

EPSTEIN-BARR VIRUS (EBV) AND LYMPHOMAGENESIS

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1. ABSTRACT

Epstein Barr virus (EBV) is a gamma herpesvirus that is associated with several specific lymphoid malignancies, some of which occur more frequently in immunocompromised individuals. EBV infection is almost ubiquitous in healthy adults, so establishing a causal role in lymphomagenesis has been difficult. Support for EBV being an oncogenic virus is derived from its ability to infect and transform normal human B-cells *in vitro*, resulting in their "immortalization" and leading to continuously growing lymphoblastoid cell lines (LCLs). In addition, viral proteins required for this EBV-mediated transformation have been identified. The presence of EBV in the neoplastic cells of specific lymphoid malignancies is quite consistent, further indicating an etiopathogenic role in their development. Nevertheless, it is clear that while important in the process of lymphomagenesis, infection by EBV is not sufficient. Important co-factors exist for the development of EBV-associated lymphomas, one of which is the lack of normal immune surveillance.

2. INTRODUCTION

The observation of an association between EBV and lymphomas dates back to the first identification of this virus. Denis Burkitt recognized and described a novel childhood tumor that was unusually common throughout equatorial Africa (1). This geographic distribution led him to suspect an infectious etiology. Subsequently he provided fresh tumor tissues to Tony Epstein and his graduate

student, Yvonne Barr, who were able to establish cell lines and identify herpesvirus-like particles by electron microscopy in a subset of cells (2,3). Since then, this virus and associated diseases have been studied extensively, and at the time of this review a bibliographic search revealed over 17,000 publications. Important general discoveries include seroepidemiologic studies that determined that EBV is widespread in all human populations (4), and biologic studies showing that EBV, like other herpesviruses, was found to target one cell type where infection was predominantly latent (B cells), and another capable of supporting lytic replication (mucosal epithelial cells). This review will focus on the role of EBV in lymphoproliferative disorders, including the diseases with which it is associated and biologic features related to lymphoid transformation.

3. PATTERNS OF EBV LATENCY IN B CELLS

EBV is generally found in its latent form in lymphomas and lymphoproliferative disorders. Although the pattern of EBV gene expression can vary significantly during latency, and probably represents a spectrum, a simplified classification establishing three patterns of latency has been provided (Figure 1) (5,6). In latency I, EBNA1 (necessary for maintaining the episomal EBV) is the major viral protein expressed. This latency pattern is established in infected B cells in healthy individuals. Latency III involves the unrestricted expression of all 9

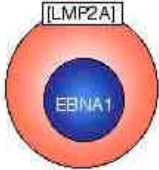
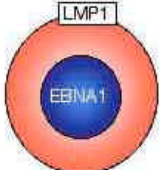

EBV Gene Expression Profile	Latency I 		Latency II 		Latency III 	
Lymphoma Type	Burkitt's (HIV+/-)	Primary Effusion	Hodgkin's	T/NK Cell	AIDS-NHL (Large Cell)	PT-LPD
Other Transforming Alterations/Infections	c-myc +/- p53	KSHV/HHV8 +/- BCL-6	+/- IκBα	?	+/- BCL-6 +/- c-myc +/- p53 +/- Ras	+/- BCL-6 +/- c-myc +/- p53 +/- N-ras
Immunogenicity	-		+ / -		+++	

Figure 1. Patterns of EBV latent gene expression in malignant lymphomas. Some of the relevant EBV genes that are expressed during Latencies I, II, and III are illustrated in the top portion of the figure. The transforming EBV gene LMP1 is expressed in Latencies II and III, and the highly immunogenic EBNA1 only in Latency III. Cellular genetic alterations and/or viral co-infection known to occur in these lesions are listed as other transforming events; those occurring in virtually all cases are shown in bold letters.

latent genes including six EBV- encoded nuclear antigens (EBNA1-6), and three latent membrane proteins (LMP1, LMP2A, and LMP2B). Latency II is an intermediate pattern with expression of EBNA1 and varying amounts of the three LMP proteins.

3.1. EBV latency in lymphomas and immune recognition

Since EBNA proteins are immunogenic, with the exception of EBNA1, an important feature of Latency III is the recognition and elimination of the EBV infected cells by the immune system. Therefore, lymphomas with unrestricted EBV latency are mainly encountered in immunodeficient individuals. In contrast, most lymphomas in immunocompetent hosts will have Latency I or II, as down-regulation of the immunogenic EBNA proteins is thought to be an important mechanism of immune evasion by EBV (5).

