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Epstein–Barr virus infection: basis of malignancy and potential for therapy

Paul G. Murray and Lawrence S. Young

The Epstein–Barr virus (EBV) is a human herpesvirus that is usually carried lifelong as an asymptomatic infection. EBV is the causative agent of infectious mononucleosis and has been linked to the development of several malignant tumours, including B-cell neoplasms such as Burkitt's lymphoma and Hodgkin's disease, certain forms of T-cell lymphoma, and some epithelial tumours, such as undifferentiated nasopharyngeal carcinoma and a proportion of gastric cancers. All these tumours are characterised by the presence of multiple extrachromosomal copies of the circular viral genome in the tumour cells and the expression of EBV-encoded latent genes, which appear to contribute to the malignant phenotype. An increasing understanding of the function of EBV latent genes and of the nature of the immune response to the virus is providing exciting new possibilities for the treatment of EBV-associated malignancies. For example, adoptive transfer of virus-specific cytotoxic T lymphocytes has already been of value in the treatment of EBV-positive B-cell lymphomas arising in post-transplant patients, and this approach is currently being investigated in other EBV-associated tumours. In addition, gene therapy offers the opportunity to deliver agents that might directly interfere with the function of specific EBV genes. This review summarises the role of EBV in malignancy. In particular, it focuses on the latent proteins as a basis for understanding how EBV might contribute to the process of transformation. Strategies to target EBV in tumours, potentially providing alternative therapeutic approaches, are also discussed.

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Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994 ©2001 Cambridge University Press

Epstein–Barr virus (EBV) is a member of the *Lymphocryptovirus* genus. These viruses are closely related members of the gamma herpesvirus family and are similar to each other with respect to genomic structure and gene organisation. The EBV genome is composed of linear, double-stranded DNA, which is approximately 172 kb in length (see Fig. 1a), and regions of the EBV genome have become known by their position on a *Bam*HI restriction endonuclease map of the genome (see Fig. 1b).

EBV is orally transmitted and infectious virus can be detected in oropharyngeal secretions from infectious mononucleosis (IM) patients, from immunosuppressed patients and, at lower levels, from healthy EBV-seropositive individuals (Ref. 1). EBV enters B cells by interaction of the major viral glycoprotein gp350/220 with the complement receptor (CR2/CD21, which is the receptor for complement component C3d). The penetration of B cells by EBV also involves the viral glycoproteins gp25 (gL) and gp42/38 in a complex with viral gp85 (gH). This complex mediates an interaction between EBV and major histocompatibility complex (MHC) class II molecules, which serve as a co-receptor for virus entry into B cells (Ref. 2).

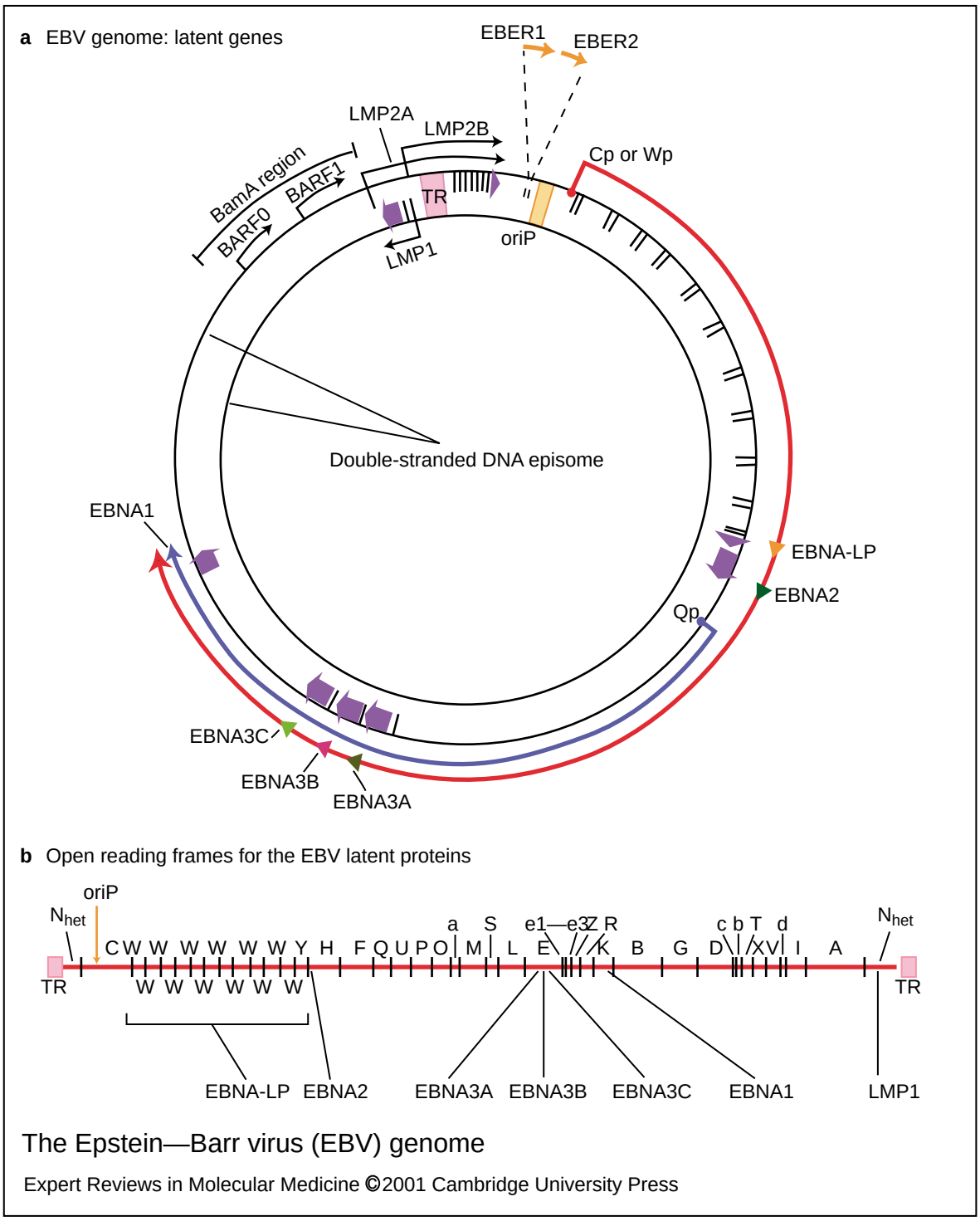
Crosslinking of CD21 activates B cells from their resting state. Some of the immediate effects that occur simply from this binding event include Lck activation and calcium (Ca^{2+}) mobilisation, which is followed by increased mRNA synthesis, homotypic cell adhesion, blast transformation, surface CD23 expression, and interleukin 6 (IL-6) production. Once the viral genome has been uncoated and transferred to the nucleus, circularisation and transcription from the Wp promoter begin a cascade of events leading to expression of all the latent genes. The EBV nuclear antigen (EBNA) leader protein (EBNA-LP) and EBNA2 are the first proteins to be detected and these are sufficient to advance the cells to early G1 phase of the cell cycle. The EBV-infected (EBV^+) cells begin to proliferate in a manner that depends on high cell density and on the autocrine production of cytokines that promote B-cell growth. Later, the EBV^+ cells evolve into cells that grow more rapidly and are less dependent on autocrine growth mechanisms. Following infection in vivo, the virus establishes a latent infection in memory B cells and this is characterised by the limited expression of a subset of virus latent genes.

