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Epstein-Barr virus gene expression in post-transplant lymphoproliferative disorders

Martin Rowe[1], Gerald Niedobitek[2], Lawrence S. Young[3]

[1] Department of Medicine, University of Wales College of Medicine, Tenovus Building, Heath Park, Cardiff, CF4 4XX, UK
[2] Pathologisches Institut, Friedrich-Alexander Universitat, Krankenhausstr. 8–10, D-91054 Erlangen, Germany
[3] Institute for Cancer Studies, University of Birmingham Medical School, Birmingham B15 2TA, UK

Introduction

The Epstein-Barr virus (EBV) contains a large, double-stranded DNA genome with the potential to express more than 80 genes [24]. The structural organisation of these genes is complex, and the regulation of their expression is to a large extent dependent upon host cell transcription factors. During lytic virus productive infection, most of the EBV genes are expressed. However, entry into the lytic cycle is tightly regulated and, at any given time, most EBV-infected cells are non-productive and express only a limited set of 'latent' genes. The notable exception to this general rule is found in oral hairy leukoplakia in AIDS patients, where the the epithelial cells appear to be fully permissive for EBV lytic cycle [71]. Before dealing specifically with the EBV gene expression in post-transplant lymphoproliferative disorders (PTLD), we will briefly review the EBV gene products and the different EBV:host cell interactions that can occur.

EBV genes

The so-called 'latent' genes of EBV are defined as the limited set of genes that are expressed in lymphoblastoid cell lines (LCL) established following experimental infection of normal peripheral blood B lymphocytes with EBV in vitro. In the context of EBV-associated malignancies, the latent genes have attracted considerably more interest than the lytic cycle genes since it is the cooperative action of several of the latent gene products that confers upon EBV the ability to transform normal resting B cells into permanently growing LCL [24]. LCL are generally non-permissive for EBV replication and express two abundant non-polyadenylated RNA species (EBERs 1 and 2), together with mRNA species encoding six EBV nuclear antigens (EBNA), and three latent membrane proteins (LMP). Multiply-spliced transcripts from the *Bam*HI A region of the viral

Correspondence to: M. Rowe

Table 1. EBV genes expressed in latent infection of normal B cells in vitro

Viral gene product	(alternative nomenclature)[a]	Characteristics and functions
EBNA1		Nuclear protein, essential for viral plasmid replication and maintenence of the episome. Binds to specific DNA sequences in *ori-P*. Transcriptional activator
EBNA2		Nuclear protein, essential for B cell transformation. Transcriptional activator that associates with sequence-specific DNA-binding cellular factors such as RBP-Jκ and PU.1. EBNA-2 activates viral gene expression (e.g. LMP-1 and LMP-2) as well as cellular gene expression (e.g. CD21 and CD23)
EBNA3A	(EBNA3)	Nuclear protein, essential for B cell transformation. Binds RBP-Jκ and antagonises EBNA-2/RBP-Jκ function
EBNA3B	(EBNA4)	Nuclear protein, not essential for B cell transformation. Binds RBP-Jκ and antagonises EBNA-2/RBP-Jκ function
EBNA3C	(EBNA6)	Nuclear protein, essential for B cell transformation. Binds RBP-Jκ transcription factor and antagonise EBNA-2/RBP-Jκ function
EBNA-LP 'Leader Protein'	(EBNA5)	Nuclear protein, not essential, but greatly enhances efficiency of B cell transformation. Cooperates with EBNA2 during primary infection of resting B cells to induce the G_0–G_1 transition. Also interacts with Rb tumour-suppressor gene product
LMP1		Membrane protein product of oncogene essential for B cell transformation. Regulates cell growth, enhances cell survival, and increases the antigen-presenting capacity of cell. Plieotropic effects on cellular genes are mediated in part by activation of the NF-κB transcription factor, and by the SEK-JNK and ras-MAPK kinase pathways
LMP2A	(TP1)	Membrane protein, not essential for B cell transformation. Blocks switch from the latent state into lytic cycle, and can modulate transmembrane signal transduction. Substrate for *src* family tyrosine kinases
LMP2B	(TP2)	Membrane protein, not essential for B cell transformation. LMP-2B is an alternatively spliced gene product that is identical to LMP-2A except that it lacks the amino-terminal kinase-interacting domains. Its function is unclear, but it may complex with and modulate LMP-2A function
EBER-1 and EBER-2		Abundantly expressed small, non-polyadenylated RNAs that are non-essential for B cell transformation. Associate with nuclear proteins, and may regulate activity of specific protein kinases
BARF transcripts		Multiple-spliced rightward transcripts from the *Bam*HI A region of the genome, that are non-essential for B cells transformation. The protein products are poorly characterised, and it is not conclusive that proteins are expressed from these transcirpts in LCL

[a] There is no agreed standard nomenclature for the EBV-encoded proteins, and two versions are in common usage. Usage of the nomenclature in parentheses is less widespread, being confined mainly to certain European research groups

genome are also detected in LCL but the protein(s) endoded by these mRNAs are, as yet, poorly characterised. The EBNA1, EBNA2, EBNA3A, EBNA3C, and LMP1 latent genes are essential for the ability of EBV to effect growth transformation of normal B cells, while EBNA-LP is not essential but does significantly enhance transformation [24]. The latent gene products and their functions, are summarised in Table 1.