4. MOLECULAR MECHANISMS OF EBV MEDIATED TRANSFORMATION

Some EBV-associated lymphomas, exemplified by Burkitt's lymphoma, express mainly EBNA1. Although EBNA1 transgenic mice have been shown to have an increased incidence of lymphomas, at least in one study (7), EBNA1 does not appear to be transforming. This may be an explanation for the observation that lymphomas with Latency I bear cellular oncogenic alterations, such as translocations involving the c-myc oncogene characteristic of Burkitt's lymphomas. Of the EBNA proteins expressed in Latency III, EBNA2 and LMP1 are essential for transformation by EBV *in vitro*. EBNA2 is thought to represent a constitutively active member of the Notch signaling pathway (8,9). The LMP1 protein is transforming

and tumorigenic *in vitro* (10), and transgenic mice expressing LMP1 under the control of immunoglobulin gene regulatory elements develop B cell lymphomas with increased frequency (11). LMP1 functions as a constitutively active CD40 receptor, a member of the TNF-receptor family. LMP1 aggregates in the membrane as its cytoplasmic tail interacts with tumor necrosis factor receptor-associated factors (TRAFs) and TNFR-1-associated death domain protein (TRADD), leading to activation of NF- κ B and the c-Jun amino-terminal kinase (JNK)(12-14), a kinase cascade activated by inflammatory cytokines and involved in bcr-abl leukemogenesis (15). NF- κ B is an important transcription factor, the activation of which leads to expression of a variety of cellular genes related to B-cell proliferation and malignancy, including ICAM-1, LFA-3, CD40, EB13, Fas and TRAF1 (16), and the matrix metalloproteinase-9 which may contribute to tumor invasion and metastasis (17). Association of LMP1 with TRAF-1 and TRAF-3, as well as activation of NF- κ B, have been recently demonstrated to occur *in vivo* in EBV-associated lymphomas expressing LMP1, suggesting that this is indeed a relevant pathobiologic pathway in EBV-related lymphomagenesis (18).

4.1. Maintenance of the transformed phenotype by EBV

Although it is clear that EBV can transform B-cells *in vitro*, its role in the maintenance of the transformed state is still under active investigation. Elimination of episomal EBV from LCL cells has been achieved by treatment with hydroxyurea, which induces an inhibition of cellular proliferation (19). Furthermore, loss of the EBV genome from the BL cell line Akata, results in loss of the malignant phenotype, although Akata is a Latency I cell line, and does not express the major EBV transforming genes, LMP1 and EBNA2 (20). Two studies show that re-

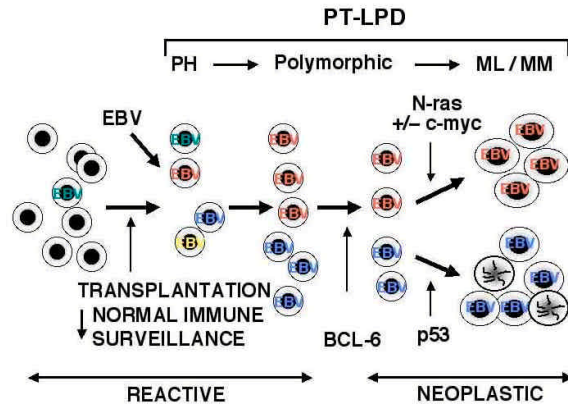


Figure 2. Model of tumor progression in EBV-positive solid organ post-transplantation lymphoproliferative disorders. An initial step in the development of PT-LPDs appears to be the polyclonal expansion of EBV infected B cells, either as a result of release from immune surveillance of latently infected cells or *de novo* infection. This expansion would have a histologic appearance of a plasmacytic hyperplasia (PH). Subsequently, some clones with unknown selective advantages outgrow others, leading to oligoclonal and then monoclonal expansions, which are still responsive to immune responses if restored. These lesions may have the histologic appearance of a polymorphic PT-LPD. However, if genetic alterations in oncogenes or tumor suppressor genes occur, a truly neoplastic process arises which may no longer be responsive to immune surveillance mechanisms. Mutation in BCL-6 alone does not appear to affect the morphologic features, but cases with structural alterations in p53, N-ras and or c-myc have the histologic appearance of a monomorphic malignant lymphoma (ML) or, more rarely, a multiple myeloma/plasmacytoma (MM).