EBV latent proteins

When peripheral blood lymphocytes from chronic virus carriers are placed in culture, the few EBV^+ B cells that are present regularly give rise to spontaneous outgrowth of EBV-transformed, immortalised cell lines, known as lymphoblastoid cell lines (LCLs), provided that immune T cells are either removed or inhibited by the addition of cyclosporin A to the culture (Ref. 3).

Every cell in an LCL carries multiple copies of circular extrachromosomal viral DNA (episomes) and produces several latent proteins, including six nuclear antigens (EBNAs 1, 2, 3A, 3B and 3C, and EBNA-LP) and three latent membrane proteins (LMPs 1, 2A and 2B) (Ref. 4). Transcripts from the *Bam*HI A region (BamA) of the viral genome are also detected, although whether these encode proteins remains controversial (Ref. 5). In addition to the latent proteins, LCLs also show abundant expression of the small non-polyadenylated (and therefore non-coding) RNAs EBERs 1 and 2; the function of these transcripts is not clear but they are believed to be expressed in all forms of latent EBV infection and have served as excellent targets to detect EBV in tumours (see later). The relative positions of these viral genes are illustrated in Figure 1b on a linearised *Bam*HI restriction map of the viral genome. The different EBNAs are encoded by individual mRNAs generated by differential splicing of the same long 'rightward' primary transcript expressed from one of two promoters (Cp or Wp) located close together in the *Bam*HI C and W regions of the genome (Ref. 6). These are illustrated in Figure 1a on the covalently closed EBV episome. A switch from Wp to Cp occurs early in B-cell infection. The LMP transcripts are expressed from separate promoters in the *Bam*HI N region of the EBV genome, with the leftward LMP1 and rightward LMP2B mRNAs apparently controlled by the same bidirectional promoter sequence (Ref. 6). This pattern of latent EBV gene expression is referred to as the 'latency III' (Lat III) form of EBV infection, and is characteristic of LCLs and most post-transplant lymphomas (see later). At least two other forms of EBV latency are observed. In Lat I, which is characteristic of Burkitt's lymphoma (BL), only EBNA1, the EBERs and the BamA transcripts are regularly detected. In Lat II, observed in EBV-associated Hodgkin's disease (HD) and undifferentiated nasopharyngeal carcinoma (UNPC), the EBERs, EBNA1 and BamA transcripts are expressed

Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994



The Epstein—Barr virus (EBV) genome

Figure 1. The Epstein–Barr virus (EBV) genome (*see next page for legend*) **(fig001lyb).**

together with both LMP1 and LMP2. In Lat I and Lat II, EBNA1 is transcribed from the Qp promoter rather than Wp or Cp. These alternative forms of latency are discussed in more detail later with respect to EBV-associated malignancies.

The Lat III pattern of EBV gene expression seen in LCLs is matched by an equally consistent and characteristic cellular phenotype, with high-level expression of the B-cell activation markers CD23, CD30, CD39 and CD70 and of

Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994 ©2001 Cambridge University Press

Figure 1. The Epstein–Barr virus (EBV) genome. (a) Diagram showing the location and transcription of the EBV latent genes on the double-stranded viral DNA episome. The origin of plasmid replication (oriP) is shown in orange. The large solid blocks (in purple) represent coding exons for each of the latent proteins and the arrows indicate the direction in which they are transcribed; the latent proteins include the six nuclear antigens (EBNAs 1, 2, 3A, 3B and 3C, and EBNA-LP) and the three latent membrane proteins (LMPs 1, 2A, 2B). EBNA-LP is transcribed from variable numbers of repetitive exons. LMP2A and LMP2B are composed of multiple exons located either side of the terminal repeat (TR) region, which is formed during the circularisation of the linear DNA to produce the viral episome. The orange arrows at the top represent the highly transcribed non-polyadenylated RNAs EBER1 and EBER2; their transcription is a consistent feature of latent EBV infection. The outer long arrowed red line represents EBV transcription during a form of latency known as latency III (Lat III), where all the EBNAs are transcribed from either the Cp or Wp promoter; the different EBNAs are encoded by individual mRNAs generated by differential splicing of the same long primary transcript. The inner shorter arrowed blue line represents the EBNA1 transcript originating from the Qp promoter during Lat I and Lat II. Transcripts from the BamA region can be detected during latent infection, but no protein arising from this region has been definitively identified. Shown here are the locations of the BARF0 and BARF1 coding regions. (b) Diagram showing the location of open reading frames for the EBV latent proteins on a *Bam*HI restriction endonuclease map of the prototype B95.8 EBV genome. The *Bam*HI fragments are named according to size, with A being the largest. Lower-case letters denote the smallest fragments. Note that the LMP2 proteins are produced from mRNAs that splice across the terminal repeats (TR) in the circularised EBV genome. This region has often been referred to as N_{het} to denote the heterogeneity in this region according to the number of terminal repeats within different virus isolates **(fig001lyb)**.

the cellular adhesion molecules leukocyte-function-associated molecule 1 (LFA-1; CD11a/18), LFA-3 (CD58) and intercellular adhesion molecule 1 (ICAM-1; CD54) (Ref. 7). These markers are usually absent or expressed at low levels on resting B cells, but are transiently induced to high levels when these cells are activated into short-term growth by antigenic or mitogenic stimulation, suggesting that EBV-induced immortalisation can be elicited through the constitutive activation of the same cellular pathways that drive physiological B-cell proliferation. The ability of EBNA2, EBNA3C and LMP1 to induce LCL-like phenotypic changes when expressed individually in human B-cell lines implicates these viral proteins as key effectors of the immortalisation process (Ref. 8).

EBV infection of B cells in vivo

Several lines of evidence support a role for the B cell as the site of EBV persistence in vivo (Refs 9, 10, 11, 12). Indeed, therapy aimed at eliminating virus replication using long-term acyclovir treatment (which, following conversion to acyclovir triphosphate, interferes with viral replication by competing with deoxyguanosine triphosphate for viral DNA polymerase) eliminates virus excretion from the oropharnyx but does not affect the level of latent infection in B cells (Ref. 9). As soon as treatment is halted, virus can be detected in the oropharyngeal secretions at pre-treatment levels (Ref. 10). In addition, studies of EBV strains in donor–recipient pairs before and after bone marrow transplantation (BMT) have shown that the recipient's strain disappeared from the oropharynx and was replaced by the donor's strain, indicating that the bone-marrow B cells harbour EBV (Ref. 11). Furthermore, patients with X-linked agammaglobulinaemia (XLA), who are deficient in mature B cells, are found to be free of EBV infection, suggesting they are not able to maintain a persistent infection (Ref. 12).

EBV exists in the peripheral blood within the IgD^- memory B-cell pool, with EBV gene expression in these cells restricted to LMP2A and possibly EBNA1 (Ref. 13). Recent work has shown that a subset of healthy tonsils contains EBV^+ naive (IgD^+) B cells and that these cells express the Lat III programme and show an activated phenotype, suggesting they have been directly infected (Ref. 14). These cells are presumably either eliminated by virus-specific cytotoxic T lymphocytes (CTLs) (Fig. 2) or differentiate to IgD^- memory B cells, which then leave the tonsil. Some of these memory B cells will pass through mucosal lymphoid tissues and terminally differentiate into plasma cells, whereupon they might enter the lytic cycle. However, a proportion also exits the cell cycle and will replenish the peripheral pool of infected memory cells.