Some LCL are partially permissive for virus production in vitro, and contain a minor subpopulation of cells in which the lytic cycle genes are expressed in a temporal fashion, i.e. initial induction of key lytic cycle genes sets off a cascade of events which leads to expression of further lytic cycle genes. Historically, and similarly to other herpesviruses, the lytic cycle antigens were classified into groups according to whether their expression was independent of DNA synthesis, i.e. "early antigens " (EAs), or blocked by inhibitors of DNA synthesis, i.e. "late antigens". The EA genes encode non-structural proteins which include transcriptional activators and enzymes, while many of the late antigens are structural proteins that are incorporated into the virus nucleocapsid (i.e. virus capsid antigens, VCA) or the virus membrane envelope (i.e. membrane antigens, MA). Not all the EBV lytic cycle gene products have been characterised, but antigens representative of the key stages of lytic cycle have been identified and monoclonal antibodies to some of these antigens are available. The lytic cycle genes are generally designated according to the *Bam*HI restriction fragment in which the open reading frame (ORF) starts, and the leftward or rightward direction of transcription from the genome. Thus, the important immediate-early switch protein that initiates the cascade of EBV gene expression in lytic cycle, is encoded by the BZLF1 gene (the ***Bam*** HI **Z** restriction fragment of EBV, beginning with the **L**eftward OR **F**rame number **1**). The gene products of BZLF1, BMLF1 and BRLF1 are all important transcriptional regulators in early lytic cycle. Other EA gene products include a dUTPase (BLLF3), an alkaline exonuclease (BGLF5), a thymidine kinase (BXLF1), a DNA polymerase (BALF3), and a bcl-2 homologue (BHRF1) with anti-apoptotic function. Late gene products include an IL-10 homologue (BCRF1), the gp340/220 membrane envelope glycoprotein (BLLF1) that is the ligand for the CD21 EBV receptor, the gp85 membrane envelope glycoprotein (BXLF2) involved in membrane fusion, and the 150-kDa major VCA component (BcLF1).

Sequence variations in different isolates of EBV

Many of the EBV latent genes show significant sequence variation between different virus isolates, the most remarkable being in the EBNA2 gene where sequence variations have been used to define two families of EBV (type 1 and type 2) between which the EBNA2 proteins show only about 53% amino acid sequence identity [1, 5]. The two types of EBV show important functional differences, with type 1 EBV strains demonstrating a greater efficiency for growth transformation of normal B cells than do type 2 EBV strains [48]. The EBNA3A, EBNA3B and EBNA3C proteins also show significant, but lesser, variations in their sequences which normally segregate with the type 1 and 2 families of EBV defined by EBNA2 sequences [52, 56]. Nevertheless, while the EBNA3 proteins play essential roles in B cell transformation, it appears that the different transforming abilities of type 1 and type 2 EBV strains are largely attributable to the different EBNA2 proteins [24].

Monoclonal antibodies have been generated that recognise EBNA2 proteins from both virus families, while other EBNA2-reactive monoclonal antibodies are type specific [27, 69]. Monoclonal antibodies that have thus far been generated to EBNA-LP and to the EBNA3 family have been of limited use in analysing EBV gene expression in biopsy material, either because they only react with a subset of sequence variants, or because they are weakly reactive, at best, with their antigens in histological specimens [8, 33, 52]. A reliable method for determining the EBV type in biopsy material

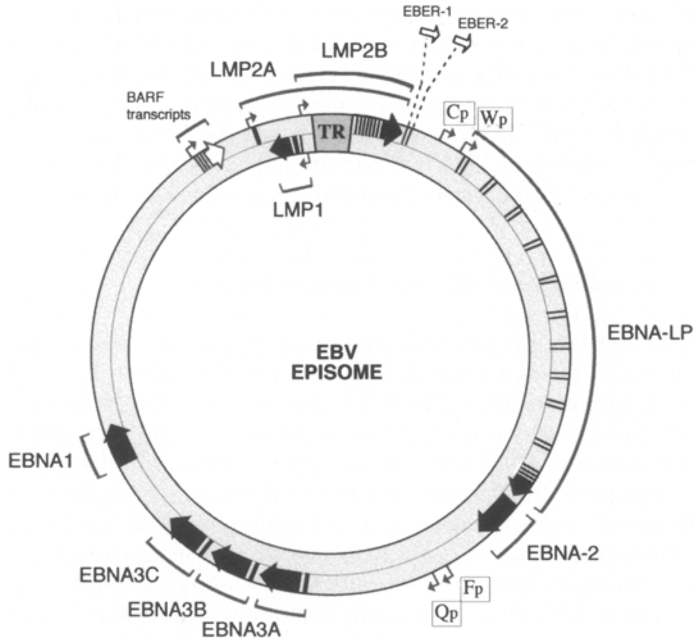
involves polymerase chain reaction (PCR) analysis of EBNA2 and EBNA3C sequences with appropriate type-specific primers and/or probes.

