.gov/ncidod/diseases/ebv.htm

Cesarman E, Chadburn A (2007). *Lymphoproliferations of immunodeficiency*. In: *Molecular Pathology in Clinical Practice*. Leonard DGB, Bagg A, Caliendo K *et al.*, editors. New York: Springer-Verlag.

Cesarman E, Chang Y, Moore PS *et al.* (1995). Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *N Engl J Med*, 332: 1186–1191. doi:10.1056/NEJM199505043321802 PMID:7700311

Cesarman E & Mesri EA (1999). Virus-associated lymphomas. *Curr Opin Oncol*, 11: 322–332. doi:10.1097/00001622-199909000-00002 PMID:10505767

Chan CK, Mueller N, Evans A *et al.* (1991). Epstein-Barr virus antibody patterns preceding the diagnosis of nasopharyngeal carcinoma. *Cancer Causes Control*, 2: 125–131. doi:10.1007/BF00053132 PMID:1651778

Chan J, Jaffe ES, Ralfkiaer E (2001a). *Extranodal NK/T cell lymphoma, nasal type*. In: *World Health Organization Classification of Tumours – Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. Lyon: IARC Press, pp. 204–207.

Chan J, Wong KF, Jaffe ES *et al.* (2001b). *Aggressive NK-cell leukemia*. In: *World Health Organization Classification*

of Tumours – Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. Lyon: IARC Press, pp. 198–200.

Chan JKC, Bray F, McCarron P *et al.* (2005). *Nasopharyngeal carcinoma*. In: *World Health Organization Classification of Tumours – Pathology and Genetics of Head and Neck Tumours*. Barnes EL, Eveson JW, Reichart P, Sidransky D, editors. Kleihues P, Sobin LH, series editors. Lyon, France: IARC Press, pp. 85–97.

Chan KH, Tam JS, Peiris JS *et al.* (2001). Epstein-Barr virus (EBV) infection in infancy. *J Clin Virol*, 21: 57–62. doi:10.1016/S1386-6532(01)00149-4 PMID:11255098

Chang ET, Zheng T, Lennette ET *et al.* (2004). Heterogeneity of risk factors and antibody profiles in epstein-barr virus genome-positive and -negative hodgkin lymphoma. *J Infect Dis*, 189: 2271–2281. doi:10.1086/420886 PMID:15181575

Chen F, Zou JZ, di Renzo L *et al.* (1995). A subpopulation of normal B cells latently infected with Epstein-Barr virus resembles Burkitt lymphoma cells in expressing EBNA-1 but not EBNA-2 or LMP1. *J Virol*, 69: 3752–3758. PMID:7745723

Chen J-Y, Chen C-J, Liu M-Y *et al.* (1987). Antibodies to Epstein-Barr virus-specific DNase in patients with nasopharyngeal carcinoma and control groups. *J Med Virol*, 23: 11–21. doi:10.1002/jmv.1890230103 PMID:2824675

Chen MR, Liu MY, Hsu SM *et al.* (2001). Use of bacterially expressed EBNA-1 protein cloned from a nasopharyngeal carcinoma (NPC) biopsy as a screening test for NPC patients. *J Med Virol*, 64: 51–57. doi:10.1002/jmv.1017 PMID:11285569

Chêne A, Donati D, Guerreiro-Cacais AO *et al.* (2007). A molecular link between malaria and Epstein-Barr virus reactivation. *PLoS Pathog*, 3: e80 doi:10.1371/journal.ppat.0030080 PMID:17559303

Cheung A & Kieff E (1982). Long internal direct repeat in Epstein-Barr virus DNA. *J Virol*, 44: 286–294. PMID:6292491

Chien YC, Chen JY, Liu MY *et al.* (2001). Serologic markers of Epstein-Barr virus infection and nasopharyngeal carcinoma in Taiwanese men. *N Engl J Med*, 345: 1877–1882. doi:10.1056/NEJMoa011610 PMID:11756578

Choy EY, Siu KL, Kok KH *et al.* (2008). An Epstein-Barr virus-encoded microRNA targets PUMA to promote host cell survival. *J Exp Med*, 205: 2551–2560. doi:10.1084/jem.20072581 PMID:18838543

Cohen JI, Wang F, Mannick J, Kieff E (1989). Epstein-Barr virus nuclear protein 2 is a key determinant of lymphocyte transformation. *Proc Natl Acad Sci USA*, 86: 9558–9562. doi:10.1073/pnas.86.23.9558 PMID:2556717

Connelly RR & Christine BW (1974). A cohort study of cancer following infectious mononucleosis. *Cancer Res*, 34: 1172–1178. PMID:4842360

Cosmopoulos K, Pegtel M, Hawkins J *et al.* (2009). Comprehensive profiling of Epstein-Barr virus microRNAs in nasopharyngeal carcinoma. *J Virol*, 83: 2357–2367. doi:10.1128/JVI.02104-08 PMID:19091858

Countryman J, Gradoville L, Bhaduri-McIntosh S *et al.* (2009). Stimulus duration and response time independently influence the kinetics of lytic cycle reactivation of Epstein-Barr virus. *J Virol*, 83: 10694–10709. doi:10.1128/JVI.01172-09 PMID:19656890

Cox C, Chang S, Karran L *et al.* (1996). Persistent Epstein-Barr virus infection in the common marmoset (Callithrix jacchus). *J Gen Virol*, 77: 1173–1180. doi:10.1099/0022-1317-77-6-1173 PMID:8683204

Curran JA, Laverty FS, Campbell D *et al.* (2001). Epstein-Barr virus encoded latent membrane protein-1 induces epithelial cell proliferation and sensitizes transgenic mice to chemical carcinogenesis. *Cancer Res*, 61: 6730–6738. PMID:11559544

Dawson CW, Laverick L, Morris MA *et al.* (2008). Epstein-Barr virus-encoded LMP1 regulates epithelial cell motility and invasion via the ERK-MAPK pathway. *J Virol*, 82: 3654–3664. doi:10.1128/JVI.01888-07 PMID:18199641

de Bruin PC, Jiwa NM, Oudejans JJ *et al.* (1995). Epstein-Barr virus in primary gastrointestinal T cell lymphomas. Association with gluten-sensitive enteropathy, pathological features, and immunophenotype. *Am J Pathol*, 146: 861–867. PMID:7717453

de Sanjosé S, Bosch R, Schouten T *et al.* (2007). Epstein-Barr virus infection and risk of lymphoma: immunoblot analysis of antibody responses against EBV-related proteins in a large series of lymphoma subjects and matched controls. *Int J Cancer*, 121: 1806–1812. doi:10.1002/ijc.22857 PMID:17557295

de Thé G, Day NE, Geser A *et al.* (1975). Sero-epidemiology of the Epstein-Barr virus: preliminary analysis of an international study - a review. *IARC Sci Publ*, 11: 3–16.

de Thé G, Lavoué M-E, Muenz L (1978b). Differences in EBV antibody titres of patients with nasopharngeal carcinoma originating from high, intermediate and low incidence areas. *IARC Sci Publ*, 20: 471–481.

Decker LL, Klaman LD, Thorley-Lawson DA (1996). Detection of the latent form of Epstein-Barr virus DNA in the peripheral blood of healthy individuals. *J Virol*, 70: 3286–3289. PMID:8627812

Delecluse HJ, Anagnostopoulos I, Dallenbach F *et al.* (1997). Plasmablastic lymphomas of the oral cavity: a new entity associated with the human immunodeficiency virus infection. *Blood*, 89: 1413–1420. PMID:9028965

Dinand V, Dawar R, Arya LS *et al.* (2007). Hodgkin's lymphoma in Indian children: prevalence and significance of Epstein-Barr virus detection in Hodgkin's and Reed-Sternberg cells. *Eur J Cancer*, 43: 161–168. doi:10.1016/j.ejca.2006.08.036 PMID:17113770

Dirmeier U, Neuhierl B, Kilger E *et al.* (2003). Latent membrane protein 1 is critical for efficient growth transformation of human B cells by epstein-barr virus. *Cancer Res*, 63: 2982–2989. PMID:12782607

Donati D, Espmark E, Kironde F *et al.* (2006). Clearance of circulating Epstein-Barr virus DNA in children with acute malaria after antimalaria treatment. *J Infect Dis*, 193: 971–977. doi:10.1086/500839 PMID:16518759