The Lat II pattern of EBV gene expression has also been detected in tonsillar memory B cells and

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ISSN 1462-3994 ©2001 Cambridge University Press

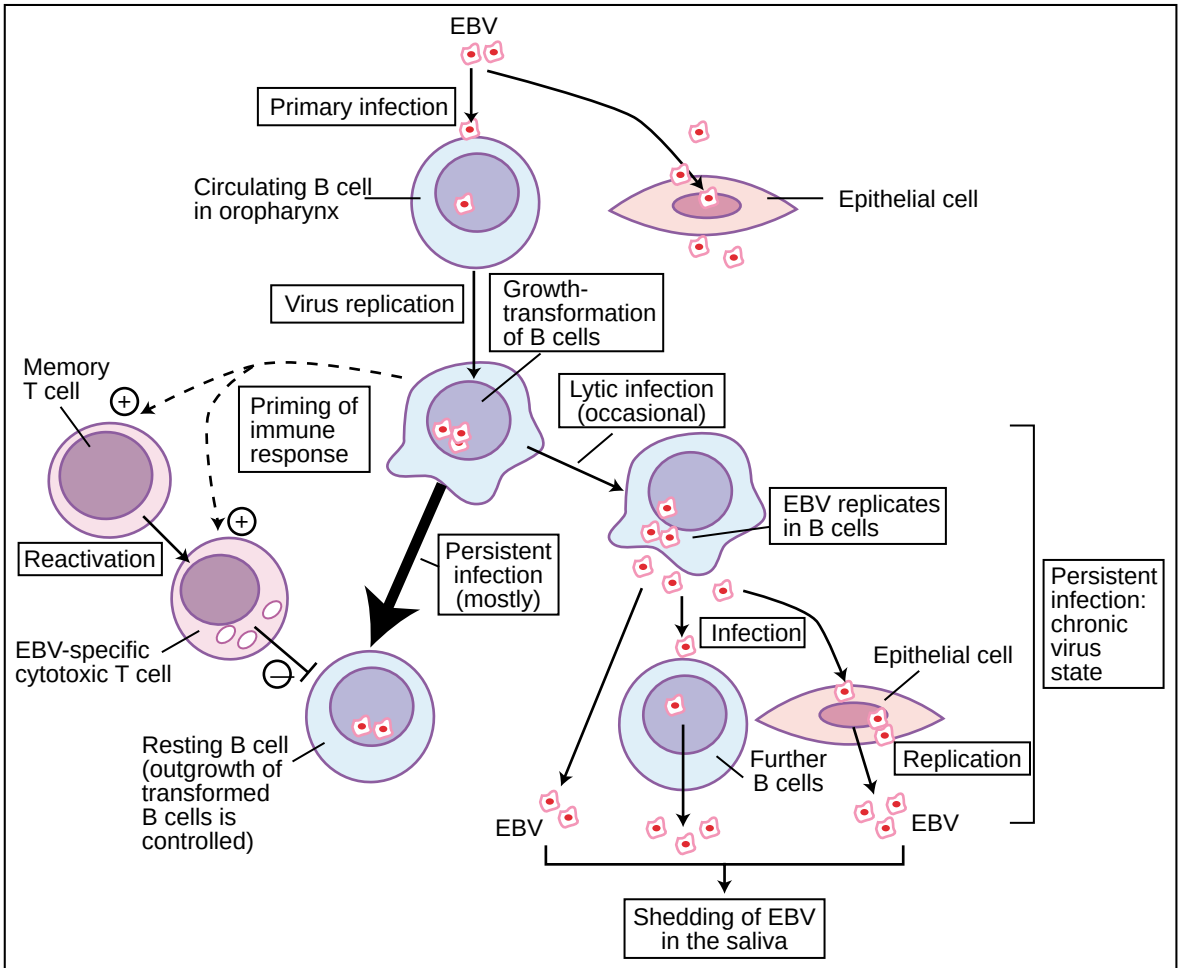

Figure 2. Epstein–Barr virus (EBV) infection in normal healthy virus carriers. Virus infection involves two cellular compartments: (1) B cells, where infection is predominantly latent and has the potential to induce growth-transformation of infected cells; and (2) epithelial cells, where infection is predominantly replicative. Although the exact mode of primary and persistent EBV infection and the relative contributions of B cells and epithelial cells are uncertain, recent data point to the B-cell compartment as the main mediator of primary as well as persistent infection. Following primary infection of B cells, a chronic virus carrier state is established in which the outgrowth of EBV-transformed B cells is controlled by an EBV-specific cytotoxic T lymphocyte response re-activated from a pool of virus-specific memory T cells. At certain sites, latently infected B cells can become permissive for lytic EBV infection. Infectious virus released from these cells can be shed directly into the saliva or might infect epithelial cells and other B cells. In this way a virus-carrier state is established that is characterised by persistent, latent infection in circulating B cells and occasional EBV replication in B cells and epithelial cells **(fig002lyb).**

germinal centre B cells (Ref. 15). LMP1 can provide surrogate T-cell help via mimicry of an activated CD40 receptor, and LMP2A can substitute for B-cell receptor engagement (see later). Thus, the virus might enter a germinal centre reaction and re-express LMP1 and LMP2, providing a mechanism for the antigen-independent expansion of EBV$^+$ B cells (Ref. 15). However, these data are not supported by studies of CD40 null mice, which are defective for isotype switching and germinal centre formation. When LMP1 is constitutively expressed from a

Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994 ©2001 Cambridge University Press

transgene in the B cells of these mice, they are still not able to form germinal centres or produce high-affinity antibodies (Ref. 16). Furthermore, germinal centres are also not formed when LMP1 is expressed in a wild-type ($CD40^+$) background, suggesting that, rather than facilitating a germinal centre reaction, LMP1 actively inhibits this process. These conflicts remain to be resolved.

EBV infection of epithelial cells in vivo

Although much of the evidence described above implicates the B-cell compartment as the site of persistence of EBV, infection of squamous epithelial cells in normal individuals might occur since EBV can be detected in oral hairy leukoplakia, a benign lesion of the oral epithelia characterised by intense lytic infection of these tissues (Ref. 17). However, EBV is not usually detectable in normal epithelial tissues, including desquamated oropharyngeal cells and tonsillar epithelium from IM patients (Ref. 18), and normal epithelium adjacent to EBV^+ UNPCs (Ref. 19) and gastric carcinomas (Ref. 20). Nevertheless, the virus can be detected in pre-invasive UNPC (Ref. 21) and dysplastic gastric epithelium (Ref. 20), suggesting that EBV might be an early trigger in the pathogenesis of these lesions – a view supported by the finding that EBV infection of epithelial cells in vitro stimulates cell proliferation and induces a malignant phenotype (Ref. 22).

Epithelial cells generally do not express CD21, suggesting that EBV enters these tissues by other cellular receptors. Various human epithelial cells can be infected in vitro either by direct contact with high-titre virus supernatant or by mixed culture with EBV-producing cells such as the BL cell line Akata, suggesting a model of EBV infection in vivo whereby epithelial tissues might be infected by virtue of their close proximity to lytically infected B cells resident near or within epithelial tissues (e.g. adjacent to the subepithelial sinus in tonsil or within nasopharyngeal mucosa). It has recently been shown that virus made by epithelial cells lacking MHC class II (E-EBV) contains increased levels of gp42 and is more infectious for B cells compared with virus made in B cells (B-EBV) (C.M. Borza and L.M. Hutt-Fletcher, pers. commun.). By contrast, B-EBV was equally or more infectious for epithelial cells than was E-EBV. These data suggest that primary EBV infection of epithelial tissues could lead to the production of virus with a particular tropism for B cells, which would facilitate the establishment of persistent infection in this compartment. Later, virus produced by B cells near to epithelia could more readily infect epithelial tissues, with subsequent release of virus into saliva.