The prevalance of type 1 and type 2 EBV strains varies with geographical location. Thus, in Western communities greater than 90% of EBV isolates are type 1, whereas type 1 and type 2 EBV isolates show roughly equal prevalence in some parts of equatorial Africa [12, 67, 70, 72]. A notable exception to this general observation is that the incidence of type 2 EBV amongst HIV-positive homosexual males in Western communities appears to be three to four times higher than in the general population; this is not a consequence of immunosuppression per se since HIV-positive hemophilic cohorts do not show the same elevated incidence of type 2 EBV [68]. Generally, the prevalence of EBV type 1 and type 2 isolates in EBV-associated tumours usually reflects the prevalence of the respective EBV types in the healthy population from the same geographic location [21, 70]. Most healthy individuals carry just one strain of EBV, but immunosuppression induced in allograft recipients, or acquired following HIV infection, increases the incidence of multiple type 1 and/or type 2 EBV infections [12, 67, 68]. In HIV-positive patients, the multiple EBV infections appear to be acquired by natural routes [68], but in allograft recipients additional strains of EBV can also be derived from the allograft itself [17]. The contribution of type 1 versus type 2 EBV infection to the development of PTLD has been analysed in several studies, and the consensus is that a single virus strain (usually type 1) is detectable in the lesions [9]. While earlier studies suggested that mixed virus infection occurs in AIDS-related lymphomas, more recent work indicates that co-infection can be detected in EBV-positive lymphadenopathies but not in the monoclonal lymphomas that subsequently develop [41].

Recently, attention has focussed on sequence variants of the LMP1 oncogene. Although LMP1 is generally well conserved amongst different EBV isolates, typically showing greater than 95% amino acid (aa) sequence identity amongst different EBV isolates, there has been considerable interest in the possibility that natural sequence variation may affect the function of the LMP1 and, thus, influence the development of EBV-associated disease. This interest was fuelled by the discovery of a deletion variant (del-LMP1) that was originally identified in tumors of Chinese patients with undifferentiated nasopharyngeal carcinoma, and which is characterised by a 30-base pair (bp) deletion which removes a 10-aa sequence near one of the two known functional domains of the protein [4, 18, 19]. The del-LMP1 variant was subsequently shown to be the dominant LMP1 sequence in Chinese populations in south east Asia, and has been detected at lower frequencies in other populations worldwide [34, 60]. The two prototype del-LMP1 variants, CAO and 1510, are widely accepted as being more oncogenic than the standard B95.8 EBV LMP1 gene in rodent fibroblasts and in a human epithelial cell line, and the deletion itself appears to be sufficient to con-

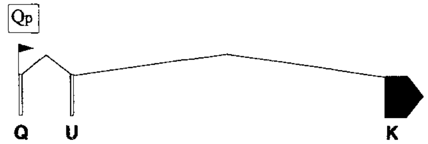
Fig. 1 A, B. Organisation and expression of the Epstein-Barr virus (EBV) latent genes. **A** Schematic representation of the EBV genome, showing the location of the coding exons for the EBV genes expressed in latently infected EBV-transformed lymphoblastoid cell lines. Within the virus capsid the viral genome is a linear double-stranded DNA, but following latent infection of normal B cells the genome circularises via the terminal repeat (TR) regions. The major promoters for the latent genes are indicated by *bent arrows* showing the direction of transcription. **B** The exon structure of spliced EBNA mRNA differs in latency I/II and latency III forms of infection. The *filled blocks* represent coding exons, while the *hollow blocks* represent the non-coding exons. The *flags* indicate the promoters used to generate the long primary transcripts; *Cp*, *Wp*, and *Qp labels* indicate the *Bam*HI restriction fragments in which the EBNA promoters reside. Similarly, the *labels Y,Q,U*, and *K* indicate the the *Bam*HI restriction fragments in which the labelled exons reside ▶