Dresang LR, Vereide DT, Sugden B (2009). Identifying sites bound by Epstein-Barr virus nuclear antigen 1 (EBNA1) in the human genome: defining a position-weighted matrix to predict sites bound by EBNA1 in viral genomes. *J Virol*, 83: 2930–2940. doi:10.1128/JVI.01974-08 PMID:19129441

Dunleavy K, Wilson WH, Jaffe ES (2007). Angioimmunoblastic T cell lymphoma: pathobiological insights and clinical implications. *Curr Opin Hematol*, 14: 348–353. doi:10.1097/MOH.0b013e328186ffbf PMID:17534160

Dupuis J, Boye K, Martin N *et al.* (2006a). Expression of CXCL13 by neoplastic cells in angioimmunoblastic T-cell lymphoma (AITL): a new diagnostic marker providing evidence that AITL derives from follicular helper T cells. *Am J Surg Pathol*, 30: 490–494. doi:10.1097/00000478-200604000-00009 PMID:16625095

Dupuis J, Emile JF, Mounier N *et al.*Groupe d'Etude des Lymphomes de l'Adulte (GELA) (2006b). Prognostic significance of Epstein-Barr virus in nodal peripheral T-cell lymphoma, unspecified: A Groupe d'Etude des Lymphomes de l'Adulte (GELA) study. *Blood*, 108: 4163–4169. doi:10.1182/blood-2006-04-017632 PMID:16902151

Dykstra ML, Longnecker R, Pierce SK (2001). Epstein-Barr virus coopts lipid rafts to block the signaling and antigen transport functions of the BCR. *Immunity*, 14: 57–67. doi:10.1016/S1074-7613(01)00089-9 PMID:11163230

Einhorn L & Ernberg I (1978). Induction of EBNA precedes the first cellular S-phase after EBV-infection of human lymphocytes. *Int J Cancer*, 21: 157–160. doi:10.1002/ijc.2910210205 PMID:203544

Eliopoulos AG, Blake SM, Floettmann JE *et al.* (1999). Epstein-Barr virus-encoded latent membrane protein 1 activates the JNK pathway through its extreme C terminus via a mechanism involving TRADD and TRAF2. *J Virol*, 73: 1023–1035. PMID:9882303

Epstein MA, Achong BG, Barr YM (1964). Virus particles in cultured lymphoblasts from Burkitt's lymphma. *Lancet*, 283: 702–703. doi:10.1016/S0140-6736(64)91524-7

Evans AS (1971). The spectrum of infections with Epstein-Barr virus: a hypothesis. *J Infect Dis*, 124: 330–337. PMID:4335371

Evans AS & Gutensohn NM (1984). A population-based case-control study of EBV and other viral antibodies among persons with Hodgkin's disease and their siblings. *Int J Cancer*, 34: 149–157. doi:10.1002/ijc.2910340203 PMID:6088401

Fan H, Nicholls J, Chua D *et al.* (2004). Laboratory markers of tumor burden in nasopharyngeal carcinoma: a comparison of viral load and serologic tests for Epstein-Barr virus. *Int J Cancer*, 112: 1036–1041. doi:10.1002/ijc.20520 PMID:15386346

Ferlay J, Shin HR, Bray F *et al.* (2010). *GLOBOCAN 2008 – Cancer Incidence and Mortality Worldwide*. Lyon, France: IARC. Available at: http://globocan.iarc.fr

Fingeroth JD, Clabby ML, Strominger JD (1988). Characterization of a T-lymphocyte Epstein-Barr virus/C3d receptor (CD21). *J Virol*, 62: 1442–1447. PMID:2831405

Fingeroth JD, Weis JJ, Tedder TF *et al.* (1984). Epstein-Barr virus receptor of human B lymphocytes is the C3d receptor CR2. *Proc Natl Acad Sci USA*, 81: 4510–4514. doi:10.1073/pnas.81.14.4510 PMID:6087328

Flavell KJ & Murray PG (2000). Hodgkin's disease and the Epstein-Barr virus. *Mol Pathol*, 53: 262–269. doi:10.1136/mp.53.5.262 PMID:11091850

Frangou P, Buettner M, Niedobitek G (2005). Epstein-Barr virus (EBV) infection in epithelial cells in vivo: rare detection of EBV replication in tongue mucosa but not in salivary glands. *J Infect Dis*, 191: 238–242. doi:10.1086/426823 PMID:15609234

Fukayama M, Hino R, Uozaki H (2008). Epstein-Barr virus and gastric carcinoma: virus-host interactions leading to carcinoma. *Cancer Sci*, 99: 1726–1733. doi:10.1111/j.1349-7006.2008.00888.x PMID:18616681

Fukuda M, Ikuta K, Yanagihara K *et al.* (2001). Effect of transforming growth factor-beta1 on the cell growth and Epstein-Barr virus reactivation in EBV-infected epithelial cell lines. *Virology*, 288: 109–118. doi:10.1006/viro.2001.1071 PMID:11543663

Gallagher A, Armstrong AA, MacKenzie J *et al.* (1999). Detection of Epstein-Barr virus (EBV) genomes in the serum of patients with EBV-associated Hodgkin's disease. *Int J Cancer*, 84: 442–448. doi:10.1002/(SICI)1097-0215(19990820)84:4<442::AID-IJC20>3.0.CO;2-J PMID:10404101

Geser A, Brubaker G, Draper CC (1989). Effect of a malaria suppression program on the incidence of African Burkitt's lymphoma. *Am J Epidemiol*, 129: 740–752. PMID:2923122

Geser A, de Thé G, Lenoir G *et al.* (1982). Final case reporting from the Ugandan prospective study of the relationship between EBV and Burkitt's lymphoma. *Int J Cancer*, 29: 397–400. doi:10.1002/ijc.2910290406 PMID:6282763

Glaser SL, Keegan TH, Clarke CA *et al.* (2005). Exposure to childhood infections and risk of Epstein-Barr virus–defined Hodgkin's lymphoma in women. *Int J Cancer*, 115: 599–605. doi:10.1002/ijc.20787 PMID:15700307

Gotleib-Stematsky T, Ramot B, Vonsover B *et al.* (1976). Antibodies to Epstein-Barr viral capsid and early

antigens associated with Burkitt's lymphoma and lymphoblastic lymphosarcoma in Israel. *J Natl Cancer Inst*, 56: 721–723. PMID:233958

Gratama JW & Ernberg I (1995). Molecular epidemiology of Epstein-Barr virus infection. *Adv Cancer Res*, 67: 197–255. doi:10.1016/S0065-230X(08)60714-9 PMID:8571815

Gruhne B, Sompallae R, Marescotti D *et al.* (2009). The Epstein-Barr virus nuclear antigen-1 promotes genomic instability via induction of reactive oxygen species. *Proc Natl Acad Sci USA*, 106: 2313–2318. doi:10.1073/pnas.0810619106 PMID:19139406

Grundhoff A, Sullivan CS, Ganem D (2006). A combined computational and microarray-based approach identifies novel microRNAs encoded by human gamma-herpesviruses. *RNA*, 12: 733–750. doi:10.1261/rna.2326106 PMID:16540699

Guasparri I, Bubman D, Cesarman E (2008). EBV LMP2A affects LMP1-mediated NF-kappaB signaling and survival of lymphoma cells by regulating TRAF2 expression. *Blood*, 111: 3813–3820. doi:10.1182/blood-2007-03-080309 PMID:18230756

Gustafsson A, Levitsky V, Zou JZ *et al.* (2000). Epstein-Barr virus (EBV) load in bone marrow transplant recipients at risk to develop posttransplant lymphoproliferative disease: prophylactic infusion of EBV-specific cytotoxic T cells. *Blood*, 95: 807–814. PMID:10648390

Gutensohn N & Cole P (1981). Childhood social environment and Hodgkin's disease. *N Engl J Med*, 304: 135–140. doi:10.1056/NEJM198101153040302 PMID:6255329

Gutensohn NM (1982). Social class and age at diagnosis of Hodgkin's disease: new epidemiologic evidence for the "two-disease hypothesis". *Cancer Treat Rep*, 66: 689–695. PMID:7074638

Hardell E, Eriksson M, Lindström G *et al.* (2001a). Case-control study on concentrations of organohalogen compounds and titers of antibodies to Epstein-Barr virus antigens in the etiology of non-Hodgkin lymphoma. *Leuk Lymphoma*, 42: 619–629. doi:10.3109/10428190109099322 PMID:11697490