Function of the EBV latent proteins

The transformation of B cells by EBV involves the co-ordinated action of several latent gene products. With the demonstration of more restricted patterns of EBV gene expression in tumours, the function of these proteins has been the focus of much interest.

EBNA1

EBNA1 is a DNA-binding protein that is required for the replication and maintenance of the episomal EBV genome; this function is achieved through the binding of EBNA1 to oriP, the plasmid origin of viral replication (Ref. 4). EBNA1 also interacts with two sites downstream of Qp to negatively regulate its own expression (Ref. 23). EBNA1 acts as a transcriptional transactivator and upregulates Cp and the LMP1 promoter (Ref. 4).

The EBNA1 protein contains a glycine-glycine-alanine (Gly-Gly-Ala) repeat sequence, which varies in size in different EBV isolates (Ref. 4). This repeat domain is a cis-acting inhibitor of MHC class I-restricted presentation and appears to function by inhibiting antigen processing via the ubiquitin–proteosome pathway (Ref. 24). Failure to present EBNA1-derived peptides results in ineffective $CD8^+$ T-cell responses to EBNA1 when expressed in target cells. Directing EBNA1 expression to B cells in transgenic mice results in B-cell lymphomas, suggesting that EBNA1 might also have a direct role in oncogenesis (Ref. 25).

EBNA2

The inability of an EBV strain, P3HR-1, carrying a deletion of the gene encoding EBNA2 and the last two exons of EBNA-LP to transform B cells in vitro was the first indication of the crucial role of the EBNA2 protein in the transformation process (Ref. 4). Restoration of the EBNA2 gene into P3HR-1 has unequivocally confirmed the importance of EBNA2 in B-cell transformation and has allowed the functionally relevant domains of the EBNA2 protein to be identified (Ref. 26).

Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994

EBNA2 is a transcriptional activator of both cellular and viral genes, and upregulates the expression of certain B-cell antigens, including CD21 and CD23, as well as LMP1 and LMP2 (Refs 4, 8). EBNA2 also transactivates the Cp promoter, thereby inducing the switch from Wp to Cp observed early in B-cell infection. EBNA2 interacts with a ubiquitous DNA-binding protein, RBP-Jκ, and this is partly responsible for targeting EBNA2 to promoters that contain cognate Jκ sequence (Ref. 27). The RBP-Jκ homologue in *Drosophila* is involved in signal transduction from the Notch receptor, a pathway that is important in cell fate determination in *Drosophila* and has also been implicated in the development of T-cell tumours in humans (Ref. 28). Recent work demonstrates that EBNA2 can functionally replace the intracellular region of Notch (Ref. 29). The c-*myc* oncogene is also a transcriptional target of EBNA2 – an effect that is likely to be important for EBV-induced B-cell proliferation (Ref. 30).

EBNA3 family

Studies with EBV recombinants have shown that EBNA3A and EBNA3C are essential for B-cell transformation in vitro, whereas EBNA3B is dispensable (Ref. 31). EBNA3C can induce the upregulation of both cellular (CD21) and viral (LMP1) gene expression (Ref. 32), and repress the Cp promoter (Ref. 33), and might interact with the retinoblastoma protein, pRb, to promote transformation (Ref. 34). Although not essential for transformation, EBNA3B has been shown to induce expression of vimentin and CD40 (Ref. 35). The EBNA3 proteins associate with the RBP-Jκ transcription factor and disrupt its binding to the cognate Jκ sequence and to EBNA2, thus repressing EBNA2-mediated transactivation (Ref. 31). Thus, EBNA2 and the EBNA3 proteins work together to precisely control RBP-Jκ activity, thereby regulating the expression of cellular and viral promoters containing cognate Jκ sequence. More recently, EBNA3C has been shown to interact with human histone deacetylase 1, which, in turn, contributes to the transcriptional repression of Cp, by RBP-Jκ (Ref. 36).

EBNA-LP

EBNA-LP is encoded by the leader of each of the EBNA mRNAs and encodes a protein of variable size depending on the number of *Bam*HI W repeats contained by a particular EBV isolate. Although not absolutely required for B-cell transformation in vitro, EBNA-LP is required for the efficient outgrowth of LCLs (Ref. 37). Transient transfection of EBNA-LP and EBNA2 into primary B cells induces G0 to G1 transition as measured by the upregulation of cyclin D2 expression (Ref. 38). EBNA-LP can also cooperate with EBNA-2 in upregulating transcriptional targets of EBNA2, including LMP1 (Ref. 39). EBNA-LP has been shown to co-localise with pRb in LCLs and in vitro biochemical studies have demonstrated an interaction of EBNA-LP with both pRb and p53 (Ref. 40). However, this interaction has not been verified in LCLs and, unlike the situation with the human papillomavirus (HPV)-encoded E6/E7 and adenovirus E1 proteins, EBNA-LP expression appears to have no effect on the regulation of the pRb and p53 pathways.

LMP1

At least four signalling pathways – namely NF-κB, JNK/AP-1, p38/MAPK and JAK/STAT – have been implicated in the function of LMP1 (Refs 41, 42, 43, 44). Within the C-terminus of LMP1 there are at least two activating regions, referred to as CTAR1 and CTAR2 (C-terminal activating regions 1 and 2). CTAR1 is located proximal to the membrane (amino acids 186–231) and is essential for EBV-mediated transformation of primary B cells. CTAR2 (amino acids 351–386) is located at the extreme C-terminus of LMP1 and is required for long-term growth of EBV^+ B cells (Ref. 45).

Activation of the transcription factor NF-κB was the first indication of the importance of LMP1 in aberrant cell signalling. Both CTAR1 and CTAR2 are able to independently activate NF-κB (Ref. 41). CTAR2 accounts for the majority (70–80%) of LMP1-mediated NF-κB activation via its interaction with the tumour necrosis factor receptor (TNFR)-associated death domain (TRADD) protein (Ref. 45). The remaining 20–30% of LMP1-mediated NF-κB activation is achieved through the CTAR1 $P^{204}\times Q^{206}\times T^{208}$ motif, which interacts with several TNFR-associated factors (TRAFs) (Ref. 46).

LMP1 activates the JNK (c-Jun N-terminal kinase) cascade [also known as the stress-activated protein kinase (SAPK) cascade] (Ref. 42) via CTAR2 alone (Ref. 47), whereas activation of the p38/MAPK pathway is mediated by both CTAR1 and CTAR2 (Ref. 43). A proline-rich

Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994

sequence within the 33 bp repeats of the LMP1 C-terminus together with surrounding sequences (i.e. between CTAR1 and CTAR2) mediates the activation of Janus kinase 3 (JAK3) (Ref. 44).