A **Organisation of EBV Latent Genes**

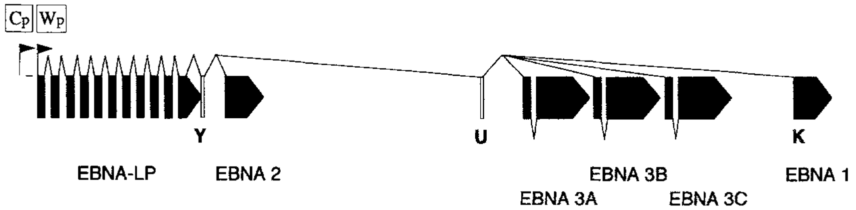


B **Structure of EBNA mRNA in different forms of Latency**

Latency I and Latency II EBNA mRNA



Latency III EBNA mRNA



fer enhanced tumourigenicity to LMP1 in rodent fibroblasts [31]. In the context of PTLD, Kingma et al. [25] reported a correlation between the of the presence of del-LMP1 variants with the aggressiveness of tumours in lymphoproliferative disease. However, a subsequent study found that the presence of del-LMP1 amongst 58 lesions from 36 heart and kidney organ transplant patients with lymphoproliferative disorders did not correlate with the aggressiveness of the lesions or with the progression of disease [59]. Furthermore, laboratory investigations do not support a major role for the deletion in affecting LMP1 function in lymphoid cells [20, 57]. Therefore, a role for LMP1 sequence variations in the pathogenesis of PTLD remains speculative.

Different patterns of EBV latent gene expression

Until the late 1980s it was widely assumed that the virus:host cell interaction displayed in cultured LCL was a model for all latent EBV infections in vivo. This view was first condradicted by studies on Burkitt's lymphoma (BL) cells, where fresh biopsy material, or early passage tumour-derived lines in culture, were shown to display a remarkably restricted pattern of EBV gene expression [50]. Even with the subsequent discovery of new latent genes, EBV gene expression in BL tumours generally appears to be restricted to just one of the six nuclear antigens, EBNA1, along with the BARF transcripts and the EBERs [14, 61], although rare cells within BL tumours may display a broader pattern of latent gene expression [37]. It was subsequently shown that many nasopharyngeal carcinomas and all EBV-positive Hodgkin's disease tumour cells display an intermediate pattern of latent gene expression; again expressing only EBNA1 of the nuclear antigens, but also expressing LMP1, LMP2, and the BARF transcripts [47]. The three patterns of EBV gene expression were defined as latency I (e.g. BL), latency II (e.g. Hodgkin's disease), and latency III (e.g. LCL) [54]. However, while this nomenclature has been useful for describing forms of latency in vitro, it has become apparent that it is too rigid for describing the range of latency patterns in vivo. It is probably more correct to consider that latency I and latency III represent two extremes of a spectrum of possible latent virus:host cell interactions, and that latency II is just one of the possible intermediate combinations. These intermediate forms may be transitory, as in the in vitro "drift" of BL lines from latency I to latency III, or stable as in the malignant cells of Hodgkin's disease which regularly display a latency II form of infection. Furthermore, heterogeneous patterns of latent EBV gene expression have been identified at the single cell level within individual tumours, both in BL and in PTLD, thus highlighting the inadequacy of the operational definitions of virus latency as defined in vitro [37, 43, 45].

Because of its value in PCR analysis of EBV gene expression, it is worth mentioning here that in latency III infections, the different EBNA proteins are the products of individual mRNA derived by differential splicing from the same long primary transcript initiated from one of two promoters located in the *Bam*HI C or W regions (Cp or Wp) of the EBV genome (Fig. 1). In contrast, in latency I and latency II infections the EBNA1 gene is transcribed from a downstream promoter (Qp) located in the *Bam*HI Q region; in lytic cycle, a neighbouring promoter (Fp) is used [40, 58]. Consequently, as depicted in Fig. 1, EBNA1 expression involves different splicing patterns and exon usage in latency III infections (i.e. a Y-U-K splice) compared to latency I/II and lytic cycle (Q-U-K splice). This has been exploited in reverse transcription (RT)-PCR analyses where sets of 5′ and 3′ primers have been designed to distinguish the different patterns of gene expression.

In cultured B cell models of EBV infection, entry into lytic cycle from the latency III state appears to result in down-regulation of all the EBNAs except EBNA1, while of the membrane proteins, at least LMP1 remains expressed. When entry into lytic cycle is induced from latency I, expression of both LMP1 and LMP2 is induced along with the EA genes [54]. It remains to be seen whether these observations also hold true for EBV-associated malignancies in vivo.