Hardell L, Lindström G, van Bavel B *et al.* (2001b). Adipose tissue concentrations of dioxins and dibenzofurans, titers of antibodies to Epstein-Barr virus early antigen and the risk for non-Hodgkin lymphoma. *Environ Res*, 87: 99–107. doi:10.1006/enrs.2001.4295 PMID:11683593

Hatzivassiliou E & Mosialos G (2002). Cellular signaling pathways engaged by the Epstein-Barr virus transforming protein LMP1. *Front Biosci*, 7: d319–d329. doi:10.2741/hatziva PMID:11779697

He YJ, Jia XS, Hasui K *et al.* (2007). Nasal and pharyngeal non-Hodgkin lymphomas and their relationship with Epstein-Barr virus: a report of 158 cases. *Zhonghua Bing Li Xue Za Zhi*, 36: 94–97. PMID:17493382

Henderson BE, Dworsky R, Pike MC *et al.* (1979). Risk factors for nodular sclerosis and other types of Hodgkin's disease. *Cancer Res*, 39: 4507–4511. PMID:498082

Henderson S, Rowe M, Gregory C *et al.* (1991). Induction of bcl-2 expression by Epstein-Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. *Cell*, 65: 1107–1115. doi:10.1016/0092-8674(91)90007-L PMID:1648447

Henle G, Henle W, Clifford P *et al.* (1969). Antibodies to Epstein-Barr virus in Burkitt's lymphoma and control groups. *J Natl Cancer Inst*, 43: 1147–1157. PMID:5353242

Henle G, Henle W, Diehl V (1968). Relation of Burkitt's tumor-associated herpes-ytpe virus to infectious mononucleosis. *Proc Natl Acad Sci USA*, 59: 94–101. doi:10.1073/pnas.59.1.94 PMID:5242134

Henle G, Henle W, Klein G *et al.* (1971b). Antibodies to early Epstein-Barr virus-induced antigens in Burkitt's lymphoma. *J Natl Cancer Inst*, 46: 861–871. PMID:4324816

Henle W & Henle G (1976). The sero-epidemiology of Epstein-Barr virus. *Adv Pathobiol*, 5: 5–17. PMID:220858

Henle W, Henle G, Andersson J *et al.* (1987). Antibody responses to Epstein-Barr virus-determined nuclear antigen (EBNA)-1 and EBNA-2 in acute and chronic Epstein-Barr virus infection. *Proc Natl Acad Sci USA*, 84: 570–574. doi:10.1073/pnas.84.2.570 PMID:3025881

Hennessy K, Fennewald S, Hummel M *et al.* (1984). A membrane protein encoded by Epstein-Barr virus in latent growth-transforming infection. *Proc Natl Acad Sci USA*, 81: 7207–7211. doi:10.1073/pnas.81.22.7207 PMID:6095274

Hennessy K, Heller M, van Santen V, Kieff E (1983). Simple repeat array in Epstein-Barr virus DNA encodes part of the Epstein-Barr nuclear antigen. *Science*, 220: 1396–1398. doi:10.1126/science.6304878 PMID:6304878

Heslop HE & Rooney CM (1997). Adoptive cellular immunotherapy for EBV lymphoproliferative disease. [Review] *Immunol Rev*, 157: 217–222. doi:10.1111/j.1600-065X.1997.tb00984.x PMID:9255632

Higgins CD, Swerdlow AJ, Macsween KF *et al.* (2007). A study of risk factors for acquisition of Epstein-Barr virus and its subtypes. *J Infect Dis*, 195: 474–482. doi:10.1086/510854 PMID:17230406

Higuchi M, Izumi KM, Kieff E (2001). Epstein-Barr virus latent-infection membrane proteins are palmitoylated and raft-associated: protein 1 binds to the cytoskeleton through TNF receptor cytoplasmic factors. *Proc Natl Acad Sci USA*, 98: 4675–4680. doi:10.1073/pnas.081075298 PMID:11296297

Hino R, Uozaki H, Inoue Y *et al.* (2008). Survival advantage of EBV-associated gastric carcinoma: survivin up-regulation by viral latent membrane protein 2A. *Cancer Res*, 68: 1427–1435. doi:10.1158/0008-5472.CAN-07-3027 PMID:18316606

Hirshaut Y, Cohen MH, Stevens DA (1973). Epstein-Barr-virus antibodies in American and African Burkitt's lymphoma. *Lancet*, 2: 114–116. doi:10.1016/S0140-6736(73)93061-4 PMID:4124041

Hjalgrim H, Askling J, Sørensen P *et al.* (2000). Risk of Hodgkin's disease and other cancers after infectious mononucleosis. *J Natl Cancer Inst*, 92: 1522–1528. doi:10.1093/jnci/92.18.1522 PMID:10995808

Hjalgrim H, Friborg J, Melbye M (2007a). *The epidemiology of EBV and its association with malignant disease.* In: *Human Herpesviruses: Biology Therapy and Immunoprophylaxis.* Arvin A, Campadelli-Fiume G, Mocarski E *et al.*, editors. Cambridge: Cambridge University Press, 929–959.

Hjalgrim H, Smedby KE, Rostgaard K *et al.* (2007b). Infectious mononucleosis, childhood social environment, and risk of Hodgkin lymphoma. *Cancer Res*, 67: 2382–2388. doi:10.1158/0008-5472.CAN-06-3566 PMID:17332371

Höfelmayr H, Strobl LJ, Marschall G *et al.* (2001). Activated Notch1 can transiently substitute for EBNA2 in the maintenance of proliferation of LMP1-expressing immortalized B cells. *J Virol*, 75: 2033–2040. doi:10.1128/JVI.75.5.2033-2040.2001 PMID:11160707

Hsu JL & Glaser SL (2000). Epstein-barr virus-associated malignancies: epidemiologic patterns and etiologic implications. *Crit Rev Oncol Hematol*, 34: 27–53. doi:10.1016/S1040-8428(00)00046-9 PMID:10781747

Hsu YC, Lu HF, Huang CC *et al.* (2006). Malignant lymphoepithelial lesions of the salivary gland. *Otolaryngol Head Neck Surg*, 134: 661–666. doi:10.1016/j.otohns.2005.10.034 PMID:16564393

Hu LF, Chen F, Zhen QF *et al.* (1995). Differences in the growth pattern and clinical course of EBV-LMP1 expressing and non-expressing nasopharyngeal carcinomas. *Eur J Cancer*, 31A: 658–660. doi:10.1016/0959-8049(94)00468-K PMID:7640034

Hu LF, Minarovits J, Cao SL *et al.* (1991). Variable expression of latent membrane protein in nasopharyngeal carcinoma can be related to methylation status of the Epstein-Barr virus BNLF-1 5′-flanking region. *J Virol*, 65: 1558–1567. PMID:1847471

IARC (1997). Epstein-barr virus and Kaposi's sarcoma herpesvirus/Human herpesvirus 8. *IARC Monogr Eval Carcinog Risks Hum*, 70: 1–492. PMID:9705682

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: 4371–4378. PMID:9557727

Isaacson P, Wright D, Ralfkiaer E *et al.* (2001). *Enteropathy type T-cell lymphoma.* In: *World Health Organization Classification of Tumours – Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues.* Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. Lyon: IARC Press, pp. 208–209.

Janz A, Oezel M, Kurzeder C *et al.* (2000). Infectious Epstein-Barr virus lacking major glycoprotein BLLF1 (gp350/220) demonstrates the existence of additional viral ligands. *J Virol*, 74: 10142–10152. doi:10.1128/JVI.74.21.10142-10152.2000 PMID:11024143

Jarrett RF, Gallagher A, Jones DB *et al.* (1991). Detection of Epstein-Barr virus genomes in Hodgkin's disease: relation to age. *J Clin Pathol*, 44: 844–848. doi:10.1136/jcp.44.10.844 PMID:1660054

Jeannel D, Hubert A, de Vathaire F *et al.* (1990). Diet, living conditions and nasopharyngeal carcinoma in Tunisia–a case-control study. *Int J Cancer*, 46: 421–425. doi:10.1002/ijc.2910460316 PMID:2394508

Jen KY, Higuchi M, Cheng J *et al.* (2005). Nucleotide sequences and functions of the Epstein-Barr virus latent membrane protein 1 genes isolated from salivary gland lymphoepithelial carcinomas. *Virus Genes*, 30: 223–235. doi:10.1007/s11262-004-5630-5 PMID:15744579