Aggregation of LMP1 within the plasma membrane is a critical prerequisite for signalling and appears to be an intrinsic property of the transmembrane domains (Ref. 48). LMP1 is palmitoylated, is enriched in lipid rafts and associates with the cytoskeleton. The transmembrane domains appear to be responsible for targeting LMP1 to lipid rafts, whereas LMP1's interaction with the TRAF molecules is important for association of LMP1 with the cytoskeleton (Ref. 49). The major difference between LMP1 and the TNFR family is that LMP1 functions as a constitutively activated receptor and therefore does not rely on the binding of an extracellular ligand. Experiments with chimaeric molecules consisting of the extracellular and transmembrane domains of CD2, CD4 or nerve growth factor receptor (NGFR) fused to the cytoplasmic C-terminus of LMP1 proved that LMP1 signalling occurs only upon aggregation of the chimaera by ligand binding or antibody-induced aggregation (Ref. 48). Conversely, the CD40 cytoplasmic tail was rendered constitutively active when linked to the N-terminal and transmembrane domains of LMP1 (Ref. 50).

The cloning and sequencing of the gene encoding LMP1 from EBV isolates derived from either a Chinese or a Taiwanese UNPC identified several mutations compared with that from the prototype B95.8 strain, including a point mutation leading to loss of an *Xho*I restriction site in the first exon, a 30 bp deletion in the C-terminus immediately upstream of CTAR2, and multiple point mutations (Ref. 51). These so-called delLMP1 variants (typified by the LMP1 encoded by EBV strain Cao; Cao-LMP1) display increased tumourigenicity in vitro (Ref. 52). Initially, the delLMP1 variant was thought to be preferentially associated with UNPC, but it can also be detected in some T-cell lymphomas, HD patients, IM patients and LCLs from healthy controls. Healthy virus carriers have been found to have a similar frequency of these mutations as the virus-infected tumour patients from the same geographical region (Ref. 53). However, there is an increased incidence of this deletion variant in human immunodeficiency virus $(HIV)^+$ HD compared with HIV^- HD, and in paediatric HD compared with normal controls (Ref. 54).

Cao-LMP1 is impaired in its ability to upregulate CD40 and CD54 compared with B95.8-LMP1 even though Cao-LMP1 can induce greater activation of NF-κB than B95.8-LMP1 does (Ref. 55). However, the 30 bp deletion is not responsible for these differences, and sequences outside CTAR2 appear to be involved (Ref. 55). Continued study of delLMP1 will help to dissect further the LMP1 signalling pathways and to assess the contribution of LMP1 sequence variation to the pathogenesis of EBV-associated tumours such as HD and NPC.

LMP2

The gene encoding LMP2 yields two distinct proteins: LMP2A and LMP2B. The structures of LMP2A and LMP2B are similar; both have 12 transmembrane domains and a 27 amino acid cytoplasmic C-terminus. In addition, LMP2A has a 119 amino acid cytoplasmic N-terminal domain. LMP2A aggregates in patches within the plasma membrane of latently infected B cells (Ref. 56). Neither LMP2A nor LMP2B is essential for B-cell transformation (Refs 57, 58).

The LMP2A N-terminal domain contains eight tyrosine residues, two of which (Y74 and Y85) form an immunoreceptor tyrosine-based activation motif (ITAM) (Ref. 58). When phosphorylated, the ITAM present in the B-cell receptor (BCR) plays a central role in mediating lymphocyte proliferation and differentiation by the recruitment and activation of the Src family of protein tyrosine kinases (PTKs) and the Syk PTK. LMP2A also interacts with these PTKs through its phosphorylated ITAM and this association appears to negatively regulate PTK activity (Ref. 58). Thus, the LMP2A ITAM has been shown to be responsible for blocking BCR-stimulated Ca^{2+} mobilisation, tyrosine phosphorylation and activation of the EBV lytic cycle in B cells (Ref. 59).

Expression of LMP2A in the B cells of transgenic mice abrogates normal B-cell development, allowing immunoglobulin-negative cells to colonise peripheral lymphoid organs (Ref. 60). This suggests that LMP2A can drive the proliferation and survival of B cells in the absence of signalling through the BCR (Ref. 60). Taken together, these data support a role for LMP2 in modifying the normal programme of B-cell development to favour the maintenance of EBV latency and to prevent inappropriate activation of the EBV lytic

Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994

cycle. A modulatory role for LMP2B in regulating LMP2A function has been suggested (Ref. 61). The consistent expression of LMP2A in HD and NPC suggests an important function for this protein in oncogenesis but this remains to be shown. A recent report demonstrates the adhesion-dependent tyrosine phosphorylation of LMP2A in an epithelial cell line, an effect mediated through C-terminal Src kinase (Csk), which is a negative regulator of Src kinase activity. LMP2A also recruits Nedd4-like ubiquitin protein ligases; this might promote Lyn and Syk ubiquitination in a fashion that contributes to a block in B-cell signalling (Ref. 62). Furthermore, recent reports show that LMP2A can transform epithelial cells; this effect is mediated, at least in part, by activation of the phosphoinositide 3-kinase-Akt pathway (Ref. 63).

EBV-associated tumours

The development of sensitive in situ hybridisation techniques and of monoclonal antibodies to specific EBV proteins has resulted in an ever-increasing number of different malignancies in which virus infection can be detected. Some of these are discussed below.

BL

BL was first recognised because of its striking clinical and epidemiological features. The so-called 'endemic' or high-incidence form of BL, which is found at an annual incidence of ~5–10 cases per 100 000 children, is restricted to areas of equatorial Africa and Papua New Guinea and coincides with areas where infection with *Plasmodium falciparum* malaria is holoendemic. By contrast, sporadic cases of BL occur worldwide but at a much lower frequency (at least 50-fold less than in the high-incidence areas). The endemic and sporadic forms of BL also differ in their association with EBV. Thus, whereas virtually every BL tumour found in the high-incidence regions is EBV^+, only 15% of sporadic BL tumours carry the virus. In addition, certain 'intermediate incidence' areas outside the regions of holoendemic malaria, such as Algeria and Egypt, have increased numbers of cases that correlate with an increased proportion of EBV^+ tumours. BL is also observed as a consequence of HIV infection, frequently occurring before the development of full-blown AIDS. Only 30–40% of these cases of AIDS-BL are associated with EBV infection. A consistent feature of all BL tumours, irrespective of geographical location or AIDS association, are chromosomal translocations involving the long arm of chromosome 8 (8q24) in the region of the c-*myc* proto-oncogene, and either chromosome 14 in the region of the immunoglobulin heavy-chain gene or, less frequently, chromosome 2 or chromosome 22 in the region of the immunoglobulin light-chain genes. This translocation results in deregulated expression of the c-*myc* oncogene.

The precise role of EBV in the pathogenesis of BL remains to be established. Monoclonal EBV episomes have been detected in virus-positive BL biopsies, suggesting that EBV infection preceded proliferation of the precursor B cells (Ref. 64). The apparent origin of BL in the germinal centre is based on phenotypic studies and is supported by the ability of BL risk factors such as holoendemic malaria and chronic HIV infection to stimulate proliferation of B cells in the germinal centre. These cells are also programmed to undergo somatic mutation of immunoglobulin genes and this event, in conjunction with the stimulation of germinal centre proliferation and EBV infection, might be responsible for the generation and selection of B cells carrying the c-*myc* translocation. Recent evidence also suggests greater involvement of EBV in sporadic BL than previously documented. Thus, defective integrated EBV genomes without the presence of EBNA1 expression have been detected in some sporadic BL tumours from the United States of America (Ref. 65), suggesting a process of viral DNA rearrangement and loss during malignant progression consistent with a 'hit and run' role for EBV in the pathogenesis of sporadic BL.