establishment of Latency I in EBV-negative Akata cells restores tumorigenicity (21,22). An increased resistance to apoptosis accompanies this effect, which was postulated to be due to a decrease in c-myc and increase in Bcl-2 protein levels. This restored tumorigenicity is not simply due to expression of the EBNA1 protein, as both studies showed that enforced expression of EBNA1 in EBV-negative Akata cells does not restore tumorigenicity or apoptosis resistance. Therefore, a restricted latency program is also important for malignancy, although the precise mechanism is unknown.

5. EBV-ASSOCIATED LYMPHOMAS

5.1. Burkitt's lymphoma (BL)

African (endemic) BLs invariably contain the EBV genome. However, EBV is found in only a subset of sporadic cases. Most of our understanding of EBV gene expression was derived from the study of BL cell lines; however, *in vivo* expression has also been determined in endemic BL tissue biopsies (23). EBV-positive BLs have Latency I EBNA1, and usually LMP2A, transcripts, in the absence of lytic transcripts or other latent transcripts. Expression of the Latency I EBNA1, and repression of Latency III transcripts is thought to result from differences in CpG methylation of the corresponding promoters (24).

Therefore, it may be possible to induce changes in the methylation status of the EBV promoters, resulting in the induction of expression of immunogenic Latency III proteins followed by anti-tumor immunity. In addition, the lack of immune recognition of EBV-associated BLs is secondary to inefficient processing of class I-restricted CTL epitopes due to a loss of peptide transporters (TAP) and MHC expression (25). A recent study showed that CD40 engagement upregulates TAP-1 and HLA class I expression in BL cells, allowing recognition by virus-specific CTLs (26). Therefore, treatment with CD40 ligand has been proposed as a potential approach to allow the use of immunotherapy for the treatment of EBV-related BLs.

5.2. Post-transplantation lymphoproliferative disorder (PT-LPD)

PT-LPDs are a heterogeneous group of lymphoid proliferations arising in the setting of therapeutic immunosuppression following organ transplantation. Only a small proportion of PT-LPDs lack EBV. These cases appear to occur later after transplantation and are associated with shorter survival (27). Clinically, some patients experience regression of all EBV-positive PT-LPD lesions with a reduction of immunosuppression (in solid organ transplant recipients) or infusion of donor T cells (in bone marrow transplant recipients), whereas others rapidly die of disease despite aggressive treatment. Unlike other malignant lymphomas, histologic classification has not been very helpful in predicting clinical outcome.

Studies in our laboratory to characterize the pathologic and molecular features of PT-LPDs arising in solid organ transplant recipients have indicated that morphological and molecular analyses do provide insights into the biology of these lesions that may help in making clinical decisions. Solid organ PT-LPDs can be divided into three biologically relevant categories (28): 1) plasmacytic hyperplasia (PH); 2) polymorphic PT-LPD (polymorphic); and 3) malignant lymphoma/multiple myeloma (ML/MM). PHs are polyclonal and regress following surgical excision and/or a reduction in immunosuppression. In contrast, ML/MM are monoclonal lesions with additional oncogene/tumor suppressor gene alterations (c-myc, p53, BCL-6 and/or N-ras) that usually progress in spite of aggressive clinical intervention (29). The clinical behavior of the monoclonal polymorphic lesions, all of which lack structural alterations in c-myc, p53 or N-ras, cannot be predicted by their histologic appearance. The only molecular alteration found to be predictive of behavior among the polymorphic lesions is the presence of BCL-6 gene mutations: when present in our series, the lesions did not regress following a reduction in immunosuppression (30). Therefore, mutations in BCL-6 may be indicative of progression from a "monoclonal hyperplasia" to a true neoplasm. A schematic representation of our model of lymphoma progression in solid organ PT-LPDs is provided in Figure 2.

PT-LPDs occurring following bone marrow transplantation are different from those associated with solid organ transplantation in that they are of donor origin. These are increased in incidence when T-cell depleted

marrow is used, as well as in patients receiving anti-T-cell therapy for graft versus host disease (GVHD) or with acute high grade GVHD (31). T-cell depletion using CAMPATH-1 (CD52) antibodies, which also deplete B-lymphocytes, does not increase the incidence of PT-LPDs (32).