Association between cellular phenotype and EBV gene expression

Investigation of biopsy specimens of BL cells and the tumour-derived cell lines, showed that the pattern of EBV latent gene expression correlated with the cellular phenotype [14, 32, 51]. Thus, early passage BL lines with a true latency I type of EBV infection, express cell surface markers typical of normal germinal centre cell populations, e.g. CD10, CD38 and CD77, that are rarely expressed on LCL displaying a latency III type of infection. Conversely, LCL express high levels of activation markers, e.g. CD23, CD30, CD39 and CD70, and cell intercellular adhesion molecules, e.g. ICAM-1 and LFA-3, that are expressed only weakly, if at all, on latency I BL lines. Upon continued culture, many BL lines drift phenotypically and acquire a pattern of cell surface markers resembling that of normal LCL, and they concurrently show a broadening of viral gene expression to a latency III type of infection [14, 51].

Gene transfection experiments with latency I BL lines and EBV-negative BL lines showed that individual EBV latent genes, most notably LMP1 and EBNA2, could induce many of the cell phenotype changes that occur when BL lines spontaneously drift from a latency I to a latency III form of infection [64]. To some extent, therefore, EBV gene expression regulates cellular gene expression during the phenotypic changes associated with the latency I to latency III drift in EBV-positive BL lines. However, cellular factors probably determine whether the cell retains a latency I form of infection or switches other key latent genes. For example, while EBNA2 can induce expression of LMP1 and LMP2 in lymphoid cells [65, 73], it is not clear what first induces EBNA2 expression; cell phenotype-dependent demethylation of the EBV genome is a possibility. Furthermore, while the LMP1/2 genes are frequently expressed in epithelial cells, this is always in the context of a latency II pattern of transcription, i.e. in the absence of EBNA2 expression. This indicates that LMP1/2 expression can be regulated by cellular factors as well as by EBNA2. Another example of EBV gene expression being regulated by cellular factors is the differentiation-associated expression of lytic cycle genes in oral hairy leukoplakia lesions in AIDS patients [71].

Cytotoxic T lymphocyte immune control of EBV-infected cells

More than 90% of adults in all communities worldwide carry EBV as a persistent and asymptomatic infection. The potentially pathogenic consequences of EBV infection are efficiently kept in check by strong cell-mediated immune responses, of which HLA class I restricted and EBV-specific cytotoxic T lymphocytes (CTL) are a major component; hence the elevated risk of lymphoproliferative disease in immunosuppressed patients [47]. The immunodominant target antigens for EBV-specific CTL are the EBNA3A, 3B, 3C family of nuclear antigens [11, 22, 36]. LMP1-derived peptides are not dominant targets for CTL responses [23], but the LMP1 protein has an important

function in upregulating various components of the cellular pathways for antigen presentation, thus enhancing the immunogenicity of other viral antigens [55]. LMP2 is not a dominant antigen for CTL, but may be important in immunotherapy because LMP2-derived CTL peptide epitopes are presented via the HLA-A2 restriction determinant represented in a large proportion of the population [29]. EBNA1 has a special structural feature that prevents processing of CTL target epitopes so that EBNA1-specific CTL cannot recognise EBV-transformed LCL [2, 30]. In immunocompetent individuals, therefore, EBV-infected cells displaying a latency III type of infection should be susceptible to recognition and lysis by EBV-specific CTL, whereas cells displaying a latency I form of infection will be immunologically silent to EBV-specific CTL.

EBV gene expression in PTLD

PTLDs represent a spectrum of diseases ranging from benign infectious mononucleosis-like lymphoproliferations to monomorphous high-grade malignant lymphomas which are morphologically indistinguishable from similar lymphomas occurring in immunocompetent individuals. This heterogeneity is apparent at the genotypic level with some PTLDs representing polyclonal and others monoclonal lymphoproliferations. This heterogeneity is believed to reflect a progression from early benign polyclonal disease to late malignant lymphoma, a model that has received support from several molecular studies [7, 26, 66].

Most PTLDs have been shown to be EBV associated, and initial analyses of EBV latent gene expression in PTLDs identified a latency III type of infection [69]. This is illustrated by the histological results shown in Fig. 2 of sections of a transplanted kindney in a patient with polymorphic B cell PTLD. The latency III pattern of EBV protein expression is also reflected at the transcriptional level since detection of C/Wp-derived EBNA mRNAs as well as of LMP1 and LMP2A mRNAs has been reported in PTLDs [43]. This has been taken as evidence that PTLDs represent an EBV-driven outgrowth of B lymphocytes which is facilitated by the iatrogenic suppression of EBV-specific T cell immunity. In support of this contention is the similar latency III pattern of EBV latent gene expression observed in the PTLD-like lesions that develop in cottontop tamarins challenged with a tumourigenic dose of EBV [69] and in SCID mice inoculated with peripheral blood lymphocytes from a EBV-positive donors [53]. However, it is now clear that more restricted patterns of EBV latency are frequently observed in PTLDs, and cases with a latency II or even a latency I pattern of EBV latent protein expression have been described [6, 13, 46, 62]. While a consistent correlation between the morphological and genotypic PTLD features and the prevalent pattern of EBV gene expression has not emerged [46, 62], it appears that the monomorphic malignant lymphomas developing in transplant patients usually display more restricted forms of EBV latency, particularly when showing a predominant plasma cell differentiation or when corresponding morphologically to BL [6, 39].