UNPC

The tumour showing the most consistent worldwide association with EBV is UNPC. UNPC is characterised by the presence of undifferentiated carcinoma cells together with a prominent lymphocytic infiltrate; the latter is believed to be important for the growth of the tumour cells. A link between EBV and UNPC was suggested as early as 1966 on the grounds of serological studies (Ref. 66), and substantiated later by the demonstration of EBV DNA and the EBNA complex in the tumour cells of UNPCs using in situ hybridisation and the anti-complement immunofluorescence (ACIF) assay (Ref. 67). Southern blot hybridisation of DNA from UNPC tissues revealed monoclonality of

Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994

the resident viral genomes, suggesting that EBV infection had taken place before clonal expansion of the malignant cell population (Ref. 68).

UNPC is particularly common in areas of China and South-East Asia, reaching a peak incidence of around 20–30 cases per 100 000. Incidence rates are also high in individuals of Chinese descent irrespective of where they live, and particularly in Cantonese males. In addition to this genetic pre-disposition, environmental co-factors such as dietary components (i.e. salted fish) are thought to be important in the aetiology of UNPC (Ref. 69). Extensive serological screening has identified elevated EBV-specific antibody titres in high-incidence areas – in particular, IgA antibodies to EBV capsid antigen (VCA) and early antigens – and these have proved useful in diagnosis and in monitoring the effectiveness of therapy (Ref. 70).

Gastric cancer

Tumours resembling UNPC can arise at other locations. Most UNPC-like tumours arising within the stomach, together with a smaller proportion of typical gastric adenocarcinomas lacking a lymphoid stroma, have been shown to harbour EBV (Ref. 71). Collectively, these EBV^+ tumours account for 3–18% of all gastric cancers.

Lymphoproliferative disease in immunodeficiency

The lymphoproliferations that arise following iatrogenic immunosuppression for transplant surgery are collectively known as post-transplant lymphoproliferative disorders (PTLDs). Similar tumours are observed in patients with certain forms of inherited immunodeficiency syndromes, such as X-linked lymphoproliferative syndrome and Wiscott–Aldrich syndrome, and in AIDS patients. They are most often of B-cell origin and represent a family of lesions ranging from atypical polyclonal B-cell proliferations, which often regress following withdrawal or reduction of immune suppression, to aggressive monomorphic non-Hodgkin's lymphomas (NHLs), which generally do not resolve following immune reconstitution.

Most lymphoproliferations that arise following solid-organ grafts are of host-cell origin, whereas those that occur after BMT are frequently derived from donor cells. Most tumours generally present as multifocal lesions in extranodal locations such as the gastrointestinal tract or in the allograft organ itself. The incidence and clinical presentation of PTLDs varies with the organ transplanted, the duration of immunosuppression and the dosage and number of agents used. The high incidence of PTLDs in the transplanted organ suggests that chronic antigen stimulation in the graft might be important in the pathogenesis of these lesions. Indeed, T cells are required for the development of PTLD-like tumours in severe combined immunodeficient (SCID) mice, suggesting an important role for T-cell help in the growth of PTLDs (Ref. 72).

Most PTLD cases are EBV^+ and many show a Lat III pattern of gene expression (Ref. 73). Thus, in many cases, PTLDs appear to represent the in vivo counterpart of in vitro immortalised LCLs and, by implication, are likely to be primarily driven by EBV. However, other forms of latency (i.e. Lat I and Lat II) are sometimes seen and EBV^- forms of PTLDs have been described, including some T-cell tumours. These EBV^- tumours tend to be monomorphic, present later than EBV^+ tumours and are more aggressive (Refs 74, 75). Interestingly, some of these tumours respond to a decrease in immunosuppression (Ref. 75).

HD

As early as 1966 MacMahon had proposed that HD might be caused by an infectious agent (Ref. 76). The first evidence that this agent might be EBV was provided by the detection of raised antibody titres to EBV antigens in HD patients when compared with other lymphoma patients (Ref. 77); furthermore, these raised levels preceded the development of HD by several years (Ref. 78). In addition, the relative risk of developing HD in individuals with a history of IM, relative to those with no prior history, was shown to range between 2.0 and 5.0 (Ref. 79). However, antibody titres to other herpesviruses, including human herpesvirus 6, are also raised in pre-diagnostic sera from HD patients (Ref. 80), although the antibody titres are higher in EBV^- as opposed to EBV^+ cases (Ref. 81). In addition, raised antibody titres to the EBV VCA do not predict EBV status in HD (Ref. 82). Immunosuppressed patients show elevations of all herpes antibodies rather than the selective elevation of EBV antibodies (Ref. 83), suggesting that depression of immunoregulation, rather than a specific disease

Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994

phenomenon, might be responsible for the elevated levels in HD patients.

EBV is regularly detectable in up to half of all HD tumours from developed countries and in a greater proportion of those cases arising in developing communities. In EBV-associated HD the viral genomes are found in monoclonal form, indicating that infection of the tumour cells occurred prior to their clonal expansion (Ref. 84). Although EBV normally persists throughout the course of HD and is also found in multiple sites of HD (Ref. 85), EBV^- HD arising as a relapse of a formerly EBV^+ HD has been reported (Ref. 86). These rare cases could represent a 'hit and run' mechanism of oncogenesis as suggested for BL (see above). However, fluorescence in situ hybridisation (FISH) analysis has found no evidence of integrated EBV genomes in EBV^- HD tumours (Ref. 87). In addition to the country of residence, the association of EBV with HD also varies with histological subtype, sex, ethnicity and age (Ref. 88). Although the incidence of HD is relatively low (1–3/100 000 per year) this tumour is not geographically restricted, making its association with EBV significant in worldwide health terms.

T-cell lymphomas

EBV has been linked to a proportion of T-cell lymphomas. In particular, a very high incidence of EBV genomes has been reported in sinonasal T-cell lymphomas occurring in Japanese, Chinese, Peruvian, European and United States patients; these tumours display peculiar phenotypic and genotypic features, including the frequent absence of T-cell antigens, expression of natural killer (NK) cell markers and the absence of T-cell receptor gene rearrangements. An intriguing aspect of EBV^+ T-cell lymphomas is the frequent detection of virus in only a fraction (5–50%) of the tumour cells, implying that EBV infection might have occurred subsequent to tumour development (Ref. 89). The documented increase in the proportion of EBV^+ tumour cells with T-cell lymphoma progression or recurrence suggests that the virus might provide an additional growth/survival advantage to the transformed T cells.

Most EBV-associated T-cell lymphomas are extranodal and have a cytotoxic phenotype, as demonstrated by immunohistochemical staining for T-cell intracytoplasmic antigen 1 (TIA-1) and granzyme B (Ref. 90), suggesting that these tumours might arise following EBV infection of CTLs during the killing of EBV^+ cells by virus-specific CTLs. Interestingly, EBV^+ B cells are frequently detectable in some EBV^- T-cell lymphomas, and in contrast to the EBV^+ small lymphocytes identifiable in some UNPC or HD tumours, these cells display a Lat III phenotype, suggesting that the presence of the neoplastic T cells might be a stimulus for EBV-induced B-cell transformation (Ref. 91). A further possibility is that the EBV^+ B cells present in T-cell lymphomas might contribute to the growth of the neoplastic T cells, possibly by the secretion of cytokines or perhaps more directly by interaction of their costimulatory molecules with partner molecules on T cells.