Most PT-LPDs have EBV Latency III, with expression of immunogenic EBNA. Therefore, PT-LPDs are often sensitive to immune-restoration therapy. While in the solid organ transplant setting this may sometimes be achieved by withdrawal or reduction of immunosuppressive agents, PT-LPDs occurring following allogeneic bone marrow transplantation are ideal candidates for adoptive immunotherapy. Unselected populations of peripheral blood lymphocytes from the donor, containing EBV-specific T-cells, have been used (33,34). However, this treatment led to complications that arise from the presence of alloreactive T cells in the infusions. Recently, preparations of EBV-specific T-cell lines from donor lymphocytes have been generated (35), and used successfully in the prophylaxis and treatment of PT-LPDs in bone marrow transplant recipients (36). More efficient and faster methods for the production of EBV-specific CTLs are being developed, such as the use of EBV-loaded dendritic cells (37).

5.3. AIDS-related non-Hodgkin's lymphomas (AIDS-NHL)

AIDS-NHL can be subdivided into several major categories, which include systemic, primary central nervous system (PCNSL), and primary effusion lymphomas (PEL). The latter, while positive for EBV, also contain KSHV (see below).

Among the systemic AIDS-NHLs are the diffuse large cell lymphomas (DLCL), which frequently have immunoblastic features and generally harbor EBV infection (80%), and Burkitt's lymphomas, which are characterized by c-myc activation, frequent p53 mutations and infection by EBV in 30% of cases (38). Cases with morphologic features intermediate between BL and DLCL often occur in the setting of HIV infection, and are classified as Burkitt-like lymphoma (BLL). At the molecular level, BLLs also have intermediate features, with c-myc rearrangements in 68% of cases and EBV in 79% (39). Patients with BL have significantly higher CD4 counts than those with DLCL and, when EBV positive, they have Latency I pattern of gene expression. In contrast, the majority of DLCLs have a Latency II or III. In addition, while DLCL express adhesion molecules that are important for immune recognition, BLs do not. These observations suggest that defective EBV immunity is involved in the pathogenesis of DLCLs (40).

PCNSL are large cell lymphomas arising from germinal center B-cells, all of which contain EBV. According to a recent study, PCNSL can be divided into two categories, those with immunoblastic features which express LMP1 in conjunction with BCL-2 but no BCL-6 expression, and those with a large noncleaved cell morphology which do not express LMP1 or BCL-2, but express BCL-6 (41).

5.4. T/NK cell lymphomas

The angiocentric (nasal and nasal-type) NK/T-cell lymphomas are invariably associated with EBV infection (42). These have a high prevalence in Asia, but cases from other countries have also shown an association with EBV (43). Studies on cell lines indicate that T/NK cell lymphomas have a Latency II (44,45). While EBV has also been reported to be present in peripheral T cell lymphomas, a recent study suggests that while present, it is preferentially localized in B-cells rather than the neoplastic T cells (46). It nevertheless may play a role in attracting and inducing the clonal expansion of antigen-driven T cells, which subsequently may undergo oncogenic genetic alterations, leading to the development of a true neoplasm that may or may not remain dependent on antigen for survival.

5.5. Hodgkin's disease (HD)

Classical HD has been found to be associated with EBV infection in approximately 40% of cases in Western countries and more frequently in developing countries and in younger patients (47). HD results from a monoclonal expansion of B-cells containing somatic hypermutation of the immunoglobulin (Ig) genes. Although Hodgkin's cells are of B cell origin, they do not express Ig, and this is thought to be due to the lack of expression of transcription factors, in particular Oct-2 and BOB-1 (OCA-B or OBF-1), that are involved in Ig gene expression (48,49). It has been suggested that the Hodgkin's Reed-Sternberg (HRS) cells are derived from germinal B-cells destined to undergo apoptosis because of lack of Ig expression, but were protected by some transforming event, such as EBV infection (50). Activation of NF- κ B has been postulated to be an essential survival signal for Hodgkin's cells. In EBV-positive HD, the virus establishes Latency II within HRS cells, with expression of LMP1 and LMP2. As mentioned above, LMP1 initiates cellular signals that lead to activation of NF- κ B. In addition, aberrant activation of NF- κ B can be found in some cases of HD as a result of loss of functional I κ B (inhibitor of NF- κ B) resulting from genetic structural alterations, or from aberrant activation of the upstream regulatory I κ B kinases (IKK's) (51-54). With respect to the immunologic control of EBV-infected cells in the contexts of HD, it has been found that HRS cells express at least two subdominant targets for CTL recognition: LMP1 and LMP2. In addition, HRS cells in EBV-associated HD do express MHC class I molecules, as well as the transporter-associated molecules TAP1 and TAP2, necessary for a CTL response (55,56). While the reasons for a lack of effective immune recognition and elimination of these cells in patients with HD are not known, these observations raise the possibility of developing immunotherapy for the treatment of EBV-positive HD.