In addition to differences in EBV gene expression between PTLD patients, there is often heterogeneity of viral gene expression within single PTLD lesions. Thus, more detailed immunohistochemical analyses at the single cell level have shown that, even in those cases in which expression of EBNA2 and of LMP1 can be detected consistent with a latency III, the proportion of cells expressing these two proteins is highly variable [6, 39, 43, 46] (Figs 2B, 3A). Oudejans et al. have demonstrated that the number of EBV-infected cells is usually greater than that of cells expressing EBNA2 and/or

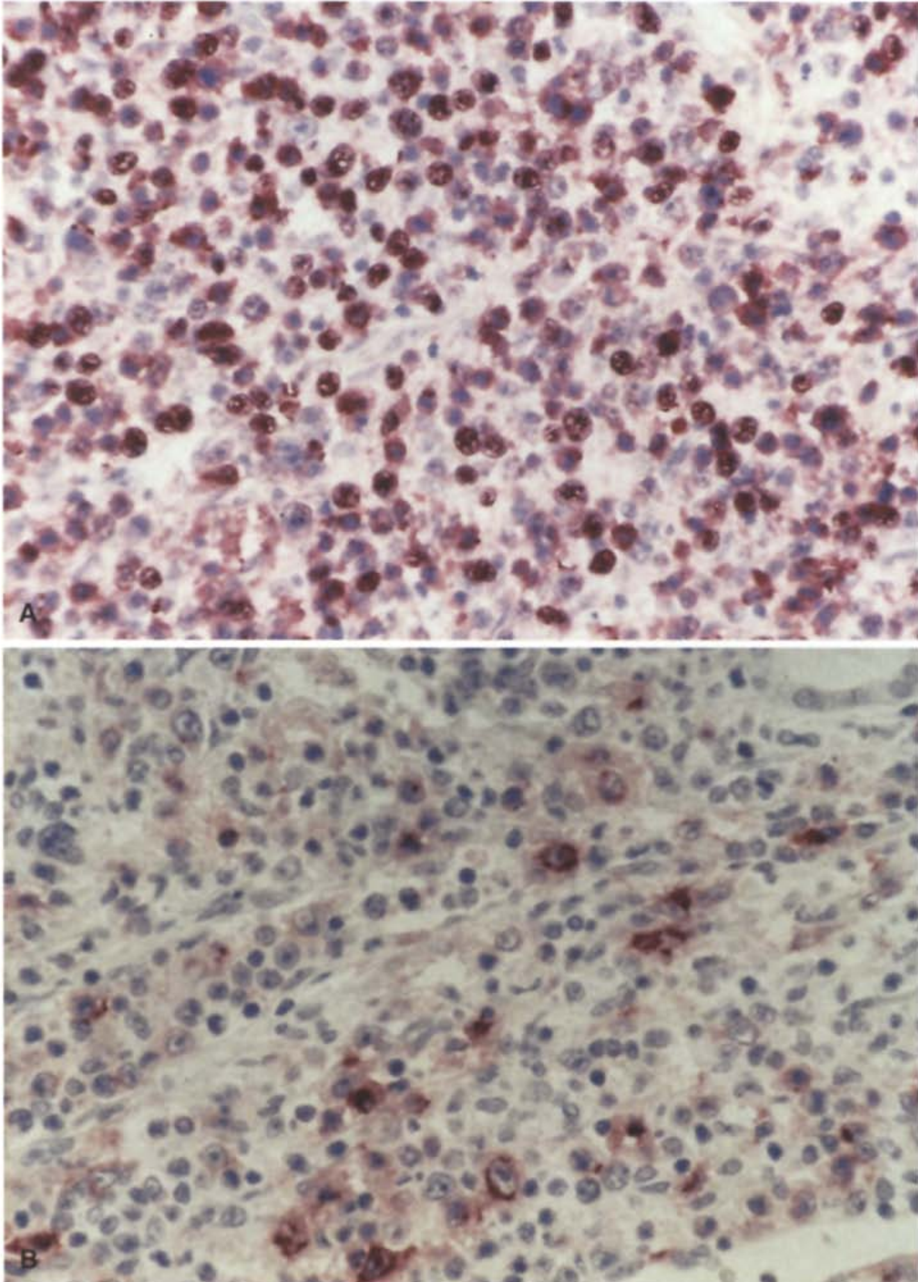


Fig. 2 A, B. Detection of EBERs and LMP1 in histological sections of a polymorphic B cell PTLD in the transplanted kidney of a renal transplant recipient. **A** In situ hybridisation for EBERs reveals latent EBV infection in the majority of infiltrating B cells. **B** Immunohistology with monoclonal antibodies, CS.1–4, shows expression of EBV-encoded LMP1 in a proportion of cells. Note the labelling of mainly large blast cells with prominent nucleoli. Immunohistochemical staining of a serial section revealed expression of EBNA2 in the majority of EBER-positive cells (not shown)