Viral gene expression in EBV-associated tumours

As mentioned earlier, BL tumour biopsy cells and early-passage BL cell lines show a Lat I pattern of viral gene expression (Ref. 92). This is characterised by abundant EBER transcription and the selective expression of EBNA1, in the absence of the other EBNA and LMP proteins, by transcription from the Qp promoter rather than the Cp or Wp promoters. In culture, BL cells grow as a carpet of dispersed cells, in contrast to the multicellular aggregates that are observed in LCL cultures. Furthermore, BL cells display a distinct cell-surface marker phenotype characterised by expression of CD10 (CALLA) and CD77 (BLA), but little or no expression of the cellular activation antigens and adhesion molecules that are regularly expressed at high levels in LCLs (Ref. 92). The Lat I form of latency observed in BL cell lines is not always stably maintained in vitro and, on serial passage, a drift to a Lat III pattern of gene expression can be observed concomitant with a change in the cellular phenotype towards that seen in LCLs (Ref. 92). A similar effect might occur in vivo as EBNA2 and LMP1 can occasionally be detected in a small proportion of BL cells in biopsy material (Ref. 93). This highlights the possibility that operational definitions of EBV latencies derived from cell lines in vitro might not readily apply to tumours in vivo.

Another form of EBV latency, Lat II, is characterised by selective expression of the Qp-driven EBNA1 mRNA, the LMP1, 2A and 2B transcripts, and the EBERs. This form of infection was first identified at the protein level in UNPC

Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994

biopsies (Ref. 94), but is clearly not restricted to epithelial cells because it is also observed in EBV$^+$ cases of HD and in certain EBV$^+$ T-cell lymphomas (Refs 95, 96).

All three forms of EBV latency can be interconverted in somatic cell hybrids between LCLs and either BL cells or certain nonlymphoid lines (Ref. 97). These transitions are influenced by the cell phenotype of the resultant hybrids, thus emphasising the complex interplay between cellular factors and the resident pattern of EBV latent gene expression.

Although the EBERs are apparently always expressed during latent infection, two recent studies suggest the possibility of EBER$^-$ forms of latency. In the first of these, the detection of EBV DNA [by polymerase chain reaction (PCR) and Southern blotting] and EBNA1 protein (by immunohistochemistry) in some invasive breast tumours was reported in the absence of EBER expression (Ref. 98). In the second study, EBV was reported in a series of hepatocellular carcinomas (HCCs), again in the absence of EBER expression (Ref. 99). Furthermore, a single terminal fragment of EBV DNA was identified in these tissues, suggesting that the EBV$^+$ cells in HCC represent clonal proliferations. Western blotting and reverse-transcription–PCR also demonstrated expression of EBNA1 and the BamA transcripts in HCC lesions. Although the results of these two studies clearly require confirmation, they suggest the possibility that tumours displaying novel patterns of EBV latency might be missed by conventional screens using in situ hybridisation to detect the EBERs.

EBV strain variation and virus-associated tumours

There are two major types of EBV isolate, originally referred to as A and B and now called types 1 and 2, which appear to be identical over the bulk of the EBV genome but show allelic polymorphism (with 50–80% sequence homology depending on the locus) in a subset of latent genes (those encoding EBNA-LP, EBNA2, EBNA3A, EBNA3B and EBNA3C) (Ref. 100). A combination of virus isolation and sero-epidemiological studies suggest that type 1 virus isolates are predominant (but not exclusively so) in many Western countries, whereas both types are widespread in equatorial Africa, New Guinea and perhaps certain other regions (Ref. 101).

In vitro studies suggest that type 1 isolates are more potent than type 2 in achieving B-cell transformation in vitro; LCLs transformed by type 2 virus characteristically show much slower growth, especially in early passage. In addition to this broad distinction between EBV types 1 and 2, there is also minor heterogeneity within each virus type, which is most easily detected as variation in the size of the EBNA proteins. These differences have been used to trace virus transmission within families and from transplant donors to recipients. The balance of evidence to date suggests that most healthy individuals are infected with only one virus type, although a small but significant percentage of healthy virus carriers do harbour multiple, perhaps sequentially acquired, EBV strains (Ref. 102). By contrast, most immunologically compromised patients are infected with multiple EBV strains.

Therapeutic strategies for EBV-associated tumours

EBV-associated tumours represent a significant proportion of all malignancies, and therapy that specifically targets EBV in these tumours represents a potentially safe and effective form of treatment. This approach could be particularly beneficial in situations where conventional treatment is either ineffective or associated with substantial systemic toxicity.

CTL-based therapies

Current research into novel therapies for EBV-associated tumours is predominantly focused on developing immunological approaches such as adoptive transfer of EBV-specific CTLs. The CTL response to EBV is a classic virus-specific response (CD8$^+$, class I-restricted), and, in any one individual, EBV-specific CTL responses are a composite of reactivities against different viral antigens. The majority of CTL responses that have been mapped to date are directed towards EBNA3A, 3B and 3C, whereas CTLs recognising EBNA2, EBNA-LP, LMP1 and LMP2 appear to be less frequent and act in the context of a limited number of MHC restrictions. Therefore, CTL therapy is likely to be most effective against those tumours expressing the Lat III pattern. Thus, transfer of donor peripheral blood mononuclear cells that contain EBV-specific T cells to patients developing a PTLD following allogeneic BMT has been shown to result in disease regression in some cases (Ref.

Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994 ©2001 Cambridge University Press

103). This method has been refined by the infusion of EBV-specific CTLs expanded in vitro from donor cells and given either at the time of tumour development or prophylactically (Ref. 104). Similar approaches are also possible in recipients of solid-organ transplants. However, in many of these cases, the donor cells are not available and the tumours usually arise in the recipient's cells. In these circumstances, CTLs must be generated from the patient's own cells, before the immunosuppression therapy is started. For practical reasons this might not always be possible, and it is also time consuming. An alternative strategy is to generate a panel of CTLs grown from healthy donors; HLA-matched CTLs can then be selected from this 'CTL bank' for infusion (Ref. 105).

EBV-specific CTLs expanded in vitro contain significant numbers of EBV-specific $CD4^+$ CTLs. Not only are these $CD4^+$ T cells cytotoxic for autologous LCLs, an effect mediated in part by CD95–CD95-ligand interactions (Ref. 106), but there is also evidence that in vitro reactivation of EBV-specific $CD8^+$ CTLs is dependent on their presence (Ref. 107). Interestingly, the ability of $CD4^+$ T cells to inhibit the long-term growth of autologous LCLs in vivo was recently shown to vary considerably between different donors (Ref. 108). Although, as mentioned earlier, endogenous EBNA1 protein cannot be processed through the MHC class I pathway, dendritic cells are able to present EBNA1 from dying EBV^+ cells to $CD4^+$ T cells (Ref. 109).

A potential hazard of the use of CTL therapy is the development of graft-versus-host disease (GVHD), because CTL lines can contain alloreactive CTLs. In addition, on rare occasions, CTL infusions can cause inflammation in patients with bulky or infiltrative disease (Ref. 110). Resistance to the infused CTLs through mutations of EBV epitopes recognised by the CTLs has also been reported (Ref. 111).