5.6. Primary Effusion Lymphomas (PEL)

Following the identification of the Kaposi's sarcoma-associated herpesvirus (KSHV), also called human herpesvirus 8 (HHV-8) (57), its presence in a small subset of malignant lymphomas was recognized (58) and subsequently confirmed by several investigators (59-63). These lymphomas possess distinctive and unusual

clinicopathologic features, including their presentation as lymphomatous effusions in body cavities, therefore being initially called body cavity-based lymphomas (BCBL), and subsequently primary effusion lymphomas (PEL). In addition to KSHV, the vast majority of PEL contain EBV, and are therefore co-infected with two different gamma herpesviruses. While these lymphomas are more frequent in HIV-positive individuals, cases of PEL occurring in HIV-negative men as well as women have been identified, and these cases also contain KSHV, but the proportion of EBV positive cases in this category is reduced (64-67).

Examination of PELs has provided information about the biology of this type of disease, and its place in the spectrum of non-Hodgkin's lymphomas. Most cases have been B cell lymphomas, as determined by the presence of clonal immunoglobulin gene rearrangements, and while they usually lack expression of B-cell associated antigens, they may express monotypic kappa or lambda immunoglobulin light chain mRNA (68) and we have found weak expression of cytoplasmic immunoglobulin in a subset of cases. Most PELs are thought to originate from post-germinal center B cells, since they commonly have hypermutation of the immunoglobulin genes (69,70). In addition to an immunoblastic morphology, PELs have a set of immunophenotypic features suggesting that they are at a preterminal stage of B cell differentiation, in particular the loss of expression of some B cell antigens, which may occur in multiple myeloma as well as in immunoblastic lymphomas. Furthermore, most PELs express CD138/Syndecan-1, an adhesion molecule that is selectively expressed by a subset of pre-B cells and by plasma cells, including myeloma plasma cells (71).

The almost invariable presence of KSHV in lymphomas having the features described above suggests that this virus is necessary for the development of PELs. However, since PELs are so uncommon, even in populations where the seroprevalence of KSHV is relatively high, it is evident that infection by this virus represents only one of several genetic events involved in their development. One such other factor appears to be EBV infection. The specific role of each of these viruses and their interaction is still poorly understood, but analysis of the genes expressed by both of them has shed some light into their possible roles. Most PEL cells, both in vivo and in cell culture, have a latent pattern of KSHV gene expression. Analysis of the pattern of EBV gene expression in PELs revealed that only EBNA1 was expressed, corresponding to type I latency (72,73). This was an unexpected finding, given the resemblance of PEL cells to immunoblastic lymphoma cells and their increased incidence in immunodeficient individuals. This observation suggests that KSHV, rather than EBV, is playing a transforming role in PELs. Supporting this hypothesis are the observations that KSHV encodes several possible viral oncogenes and that PELs do not contain structural alterations in most of the cellular transforming genes frequently involved in malignant lymphomas, in particular c-myc (reviewed in 74,75).

6. PERSPECTIVE

Following many years of arduous investigation, we are beginning to acquire a basic understanding of the mechanisms utilized by EBV in the development of lymphoproliferative disorders. We now know that EBV subverts cellular signaling pathways that result in the proliferation and survival of infected lymphocytes. In addition, we are now aware of many relevant aspects of immune recognition of EBV by the host, as well as viral mechanisms for evasion of such recognition. The medical and scientific community is now in the position to start manipulating both viral and cellular responses to develop rational approaches for the treatment and prevention of this group of common and aggressive malignancies.

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