Ji MF, Wang DK, Yu YL *et al.* (2007). Sustained elevation of Epstein-Barr virus antibody levels preceding clinical onset of nasopharyngeal carcinoma. *Br J Cancer*, 96: 623–630. doi:10.1038/sj.bjc.6603609 PMID:17285127

Kafuko GW, Henderson BE, Kirya BG *et al.* (1972). Epstein-Barr virus antibody levels in children from the West Nile District of Uganda. Report of a field study. *Lancet*, 1: 706–709. doi:10.1016/S0140-6736(72)90228-0 PMID:4111191

Kaiser C, Laux G, Eick D *et al.* (1999). The proto-oncogene c-myc is a direct target gene of Epstein-Barr virus nuclear antigen 2. *J Virol*, 73: 4481–4484. PMID:10196351

Kashuba E, Mattsson K, Pokrovskaja K *et al.* (2003). EBV-encoded EBNA-5 associates with P14ARF in extranucleolar inclusions and prolongs the survival of P14ARF-expressing cells. *Int J Cancer*, 105: 644–653. doi:10.1002/ijc.11124 PMID:12740913

Kelly GL, Milner AE, Baldwin GS *et al.* (2006). Three restricted forms of Epstein-Barr virus latency counteracting apoptosis in c-myc-expressing Burkitt lymphoma cells. *Proc Natl Acad Sci USA*, 103: 14935–14940. doi:10.1073/pnas.0509988103 PMID:17001014

Kelly GL & Rickinson AB (2007). Burkitt lymphoma: revisiting the pathogenesis of a virus-associated malignancy. *Hematology Am Soc Hematol Educ Program*, 2007: 277–284. PMID:18024641

Khalidi HS, Lones MA, Zhou Y *et al.* (1997). Detection of Epstein-Barr virus in the L & H cells of nodular lymphocyte predominance Hodgkin's disease: report of a case documented by immunohistochemical, in situ hybridization, and polymerase chain reaction methods. *Am J Clin Pathol*, 108: 687–692. PMID:9384451

Khanna R, Burrows SR, Moss DJ (1995). Immune regulation in Epstein-Barr virus-associated diseases. *Microbiol Rev*, 59: 387–405. PMID:7565411

Khanna R, Tellam J, Duraiswamy J, Cooper L (2001). Immunotherapeutic strategies for EBV-associated malignancies. *Trends Mol Med*, 7: 270–276. doi:10.1016/S1471-4914(01)02002-0 PMID:11378517

Kieff E (1996). *Epstein-Barr virus and its replication.* In: *Fields Virology.* Fields BN, Knipe DM, Howley P

et al., editors. Philadelphia, PA: Lippincott–Raven, pp. 2343–2396.

Kieff E, Given D, Powell AL *et al.* (1979). Epstein-Barr virus: structure of the viral DNA and analysis of viral RNA in infected cells. *Biochim Biophys Acta*, 560: 355–373. PMID:228726

Kieff E, Rickinson AB (2001). *Epstein-Barr virus and its replication.* In: *Fields Virology.* Fields BN, Knipe DM, Howley P *et al.*, editors. Philadelphia, PA: Lippincott–Raven, pp. 2511–2575.

Kieser A, Kaiser C, Hammerschmidt W (1999). LMP1 signal transduction differs substantially from TNF receptor 1 signaling in the molecular functions of TRADD and TRAF2. *EMBO J*, 18: 2511–2521. doi:10.1093/emboj/18.9.2511 PMID:10228165

Kim KI, Kim YS, Kim HK *et al.* (1999). The detection of Epstein-Barr virus in the lesions of salivary glands. *Pathol Res Pract*, 195: 407–412. PMID:10399181

Kim YC, Yang WI, Lee MG *et al.* (2006). Epstein-Barr virus in CD30 anaplastic large cell lymphoma involving the skin and lymphomatoid papulosis in South Korea. *Int J Dermatol*, 45: 1312–1316. doi:10.1111/j.1365-4632.2006.02951.x PMID:17076712

Kirchmaier AL & Sugden B (1997). Dominant-negative inhibitors of EBNA-1 of Epstein-Barr virus. *J Virol*, 71: 1766–1775. PMID:9032305

Kis LL, Takahara M, Nagy N *et al.* (2006). Cytokine mediated induction of the major Epstein-Barr virus (EBV)-encoded transforming protein, LMP-1. *Immunol Lett*, 104: 83–88. doi:10.1016/j.imlet.2005.11.003 PMID:16386314

Klein E, Kis LL, Klein G (2007). Epstein-Barr virus infection in humans: from harmless to life endangering virus-lymphocyte interactions. *Oncogene*, 26: 1297–1305. doi:10.1038/sj.onc.1210240 PMID:17322915

Klein G (1983). Specific chromosomal translocations and the genesis of B-cell-derived tumors in mice and men. *Cell*, 32: 311–315. doi:10.1016/0092-8674(83)90449-X PMID:6402307

Klein G, Ernberg I (2007). *Comparative aspects of EBV, HHV-8, and previously known DNA tumor viruses in experimental systems.* In: *Human Tumor Viruses.* Moore P, editor, Ch 29.

Klein G, Geering G, Old LJ *et al.* (1970). Comparison of the anti-EBV titer and the EBV-associated membrane reactive and precipitating antibody levels in the sera of Burkitt lymphoma and nasopharyngeal carcinoma patients and controls. *Int J Cancer*, 5: 185–194. doi:10.1002/ijc.2910050204 PMID:4915449

Knecht H, Sahli R, Shaw P *et al.* (1990). Detection of Epstein-Barr virus DNA by polymerase chain reaction in lymph node biopsies from patients with angioimmunoblastic lymphadenopathy. *Br J Haematol*, 75: 610–614. doi:10.1111/j.1365-2141.1990.tb07807.x PMID:2169858

Knight JS, Sharma N, Robertson ES (2005). Epstein-Barr virus latent antigen 3C can mediate the degradation of the retinoblastoma protein through an SCF cellular ubiquitin ligase. *Proc Natl Acad Sci USA*, 102: 18562–18566. doi:10.1073/pnas.0503886102 PMID:16352731

Koshiol J, Qiao YL, Mark SD *et al.* (2007). Epstein-Barr virus serology and gastric cancer incidence and survival. *Br J Cancer*, 97: 1567–1569. doi:10.1038/sj.bjc.6604063 PMID:17987041

Kulwichit W, Edwards RH, Davenport EM *et al.* (1998). Expression of the Epstein-Barr virus latent membrane protein 1 induces B cell lymphoma in transgenic mice. *Proc Natl Acad Sci USA*, 95: 11963–11968. doi:10.1073/pnas.95.20.11963 PMID:9751773

Kvåle G, Høiby EA, Pedersen E (1979). Hodgkin's disease in patients with previous infectious mononucleosis. *Int J Cancer*, 23: 593–597. doi:10.1002/ijc.2910230502 PMID:378865

Laichalk LL & Thorley-Lawson DA (2005). Terminal differentiation into plasma cells initiates the replicative cycle of Epstein-Barr virus in vivo. *J Virol*, 79: 1296–1307. doi:10.1128/JVI.79.2.1296-1307.2005 PMID:15613356

Lam N & Sugden B (2003a). LMP1, a viral relative of the TNF receptor family, signals principally from intracellular compartments. *EMBO J*, 22: 3027–3038. doi:10.1093/emboj/cdg284 PMID:12805217

Lam N & Sugden B (2003b). CD40 and its viral mimic, LMP1: similar means to different ends. *Cell Signal*, 15: 9–16. doi:10.1016/S0898-6568(02)00083-9 PMID:12401515

Lanier AP, Henle W, Bender TR *et al.* (1980a). Epstein-Barr virus-specific antibody titers in seven Alaskan natives before and after diagnosis of nasopharyngeal carcinoma. *Int J Cancer*, 26: 133–137. doi:10.1002/ijc.2910260203 PMID:6259065

Lehtinen T, Lumio J, Dillner J *et al.* (1993). Increased risk of malignant lymphoma indicated by elevated Epstein-Barr virus antibodies–a prospective study. *Cancer Causes Control*, 4: 187–193. PMID:8391336

Lei KI, Chan LY, Chan WY *et al.* (2000). Quantitative analysis of circulating cell-free Epstein-Barr virus (EBV) DNA levels in patients with EBV-associated lymphoid malignancies. *Br J Haematol*, 111: 239–246. doi:10.1046/j.1365-2141.2000.02344.x PMID:11091207