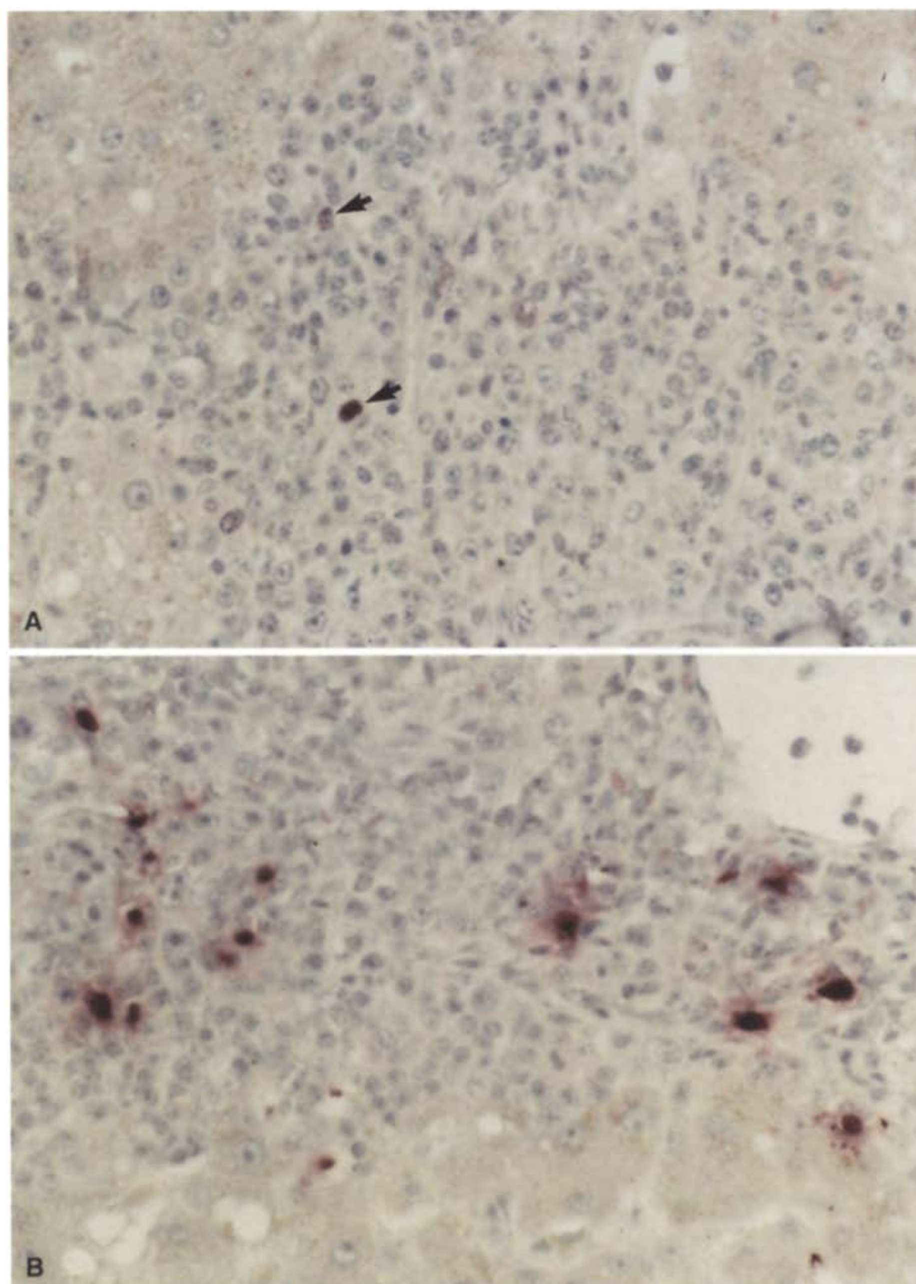


Fig. 3 A, B. Detection of latent and lytic cycle EBV proteins in histological sections of a polymorphic B cell PTLD in a bone marrow recipient. **A** Immunohistology with monoclonal antibody, PE2, shows expression of the EBNA2 latent protein in the nuclei of rare scattered cells (*arrowed*). Staining of a serial section with CS.1–4 antibodies identified similarly rare LMP1-expressing cells with large blastoid morphology (not shown). **B** Immunohistology with monoclonal antibody, BZ.1, shows expression of the early lytic cycle antigen in the nuclei of numerous cells