The general applicability of CTL-based therapies relies on the ability of the immune response to recognise and destroy tumour cells efficiently and might be compromised in situations where EBV^+ tumour cells can evade immunosurveillance. Thus, in HD, it has been suggested that IL-10 production by EBV^+ Hodgkin/Reed–Sternberg (HRS) cells might be responsible for the failure of these cells to be recognised by EBV-specific CTLs, pointing to the existence of immunosuppression that is limited to the vicinity of the tumour (Ref. 112). This is supported by the observation that tumour-derived T cells from EBV^- HD show EBV-specific cytotoxicity, whereas corresponding lymphocytes from EBV^+ HD lesions do not (Ref. 113). Despite these potential caveats, the use of donor-derived EBV-specific CTLs has been investigated in the treatment of EBV^+ HD patients (Ref. 114). In this study, EBV-specific CTLs could be generated from patients with advanced HD, albeit at lower frequency than normal controls. EBV-specific CTLs survived and had antiviral activity in vivo. These results provide some encouragement for the pursuit of CTL therapy for EBV-associated HD. However, additional work is required to establish whether the microenvironment of EBV^+ HD is likely to compromise immunotherapeutic strategies targeted at EBV^+ HD patients.

Other approaches

An alternative approach to the treatment of EBV-associated tumours is the use of gene therapy to deliver cytotoxic proteins or proteins that interfere with EBV gene function. In one study, the virus Cp promoter was used to direct expression of a suicide gene (thymidine kinase) to LCLs (Ref. 115); following ganciclovir treatment, EBNA2-expressing cells were selectively killed. In a related approach, induction of the EBV lytic cycle by either gamma-irradiation or sodium butyrate resulted in the expression of virus-encoded kinases that were able to phosphorylate ganciclovir and zidovudine (AZT) into their active forms, thereby killing the induced cells (Ref. 116). Pharmacological induction of the lytic cycle in latently infected tumour cells using demethylating agents such as 5-azacytidine is also suggested as a possible therapeutic option (Ref. 117).

Another possible therapy for EBV-induced lymphoproliferative disease and other $LMP1^+$ tumours is the use of a non-degradable IκBα mutant, which inhibits the NF-κB pathway, resulting in apoptosis in LCLs (Ref. 118). This approach might also be useful for the treatment of HD in general because constitutive NF-κB activation has been reported to be a consistent feature of this tumour, irrespective of EBV status. LMP1 has also been targeted using a single-chain anti-LMP1 antibody. Intracellular expression of this antibody markedly reduced LMP1 protein levels, which correlated with a marked reduction

Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994

of Bcl-2 expression in EBV-transformed B cells. Since Bcl-2 can protect cells from drug-induced apoptosis, reduction of Bcl-2 levels led to an increased sensitivity of these cells to drug-induced cell death (Ref. 119). A further study using low-dose hydroxyurea, which can result in EBV episomal loss in vitro, showed good responses in two patients with EBV$^+$ AIDS-associated primary central nervous system lymphoma (Ref. 120).

Paradoxically, the potential to use EBV as a gene therapy vector is being investigated. This approach involves the incorporation of therapeutic genes into a transformation-defective EBV that has the ability to infect cells in the usual way but lacks genes essential for transformation (e.g. EBNA2, LMP1). Such EBV-derived vectors have the potential to treat cancers such as B-cell lymphomas or leukaemias that are derived from cell types that can normally be infected with EBV (Ref. 121).

No vaccine is currently available for EBV and yet the development of such a vaccine could either protect individuals against primary infection (and hence presumably reduce the burden of EBV-associated cancers) or could be used to boost immunity in patients already harbouring an EBV-associated tumour. A variety of vaccines aimed at preventing primary infection are already in clinical trials. These include the testing of a gp350-based subunit vaccine (Ref. 122); a randomised double-blind Phase I study in 67 young adults indicated that the vaccine appeared to be safe and well tolerated in seronegative individuals. Although the study was not designed to evaluate efficacy, laboratory tests indicated evidence of a neutralising antibody response in vaccine recipients. Phase II trials in patients at risk of developing PTLDs are currently in development. Alternative vaccine approaches aimed at generating a therapeutic CTL response in patients with virus-associated tumours are already in development and these include peptide-based vaccines (e.g. to EBNA3A) as well as the use of recombinant viruses (e.g. modified vaccinia virus Ankara that expresses LMP2A).

Conclusions

Substantial evidence implicates EBV in the pathogenesis of tumours arising in both lymphoid and epithelial tissues. The virus appears to adopt different forms of latent infection in different tumour types, reflecting the complex interplay between EBV and the host cell environment. Another important factor influencing EBV gene expression is the immune response, in that those viral latent proteins to which immunodominant CTL responses are directed, namely the EBNA3 family of proteins, are downregulated in virus-associated tumours arising in overtly immunocompetent individuals. EBNA1, an essential protein for the maintenance of EBV infection that is expressed in all currently known forms of EBV latency, has evolved to evade immunosurveillance by developing a strategy that prevents the protein being processed through the MHC class I pathway. Studies of the function of individual EBV latent genes have highlighted the ability of these proteins to target specific cell signalling pathways. Therefore, as clearly demonstrated by studies with proteins encoded by other viruses, a knowledge of the functions of EBV latent proteins will not only be relevant to our understanding of the role of the virus in transformation but also help to elucidate the mechanisms regulating cell growth, survival and differentiation. It is hoped that this work will also provide novel approaches to therapy.

Acknowledgements and funding

The Cancer Research Campaign, Leukemia Research Fund and the Medical Research Council, UK support work in the authors' laboratory. Dr R. Tierney is thanked for help with the figures. We are grateful to Dr Andy Morgan (University of Bristol, UK) and Dr John Arrand (University of Manchester, UK) for their valuable peer review.

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Further reading, resources and contacts

The journal Epstein–Barr Virus Report covers all aspects of Epstein–Barr virus (EBV) infection.

http://www.leeds.ac.uk/lmi/ebv/ebvmain.html

The National Center for Infectious Diseases provides background information on EBV infection.

http://www.cdc.gov/ncidod/diseases/ebv.htm

BioSpace News on Epstein–Barr virus accesses relevant press releases and abstracts.

http://www.biospace.com/news_rxtarget.cfm?RxTargetID=82

The Australian Academy of Science Nova service posted an accessible introduction to Epstein–Barr virus ('Kissing the Epstein–Barr virus goodbye?') aimed at students.

http://pandora.nla.gov.au/nph-arch/O1998-Nov-20/http://www.science.org.au/nova/026/026key.htm

Introductory text on tumour virology:

Baumforth, K.R.N., Murray, P.G. and Young, L.S. (2001) Tumour viruses and human cancer. In The Biology of Disease (2nd edn) (Phillips, J., Murray, P. and Kirk, P., eds), pp. 204-213, Blackwell Science, Oxford, UK

Publisher site: http://www.blackwell-science.com/~cgilib/bookpage.bin?File=5955

Case study of post-transplant lymphoproliferative disease:

Ambinder, R.F. (2001) Case Study 12 – Fever and cervical lymph node enlargement. In The Biology of Disease (2nd edn) (Phillips, J., Murray, P. and Kirk, P., eds), pp. 246-247, Blackwell Science, Oxford, UK

Publisher site: http://www.blackwell-science.com/~cgilib/bookpage.bin?File=5955

Features associated with this article

Figures

Figure 1. The Epstein–Barr virus (EBV) genome (fig001lyb).
Figure 2. Epstein–Barr virus (EBV) infection in normal healthy virus carriers (fig002lyb).

Citation details for this article

Paul G. Murray and Lawrence S. Young (2001) Epstein–Barr virus infection: basis of malignancy and potential for therapy. Exp. Rev. Mol. Med. 15 November, http://www-ermm.cbcu.cam.ac.uk/01003842h.htm

Accession information: (01)00384-2a.pdf (short code: txt001lyb); 15 November 2001
ISSN 1462-3994