Lei KI, Chan LY, Chan WY *et al.* (2002). Diagnostic and prognostic implications of circulating cell-free Epstein-Barr virus DNA in natural killer/T-cell lymphoma. *Clin Cancer Res*, 8: 29–34. PMID:11801537

Leung SF, Tam JS, Chan AT *et al.* (2004). Improved accuracy of detection of nasopharyngeal carcinoma by combined application of circulating Epstein-Barr virus DNA and anti-Epstein-Barr viral capsid antigen IgA antibody. *Clin Chem*, 50: 339–345. doi:10.1373/clinchem.2003.022426 PMID:14684618

Leung SY, Chung LP, Yuen ST *et al.* (1995). Lymphoepithelial carcinoma of the salivary gland: in situ detection

of Epstein-Barr virus. *J Clin Pathol*, 48: 1022–1027. doi:10.1136/jcp.48.11.1022 PMID:8543624

Levitskaya J, Coram M, Levitsky V *et al.* (1995). Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1. *Nature*, 375: 685–688. doi:10.1038/375685a0 PMID:7540727

Li Q-X, Spriggs MK, Kovats S *et al.* (1997). Epstein-Barr virus uses HLA class II as a cofactor for infection of B lymphocytes. *J Virol*, 71: 4657–4662. PMID:9151859

Li Q-X, Turk SM, Hutt-Fletcher LM (1995). The Epstein-Barr virus (EBV) BZLF2 gene product associates with the gH and gL homologs of EBV and carries an epitope critical to infection of B cells but not of epithelial cells. *J Virol*, 69: 3987–3994. PMID:7539502

Liebowitz D, Kieff E (1993). *Epstein-Barr virus.* In*: The Human Herpesvirus.* Roizman B, Whitley RJ, Lopez C, editors, New York, pp. 107–172.

Liebowitz D, Wang D, Kieff E (1986). Orientation and patching of the latent infection membrane protein encoded by Epstein-Barr virus. *J Virol*, 58: 233–237. PMID:3005654

Lin JC, Wang WY, Chen KY *et al.* (2004). Quantification of plasma Epstein-Barr virus DNA in patients with advanced nasopharyngeal carcinoma. *N Engl J Med*, 350: 2461–2470. doi:10.1056/NEJMoa032260 PMID:15190138

Lin SY, Tsang NM, Kao SC *et al.* (2001). Presence of Epstein-Barr virus latent membrane protein 1 gene in the nasopharyngeal swabs from patients with nasopharyngeal carcinoma. *Head Neck*, 23: 194–200. doi:10.1002/1097-0347(200103)23:3<194::AID-HED1018>3.0.CO;2-X PMID:11428449

Liu MT, Chen YR, Chen SC *et al.* (2004). Epstein-Barr virus latent membrane protein 1 induces micronucleus formation, represses DNA repair and enhances sensitivity to DNA-damaging agents in human epithelial cells. *Oncogene*, 23: 2531–2539. doi:10.1038/sj.onc.1207375 PMID:14716302

Lo S, Ho WK, Wei WI (2004). Outcome of patients with positive Epstein-Barr virus serologic status in the absence of nasopharyngeal carcinoma in Hong Kong. *Arch Otolaryngol Head Neck Surg*, 130: 770–772. doi:10.1001/archotol.130.6.770 PMID:15210561

Lo YM, Chan LY, Lo KW *et al.* (1999). Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. *Cancer Res*, 59: 1188–1191. PMID:10096545

Lo YM, Chan WY, Ng EK *et al.* (2001). Circulating Epstein-Barr virus DNA in the serum of patients with gastric carcinoma. *Clin Cancer Res*, 7: 1856–1859. PMID:11448896

Low WK, Leong JL, Goh YH, Fong KW (2000). Diagnostic value of Epstein-Barr viral serology in nasopharyngeal carcinoma. *Otolaryngol Head Neck Surg*, 123: 505–507. doi:10.1067/mhn.2000.108201 PMID:11020195

Luo B, Wang Y, Wang XF *et al.* (2005). Expression of Epstein-Barr virus genes in EBV-associated gastric carcinomas. *World J Gastroenterol*, 11: 629–633. PMID:15655811

Lynch DT, Zimmerman JS, Rowe DT (2002). Epstein-Barr virus latent membrane protein 2B (LMP2B) co-localizes with LMP2A in perinuclear regions in transiently transfected cells. *J Gen Virol*, 83: 1025–1035. PMID:11961256

Macsween KF & Crawford DH (2003). Epstein-Barr virus-recent advances. *Lancet Infect Dis*, 3: 131–140. doi:10.1016/S1473-3099(03)00543-7 PMID:12614729

Maeda E, Akahane M, Kiryu S *et al.* (2009). Spectrum of Epstein-Barr virus-related diseases: a pictorial review. *Jpn J Radiol*, 27: 4–19. doi:10.1007/s11604-008-0291-2 PMID:19373526

Mancao C & Hammerschmidt W (2007). Epstein-Barr virus latent membrane protein 2A is a B-cell receptor mimic and essential for B-cell survival. *Blood*, 110: 3715–3721. doi:10.1182/blood-2007-05-090142 PMID:17682125

Masucci MG & Ernberg I (1994). Epstein-Barr virus: adaptation to a life within the immune system. *Trends Microbiol*, 2: 125–130. doi:10.1016/0966-842X(94)90599-1 PMID:8012755

McKhann CF (1969). Primary malignancy in patients undergoing immunosuppression for renal transplantation. *Transplantation*, 8:209–212.doi:10.1097/00007890-196908000-00033 PMID:4896335

Merchant M, Caldwell RG, Longnecker R (2000). The LMP2A ITAM is essential for providing B cells with development and survival signals in vivo. *J Virol*, 74: 9115–9124. doi:10.1128/JVI.74.19.9115-9124.2000 PMID:10982358

Merlo A, Turrini R, Dolcetti R *et al.* (2008). Adoptive cell therapy against EBV-related malignancies: a survey of clinical results. *Expert Opin Biol Ther*, 8: 1265–1294. doi:10.1517/14712598.8.9.1265 PMID:18694349

Miller CL, Lee JH, Kieff E, Longnecker R (1994). An integral membrane protein (LMP2) blocks reactivation of Epstein-Barr virus from latency following surface immunoglobulin crosslinking. *Proc Natl Acad Sci USA*, 91: 772–776. doi:10.1073/pnas.91.2.772 PMID:8290598

Miller RW & Beebe GW (1973). Infectious mononucleosis and the empirical risk of cancer. *J Natl Cancer Inst*, 50: 315–321. PMID:4702109

Mitarnun W, Suwiwat S, Pradutkanchana J *et al.* (2002). Epstein-Barr virus-associated peripheral T-cell and NK-cell proliferative disease/lymphoma: clinicopathologic, serologic, and molecular analysis. *Am J Hematol*, 70: 31–38. doi:10.1002/ajh.10094 PMID:11994979

Miyashita EM, Yang B, Lam KM *et al.* (1995). A novel form of Epstein-Barr virus latency in normal B cells in vivo. *Cell*, 80: 593–601. doi:10.1016/0092-8674(95)90513-8 PMID:7532548

Moody CA, Scott RS, Amirghahari N *et al.* (2005). Modulation of the cell growth regulator mTOR by Epstein-Barr virus-encoded LMP2A. *J Virol*, 79: 5499–5506. doi:10.1128/JVI.79.9.5499-5506.2005 PMID:15827164

Moormann AM, Chelimo K, Sumba OP *et al.* (2005). Exposure to holoendemic malaria results in elevated Epstein-Barr virus loads in children. *J Infect Dis*, 191: 1233–1238. doi:10.1086/428910 PMID:15776368

Moormann AM, Chelimo K, Sumba PO *et al.* (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. doi:10.1086/511984 PMID:17299709

Moormann AM, Heller KN, Chelimo K *et al.* (2009). Children with endemic Burkitt lymphoma are deficient in EBNA1-specific IFN-gamma T cell responses. *Int J Cancer*, 124: 1721–1726. doi:10.1002/ijc.24014 PMID:19089927

Mosier DE, Gulizia RJ, Baird SM, Wilson DB (1988). Transfer of a functional human immune system to mice with severe combined immunodeficiency. *Nature*, 335: 256–259. doi:10.1038/335256a0 PMID:2970594

Moss DJ, Burrows SR, Khanna R *et al.* (1992). Immune surveillance against Epstein-Barr virus. *Semin Immunol*, 4: 97–104. PMID:1377517

Mueller N, Mohar A, Evans A *et al.* (1991). Epstein-Barr virus antibody patterns preceding the diagnosis of non-Hodgkin's lymphoma. *Int J Cancer*, 49: 387–393. doi:10.1002/ijc.2910490313 PMID:1655660

Mueller NE, Evans A, Harris NL *et al.* (1989). Hodgkin's disease and Epstein-Barr virus. Altered antibody pattern before diagnosis. *N Engl J Med*, 320: 689–695. doi:10.1056/NEJM198903163201103 PMID:2537928

Muñoz N, Davidson RJL, Witthoff B *et al.* (1978). Infectious mononucleosis and Hodgkin's disease. *Int J Cancer*, 22: 10–13. doi:10.1002/ijc.2910220104 PMID:681023

Murray PG, Young LS (2005). *Hodgkin's lymphoma: molecular pathogenesis and the contribution of the Epstein-Barr virus.* In: *Epstein-Barr virus.* Robertson ES, editor. Norfolk: Caiser Academic Press.