LMP1 [43]. This suggests that a proportion of cells expresses a latency I pattern of EBV latent genes. Moreover, it appears that a fraction of the PTLD cells may express LMP1 in the absence of EBNA2, suggesting a latency II [43]. These conclusions are supported by the detection of Qp-derived EBNA1 mRNA in some PTLDs in addition to transcripts originating from C/Wp [43]. In some cases, EBNA2-positive cells outnumber LMP1-expressing cells [39, 43, 46]. Thus, some cells express EBNA2 in the absence of LMP1; this pattern of EBV latent protein expression has been reported previously in infectious mononucleosis and some EBV-associated lymphomas [15, 37, 38]. It is uncertain, however, whether this is merely a transitory phenomenon or whether it represents a stable fourth type of EBV latency. The propensity for EBNA2 and LMP1 expression to diminish during progression from benign polyclonal lesions to monoclonal lymphoma is consistent with the accumulation of alterations in cellular genes such as p53, N-ras and c-myc [26]. Thus, a scenario whereby a single EBV-infected B cell clone gains proliferative advantage over other virus-infected cells by gradually accumulating genetic abnormalities, is a useful model to explain the evolution of a malignant lymphoma from a polyclonal lymphoproliferation.

In addition to the expression of latent EBV proteins, viral gene products associated with replicative infection are often detected in PTLDs [35, 39, 43, 46] (Fig. 3B) . This is also supported by the detection of replicating viral DNA in PTLDs by Southern blot hybridisation [44]. Moreover, entry into the lytic cycle can be observed in polymorphic as well as in monomorphic PTLDs [39]. Similarly, cells expressing lytic cycle antigens have been observed in PTLD biopsy specimens containing EBV-positive cells with different forms of EBV latency [39, 46]. However, the proportion of cells entering into the lytic cycle is usually small, and it is unclear whether EBV replication is of significance in the pathogenesis of PTLD. Although there have been occasional reports that acyclovir may be beneficial in the treatment of PTLDs [16], this issue is still controversial and the present balance of evidence would seem to favour the idea that viral replication in PTLD cells is an epiphenomenon reflecting the underlying immune defect.

While most PTLDs are of B cell lineage, a few T cell lymphomas [28, 63] and some CD30-positive anaplastic large cell lymphomas [3] have been reported. Such cases are rare and EBV gene expression has not been investigated thoroughly. Some of these cases, and even some B cell lymphomas developing in transplant patients, have been reported to be EBV negative and may, therefore, represent sporadic lymphomas developing independently of immunosuppression [3, 39, 63]. Cases of Hodgkin's disease have also been reported in transplant recipients [10]. Similar to Hodgkin's disease cases arising in the context of AIDS, the transplantation-associated cases are invariably EBV positive and express LMP1 in the absence of EBNA2 [10]. This is consistent with a type II EBV latency as also seen in EBV-positive cases of Hodgkin's disease developing in patients without an underlying immune defect.

Conclusions

A heterogeneous pattern of EBV gene expression is observed in PTLD, both between different patients and within the same lesion, and this serves to emphasise that the operational definition of EBV latencies as determined in vitro is not easily applied to human tumours. A tendency towards more restricted EBV latent gene expression in the more malignant lymphomas is consistent with the accumulation of genetic abnormalities which may obviate the requirement for continued expression of EBNA2 and

LMP1. In this regard, the recent successful application of adoptive EBV-specific CTLs for the treatment of lymphoproliferative disease is worthy of mention. An important consequence of the latency III pattern of EBV gene expression in PTLD is the provision of immunogenic antigens, particularly the so-called immunodominant EBNA3 family, for efficient recognition by specific CTL. The ease with which EBV-specific CTL can be expanded in vitro, particularly in the context of bone marrow transplantation where donor lymphocytes can be accessed, together with the initial success of adoptive CTL therapy in the context of lymphoproliferative disease, has prompted the extension of this approach to PTLD in organ allograft recipients and to other EBV-associated tumours such as Hodgkin's disease [42, 49]. The down-regulation in monomorphic malignant lymphomas both of the immunodominant EBNAs and of LMP1, which enhances immune recognition through up-regulation of components of the antigen-processing pathway, raises concerns about the therapeutic application of polyclonal EBV-specific CTL in this setting. It is likely that prophylactic CTL treatment will prove to be more efficient than administering CTL to patients with established disease. A more thorough analysis of both EBV gene expression and immune phenotype in relation to the progression of PTLD will help to establish the feasibility of adoptive CTL therapy.

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