Musacchio JG, Carvalho MG, Morais JC *et al.* (2006). Detection of free circulating Epstein-Barr virus DNA in plasma of patients with Hodgkin's disease. *Sao Paulo Med J*, 124: 154–157. doi:10.1590/S1516-31802006000300009 PMID:17119693

Mutalima N, Molyneux E, Jaffe H *et al.* (2008). Associations between Burkitt lymphoma among children in Malawi and infection with HIV, EBV and malaria: results from a case-control study. *PLoS ONE*, 3: e2505 doi:10.1371/journal.pone.0002505 PMID:18560562

Mutirangura A, Pornthanakasem W, Theamboonlers A *et al.* (1998). Epstein-Barr viral DNA in serum of patients with nasopharyngeal carcinoma. *Clin Cancer Res*, 4: 665–669. PMID:9533535

Newton R, Carpenter L, Casabonne D *et al.* (2006). A prospective study of Kaposi's sarcoma-associated herpesvirus and Epstein-Barr virus in adults with human immunodeficiency virus-1. *Br J Cancer*, 94: 1504–1509. doi:10.1038/sj.bjc.6603100 PMID:16705315

Niederman J, Evans A (1997). *Epstein–Barr virus.* In: *Viral Infections of Humans: Epidemiology and Control.* Evans AS, Kaslow RA, editors. New York: Plenum, pp. 253–83.

Niedobitek G, Young LS, Herbst H (1997). Epstein-Barr virus infection and the pathogenesis of malignant lymphomas. *Cancer Surv*, 30: 143–162. PMID:9547991

Nilsson K, Klein G, Henle W, Henle G (1971). The establishment of lymphoblastoid lines from adult and fetal human lymphoid tissue and its dependence on EBV. *Int J Cancer*, 8: 443–450. PMID:4332899

Nkrumah F, Henle W, Henle G *et al.* (1976). Burkitt's lymphoma: its clinical course in relation to immunologic reactivities to Epstein-Barr virus and tumor-related antigens. *J Natl Cancer Inst*, 57: 1051–1056. PMID:187787

Nolan LA & Morgan AJ (1995). The Epstein-Barr virus open reading frame BDLF3 codes for a 100–150 kDa glycoprotein. *J Gen Virol*, 76: 1381–1392. doi:10.1099/0022-1317-76-6-1381 PMID:7782767

Ohshima K, Haraoka S, Harada N *et al.* (2000). Hepatosplenic gammadelta T-cell lymphoma: relation to Epstein-Barr virus and activated cytotoxic molecules. *Histopathology*, 36: 127–135. doi:10.1046/j.1365-2559.2000.00804.x PMID:10672057

Oyama T, Yamamoto K, Asano N *et al.* (2007). Age-related EBV-associated B-cell lymphoproliferative disorders constitute a distinct clinicopathologic group: a study of 96 patients. *Clin Cancer Res*, 13: 5124–5132. doi:10.1158/1078-0432.CCR-06-2823 PMID:17785567

Pathmanathan R, Prasad U, Sadler R *et al.* (1995). Clonal proliferations of cells infected with Epstein-Barr virus in preinvasive lesions related to nasopharyngeal carcinoma. *N Engl J Med*, 333: 693–698. doi:10.1056/NEJM199509143331103 PMID:7637746

Pearson GR, Weiland LH, Neel HB 3rd *et al.* (1983b). Application of Epstein-Barr virus (EBV) serology to the diagnosis of North American nasopharyngeal carcinoma. *Cancer*, 51: 260–268. doi:10.1002/1097-0142(19830115)51:2<260::AID-CNCR2820510216>3.0.CO;2-# PMID:6295597

Pegtel DM, Subramanian A, Sheen TS *et al.* (2005). Epstein-Barr-virus-encoded LMP2A induces primary epithelial cell migration and invasion: possible role in nasopharyngeal carcinoma metastasis. *J Virol*, 79: 15430–15442. doi:10.1128/JVI.79.24.15430-15442.2005 PMID:16306614

Penn I, Hammond W, Brettschneider L, Starzl TE (1969). Malignant lymphomas in transplantation patients. *Transplant Proc*, 1: 106–112. PMID:4944206

Pfeffer S, Zavolan M, Grässer FA *et al.* (2004). Identification of virus-encoded microRNAs. *Science*, 304: 734–736. doi:10.1126/science.1096781 PMID:15118162

Pokrovskaja K, Mattsson K, Kashuba E *et al.* (2001). Proteasome inhibitor induces nucleolar translocation of Epstein-Barr virus-encoded EBNA-5. *J Gen Virol*, 82: 345–358. PMID:11161273

Polack A, Hörtnagel K, Pajic A *et al.* (1996). c-myc activation renders proliferation of Epstein-Barr virus (EBV)-transformed cells independent of EBV nuclear antigen 2 and latent membrane protein 1. *Proc Natl Acad Sci USA*, 93: 10411–10416. doi:10.1073/pnas.93.19.10411 PMID:8816814

Quintanilla-Martinez L, Kumar S, Fend F *et al.* (2000). Fulminant EBV(+) T-cell lymphoproliferative disorder following acute/chronic EBV infection: a distinct clinicopathologic syndrome. *Blood*, 96: 443–451. PMID:10887104

Quintanilla-Martínez L, Lome-Maldonado C, Ott G *et al.* (1998). Primary intestinal non-Hodgkin's lymphoma and Epstein-Barr virus: high frequency of EBV-infection in T-cell lymphomas of Mexican origin. *Leuk Lymphoma*, 30: 111–121. PMID:9669681

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: 883–889. doi:10.1016/0092-8674(86)90803-2 PMID:3022942

Radkov SA, Touitou R, Brehm A *et al.* (1999). Epstein-Barr virus nuclear antigen 3C interacts with histone deacetylase to repress transcription. *J Virol*, 73: 5688–5697. PMID:10364319

Raphael M, Borisch B, Jaffe ES (2001) *Lymphomas associated with infection by the human immune deficiency virus (VIH).* In: *World Health Organization Classification of Tumours – Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues.* Jaffe ES, Harris NL, Stein H, editors. Lyon: IARC Press, pp. 260–263.

Raphael M, Said J, Borisch B *et al.* (2008) *Lymphomas associated with HIV infection.* In: *World Health Organization Classification of Tumour –, Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues.* Swerdlow SH, Campo E, Harris NL *et al.*, editors. Lyon: IARC.

Rasti N, Falk KI, Donati D *et al.* (2005). Circulating epstein-barr virus in children living in malaria-endemic areas. *Scand J Immunol*, 61: 461–465. doi:10.1111/j.1365-3083.2005.01589.x PMID:15882438

Rickinson AB, Kieff E (1996). *Epstein–Barr virus.* In: *Field's Virology.* Fields BN, Knipe DM, Howley PM *et al.*, editors. Philadelphia, PA: Lippincott-Raven, pp. 2397–2446.

Rickinson AB, Young LS, Rowe M (1987). Influence of the Epstein-Barr virus nuclear antigen EBNA 2 on the growth phenotype of virus-transformed B cells. *J Virol*, 61: 1310–1317. PMID:3033261

Rochford R, Cannon MJ, Moormann AM (2005). Endemic Burkitt's lymphoma: a polymicrobial disease? *Nat Rev Microbiol*, 3: 182–187. doi:10.1038/nrmicro1089 PMID:15685227

Rooney CM, Smith CA, Ng CY *et al.* (1995). Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. *Lancet*, 345: 9–13. doi:10.1016/S0140-6736(95)91150-2 PMID:7799740

Rosdahl N, Larsen SO, Clemmesen J (1974). Hodgkin's disease in patients with previous infectious mononucleosis: 30 years' experience. *BMJ*, 2: 253–256. doi:10.1136/bmj.2.5913.253 PMID:4406463

Rovedo M & Longnecker R (2007). Epstein-barr virus latent membrane protein 2B (LMP2B) modulates LMP2A activity. *J Virol*, 81: 84–94. doi:10.1128/JVI.01302-06 PMID:17035319

Saku T, Cheng J, Jen KY *et al.* (2003). Epstein-Barr virus infected lymphoepithelial carcinomas of the salivary gland in the Russia-Asia area: a clinicopathologic study of 160 cases. *Arkh Patol*, 65: 35–39. PMID:15357246

Samanta M, Iwakiri D, Takada K (2008). Epstein-Barr virus-encoded small RNA induces IL-10 through RIG-I-mediated IRF-3 signaling. *Oncogene*, 27: 4150–4160. doi:10.1038/onc.2008.75 PMID:18362887

Sample J, Young L, Martin B *et al.* (1990). Epstein-Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B, and EBNA-3C genes. *J Virol*, 64: 4084–4092. PMID:2166806

Sample JT, Sample CE (2008). *Epstein-Barr Virus: Molecular Biology.* In: *Encyclopedia of Virology.* Mahy BWJ, van Regenmortel MHV, editors. Oxford: Academic Press, pp 157–167.

Saqui-Salces M, Martinez-Benitez B, Gamboa-Dominguez A (2006). EBV+ lymphoepithelial carcinoma of the parotid gland in Mexican Mestizo patients with chronic autoimmune diseases. *Pathol Oncol Res*, 12: 41–45. doi:10.1007/BF02893430 PMID:16554915

Sawyer RN, Evans AS, Niederman JC, McCollum RW (1971). Prospective studies of a group of Yale University freshmen. I. Occurrence of infectious mononucleosis. *J Infect Dis*, 123: 263–270. PMID:4329526

Sbih-Lammali F, Djennaoui D, Belaoui H *et al.* (1996). Transcriptional expression of Epstein-Barr virus genes and proto-oncogenes in north African nasopharyngeal carcinoma. *J Med Virol*, 49: 7–14. doi:10.1002/(SICI)1096-9071(199605)49:1<7::AID-JMV2>3.0.CO;2-A PMID:8732865

Serraino D, Franceschi S, Talamini R *et al.* (1991). Socio-economic indicators, infectious diseases and Hodgkin's disease. *Int J Cancer*, 47: 352–357. doi:10.1002/ijc.2910470307 PMID:1993542

Shair KH, Bendt KM, Edwards RH *et al.* (2007). EBV latent membrane protein 1 activates Akt, NFkappaB, and Stat3 in B cell lymphomas. *PLoS Pathog*, 3: e166 doi:10.1371/journal.ppat.0030166 PMID:17997602

Shao JY, Li YH, Gao HY *et al.* (2004). Comparison of plasma Epstein-Barr virus (EBV) DNA levels and serum EBV immunoglobulin A/virus capsid antigen antibody titers in patients with nasopharyngeal carcinoma. *Cancer*, 100: 1162–1170. doi:10.1002/cncr.20099 PMID:15022282

Shao YM, Poirier S, Ohshima H *et al.* (1988). Epstein-Barr virus activation in Raji cells by extracts of preserved food from high risk areas for nasopharyngeal carcinoma. *Carcinogenesis*, 9: 1455–1457. doi:10.1093/carcin/9.8.1455 PMID:2841048

Shinkura R, Yamamoto N, Koriyama C *et al.* (2000). Epstein-Barr virus-specific antibodies in Epstein-Barr virus-positive and -negative gastric carcinoma cases in Japan. *J Med Virol*, 60: 411–416. doi:10.1002/(SICI)1096-9071(200004)60:4<411::AID-JMV8>3.0.CO;2-8 PMID:10686024

Sinclair AJ, Palmero I, Peters G, Farrell PJ (1994). EBNA-2 and EBNA-LP cooperate to cause G0 to G1 transition during immortalization of resting human B lymphocytes by Epstein-Barr virus. *EMBO J*, 13: 3321–3328. PMID:8045261

Sousa H, Pinto-Correia AL, Medeiros R, Dinis-Ribeiro M (2008). Epstein-Barr virus is associated with gastric carcinoma: the question is what is the significance? *World J Gastroenterol*, 14: 4347–4351. doi:10.3748/wjg.14.4347 PMID:18666324

Squillaci S, Bertalot G, Vago L *et al.* (2000). Lympho-epithelial carcinoma (malignant lympho-epithelial lesions) of the parotid gland. Description of a case with detection of EBV by in situ hybridization. *Pathologica*, 92: 189–194. PMID:10902430

Stevens SJ, Verkuijlen SA, Hariwiyanto B *et al.* (2006). Noninvasive diagnosis of nasopharyngeal carcinoma: nasopharyngeal brushings reveal high Epstein-Barr virus DNA load and carcinoma-specific viral BARF1 mRNA. *Int J Cancer*, 119: 608–614. doi:10.1002/ijc.21914 PMID:16572427

Strobl LJ, Höfelmayr H, Stein C *et al.* (1997). Both Epstein-Barr viral nuclear antigen 2 (EBNA2) and activated Notch1 transactivate genes by interacting with the cellular protein RBP-J kappa. *Immunobiology*, 198: 299–306. PMID:9442401

Sumaya CV, Henle W, Henle G *et al.* (1975). Seroepidemiologic study of Epstein-Barr virus infections in a rural community. *J Infect Dis*, 131: 403–408. PMID:163869

Suwiwat S, Pradutkanchana J, Ishida T, Mitarnun W (2007). Quantitative analysis of cell-free Epstein-Barr virus DNA in the plasma of patients with peripheral T-cell and NK-cell lymphomas and peripheral T-cell proliferative diseases. *J Clin Virol*, 40: 277–283. doi:10.1016/j.jcv.2007.08.013 PMID:17996493

Suzuki R, Takeuchi K, Ohshima K, Nakamura S (2008). Extranodal NK/T-cell lymphoma: diagnosis and treatment cues. *Hematol Oncol*, 26: 66–72. doi:10.1002/hon.847 PMID:18283711

Swart R, Ruf IK, Sample J, Longnecker R (2000). Latent membrane protein 2A-mediated effects on the phosphatidylinositol 3-Kinase/Akt pathway. *J Virol*, 74: 10838–10845. doi:10.1128/JVI.74.22.10838-10845.2000 PMID:11044134

Swerdlow SH, Campo E, Harris NL *et al.*, editors (2008). *World Health Organization Classification of Tumours – Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon: IARC Press.

Takada K (2001). Role of Epstein-Barr virus in Burkitt's lymphoma. *Curr Top Microbiol Immunol*, 258: 141–151. PMID:11443859

Tan BT, Warnke RA, Arber DA (2006). The frequency of B- and T-cell gene rearrangements and epstein-barr virus in T-cell lymphomas: a comparison between angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma, unspecified with and without associated B-cell proliferations. *J Mol Diagn*, 8: 466–475, quiz 527. doi:10.2353/jmoldx.2006.060016 PMID:16931587

Tanner J, Weis J, Fearon D *et al.* (1987). Epstein-Barr virus gp350/220 binding to the B lymphocyte C3d receptor mediates adsorption, capping, and endocytosis. *Cell*, 50: 203–213. doi:10.1016/0092-8674(87)90216-9 PMID:3036369

Tepper CG & Seldin MF (1999). Modulation of caspase-8 and FLICE-inhibitory protein expression as a potential mechanism of Epstein-Barr virus tumorigenesis in Burkitt's lymphoma. *Blood*, 94: 1727–1737. PMID:10477698

Terrin L, Dal Col J, Rampazzo E *et al.* (2008). Latent membrane protein 1 of Epstein-Barr virus activates the hTERT promoter and enhances telomerase activity in B lymphocytes. *J Virol*, 82: 10175–10187. doi:10.1128/JVI.00321-08 PMID:18684838

Thompson MP & Kurzrock R (2004). Epstein-Barr virus and cancer. *Clin Cancer Res*, 10: 803–821. doi:10.1158/1078-0432.CCR-0670-3 PMID:14871955

Thorley-Lawson DA (2005). EBV the prototypical human tumor virus–just how bad is it? *J Allergy Clin Immunol*, 116: 251–261, quiz 262. doi:10.1016/j.jaci.2005.05.038 PMID:16083776

Thorley-Lawson DA & Allday MJ (2008). The curious case of the tumour virus: 50 years of Burkitt's lymphoma. *Nat Rev Microbiol*, 6: 913–924. doi:10.1038/nrmicro2015 PMID:19008891

Thorley-Lawson DA & Gross A (2004). Persistence of the Epstein-Barr virus and the origins of associated lymphomas. *N Engl J Med*, 350: 1328–1337. doi:10.1056/NEJMra032015 PMID:15044644

Thornburg NJ & Raab-Traub N (2007). Induction of epidermal growth factor receptor expression by Epstein-Barr virus latent membrane protein 1 C-terminal-activating region 1 is mediated by NF-kappaB p50

homodimer/Bcl-3 complexes. *J Virol*, 81: 12954–12961. doi:10.1128/JVI.01601-07 PMID:17881446

Tischendorf P, Shramek GJ, Balagtas RC *et al.* (1970). Development and persistence of immunity to Epstein-Barr virus in man. *J Infect Dis*, 122: 401–409. PMID:4320006

Tiwawech D, Srivatanakul P, Karalak A, Ishida T (2008). Association between EBNA2 and LMP1 subtypes of Epstein-Barr virus and nasopharyngeal carcinoma in Thais. *J Clin Virol*, 42: 1–6. doi:10.1016/j.jcv.2007.11.011 PMID:18180201

Tomkinson B, Robertson E, Kieff E (1993). Epstein-Barr virus nuclear proteins EBNA-3A and EBNA-3C are essential for B-lymphocyte growth transformation. *J Virol*, 67: 2014–2025. PMID:8445720

Tsang WYW, Chan JKC (2005). *Lymphoepithelial carcinoma*. In: *World Health Organization Classification of Tumours – Pathology and Genetics of Head and Neck Tumours*. Barnes EL, Eveson JW, Reichart P, Sidransky D, editors. Kleihues P, Sobin LH, series editors. Lyon, France: IARC Press, pp. 85–97.

Tsimbouri P, Drotar ME, Coy JL, Wilson JB (2002). bcl-xL and RAG genes are induced and the response to IL-2 enhanced in EmuEBNA-1 transgenic mouse lymphocytes. *Oncogene*, 21: 5182–5187. doi:10.1038/sj.onc.1205490 PMID:12140768

van Baarle D, Hovenkamp E, Dukers NH *et al.* (2000). High prevalence of Epstein-Barr virus type 2 among homosexual men is caused by sexual transmission. *J Infect Dis*, 181: 2045–2049. doi:10.1086/315521 PMID:10837190

Wang CP, Chang YL, Ko JY *et al.* (2004). Lymphoepithelial carcinoma versus large cell undifferentiated carcinoma of the major salivary glands. *Cancer*, 101: 2020–2027. doi:10.1002/cncr.20614 PMID:15389474

Wang F, Gregory C, Sample C *et al.* (1990a). Epstein-Barr virus latent membrane protein (LMP1) and nuclear proteins 2 and 3C are effectors of phenotypic changes in B lymphocytes: EBNA-2 and LMP1 cooperatively induce CD23. *J Virol*, 64: 2309–2318. PMID:2157887

Wang F, Tsang SF, Kurilla MG *et al.* (1990b). Epstein-Barr virus nuclear antigen 2 transactivates latent membrane protein LMP1. *J Virol*, 64: 3407–3416. PMID:2352328

Webster-Cyriaque J & Raab-Traub N (1998). Transcription of Epstein-Barr virus latent cycle genes in oral hairy leukoplakia. *Virology*, 248: 53–65. doi:10.1006/viro.1998.9268 PMID:9705255

Weiss LM (2000). Epstein-Barr virus and Hodgkin's disease. *Curr Oncol Rep*, 2: 199–204. doi:10.1007/s11912-000-0094-9 PMID:11122844

Wilson JB, Bell JL, Levine AJ (1996). Expression of Epstein-Barr virus nuclear antigen-1 induces B cell neoplasia in transgenic mice. *EMBO J*, 15: 3117–3126. PMID:8670812

Winberg G, Matskova L, Chen F *et al.* (2000). Latent membrane protein 2A of Epstein-Barr virus binds WW domain E3 protein-ubiquitin ligases that ubiquitinate B-cell tyrosine kinases. *Mol Cell Biol*, 20: 8526–8535. doi:10.1128/MCB.20.22.8526-8535.2000 PMID:11046148

Woodman CB, Collins SI, Vavrusova N *et al.* (2005). Role of sexual behavior in the acquisition of asymptomatic Epstein-Barr virus infection: a longitudinal study. *Pediatr Infect Dis J*, 24: 498–502. doi:10.1097/01.inf.0000164709.40358.b6 PMID:15933558

Wu LY, Cheng J, Lu Y *et al.* (2004). Epstein-Barr virus infection in lymphoepithelial carcinoma of salivary glands in Sichuan Chinese. *Sichuan Da Xue Xue Bao Yi Xue Ban*, 35: 506–507. PMID:15291112

Wu TC, Mann RB, Charache P *et al.* (1990). Detection of EBV gene expression in Reed-Sternberg cells of Hodgkin's disease. *Int J Cancer*, 46: 801–804. doi:10.1002/ijc.2910460509 PMID:2172169

Wu Y, Maruo S, Yajima M *et al.* (2007). Epstein-Barr virus (EBV)-encoded RNA 2 (EBER2) but not EBER1 plays a critical role in EBV-induced B-cell growth transformation. *J Virol*, 81: 11236–11245. doi:10.1128/JVI.00579-07 PMID:17686859

Xia T, O'Hara A, Araujo I *et al.* (2008). EBV microRNAs in primary lymphomas and targeting of CXCL-11 by ebv-mir-BHRF1–3. *Cancer Res*, 68: 1436–1442. doi:10.1158/0008-5472.CAN-07-5126 PMID:18316607

Yajima M, Imadome K, Nakagawa A *et al.* (2008). A new humanized mouse model of Epstein-Barr virus infection that reproduces persistent infection, lymphoproliferative disorder, and cell-mediated and humoral immune responses. *J Infect Dis*, 198: 673–682. doi:10.1086/590502 PMID:18627269

Yao QY, Rickinson AB, Epstein MA (1985). A re-examination of the Epstein-Barr virus carrier state in healthy seropositive individuals. *Int J Cancer*, 35: 35–42. doi:10.1002/ijc.2910350107 PMID:2981780

Yap YY, Hassan S, Chan M *et al.* (2007). Epstein-Barr virus DNA detection in the diagnosis of nasopharyngeal carcinoma. *Otolaryngol Head Neck Surg*, 136: 986–991. doi:10.1016/j.otohns.2006.11.027 PMID:17547993

Young LS (2008) *Epstein-Barr Virus: General Features*. In: *Encyclopedia of Virology*. Mahy BWJ, van Regenmortel MHV, editors-in-chief. Oxford: Academic Press, pp. 148–157.

Young LS, Finerty S, Brooks L *et al.* (1989). Epstein-Barr virus gene expression in malignant lymphomas induced by experimental virus infection of cottontop tamarins. *J Virol*, 63: 1967–1974. PMID:2539497

Young LS & Rickinson AB (2004). Epstein-Barr virus: 40 years on. *Nat Rev Cancer*, 4: 757–768. doi:10.1038/nrc1452 PMID:15510157

Yuan JM, Wang XL, Xiang YB *et al.* (2000). Preserved foods in relation to risk of nasopharyngeal carcinoma in Shanghai, China. *Int J Cancer*, 85: 358–363. doi:10.1002/(SICI)1097-0215(20000201)85:3<358::AID-IJC11>3.0.CO;2-E PMID:10652427

Zhao B & Sample CE (2000). Epstein-barr virus nuclear antigen 3C activates the latent membrane protein 1 promoter in the presence of Epstein-Barr virus nuclear antigen 2 through sequences encompassing an spi-1/Spi-B binding site. *J Virol*, 74: 5151–5160. doi:10.1128/JVI.74.11.5151-5160.2000 PMID:10799590

Zheng X, Yan L, Nilsson B *et al.* (1994a). Epstein-Barr virus infection, salted fish and nasopharyngeal carcinoma. A case-control study in southern China. *Acta Oncol*, 33: 867–872. doi:10.3109/02841869409098448 PMID:7818917