

Epstein-Barr Virus Lymphomagenesis and Therapeutic Targets

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Abstract The pathogenic role of the Epstein-Barr virus (EBV) has been suggested because it is consistently found in some lymphoproliferative disorders. More than 90% of the population is exposed to the virus, usually during childhood, and after initial exposure, the virus survives for the lifetime of individuals as an episome, and expresses a limited set of proteins depending on the immune status of the host. The variable expression of viral proteins is known as latency pattern with minimal expression of viral products in immunocompetent hosts, but expression of numerous viral products in the immunocompromised host. Thus, the more virus products that are expressed, the more targets for an immune reaction. Since it is acknowledged that the neoplastic process including lymphoproliferative disorders result from a multi-step chain of events, multiple pathways are affected, and in particular in EBV-positive lymphoproliferations, EBV products interact with abnormally activated pathways in the context of the immune response of the host. In this review we analyze the structure of the virus and its interaction in the immunocompetent as well as in the immunocompromised host, and discuss the possible roles of EBV products in various lymphoproliferative disorders. Finally, we discuss the potential role of expressed EBV viral proteins and activation of pathogenic pathways in the setting of specific diagnostic categories with the identification of potential molecular targets.

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Epstein-Barr Virus Structure, Expressed Genes, and Pathogenesis

Epstein-Barr virus (EBV) or human herpes virus 4 is a ubiquitous virus that infects more than 90% of the human population and usually establishes a persistent latent infection in the host. Primary infection occurs via salivary contact in infancy and is usually asymptomatic, however when primary infection occurs in adolescents or young adults it may manifest as infectious mononucleosis. After primary infection, the virus usually remains in an asymptomatic latent state within resting memory B-cells for the lifetime of the host. Cytotoxic T-cells, both CD8+ and CD4+, and natural killer (NK) cells are in charge to recognize and eliminate the virus-infected cells [1]. When infected B-cells evade or suppress the T-cell immune response, or the host's cellular immune system fails to control EBV-induced B-cell proliferations, the infected B-cells can transform into neoplastic cells and eventually manifest as a lymphoproliferative disorder. EBV-associated lymphoproliferative disorders encompass a heterogeneous group of disorders, including chronic active EBV infection, B-cell lymphoma, T/NK-cell lymphoma or leukemia, classical Hodgkin lymphoma, as well as immunodeficiency and posttransplant lymphoproliferative disorders.

EBV infection occurs through the binding of the major viral envelope glycoprotein gp350 to the CD21 receptor on the surface of B cells and through the binding of glycoprotein gp42 to human leukocyte antigen (HLA) as a coreceptor [2]. The viral genome is linear and becomes circular by fusion of either end of the genome, and the virus persists within cells as an episome. EBV virus expresses genes in two distinct programs known as the lytic cycle and the latent cycle [3, 4]. During the lytic cycle, there is production of infectious virus and expression of viral genes that encode proteins involved in DNA replication and in assembly of viral particles. In contrast, during the latent cycle only a limited number of viral genes are expressed, and these include six nuclear proteins, referred to as *EBV nuclear antigens* (EBNA 1, 2, 3A, 3B, 3C, and EBNA-LP), and three latent membrane proteins (LMP 1, 2A and 2B), which are associated with transforming activity [5]. There are two nontranslated RNA molecules known as the EBV-encoded RNAs designated as EBER1 and EBER2. In addition, there are EBV microRNAs (miRNAs), which are encoded in two regions: BHRF1 (Bam HI fragment H rightward open reading frame 1) and BART (Bam HI-A region rightward transcript). miRNAs are the only known functional products of the BARTs transcripts [6]. BHRF1 miRNAs have been found highly expressed in immortalized lymphoblastoid cell lines (LCL), whereas BARTs miRNAs have been found in all EBV-infected cell lines as well as in biopsies of lymphoma cases [6].

There are four types of latent infection, designated as latency 0, I, II, and III, that reflect the extent of viral protein expression allowed by the host in vivo. Latency 0 refers to infection of cells where most viral genes are not expressed and therefore cells are not recognized or targeted by EBV-specific T lymphocytes; this stage is also called "in vivo latency" [1, 7]. EBV resides in memory B cells, which constitute a long-term reservoir for EBV. Thus, in the absence of production of viral proteins or antigens, there is no EBV-specific T cells neither is there an immune response

Table 1 Latency patterns of infection with Epstein-Barr virus and associated lymphoproliferative disorders

Latency type	Viral gene expressed					Lymphomas and cells affected
	EBERs	BARTs	EBNA1	EBNA2/3A, 3B, 3C/LP	LMP1/2	
0	+	+	-	-	-	Memory B-cells
I	+	+	+	-	-	Memory B-cells, BL
II	+	+	+	-	+	GCB, CHL, PTCL, AITL, NK/T
III	+	+	+	+	+	LCL, PTLD, AIDS-related lymphoma, EBV+ DLBCL of the elderly

AIDS acquired immunodeficiency syndrome, *AITL* angioimmunoblastic T-cell lymphoma, *BART* EBV microRNA, *BL* Burkitt lymphoma, *CHL* classical Hodgkin lymphoma, *DLBCL* diffuse large B-cell lymphoma, *EBER* Epstein-Barr virus encoded RNA, *GCB* germinal center B-cells, *LCL* lymphoblastoid cell lines, *NKT* NK/T-cell lymphoma, *PTCL* peripheral T-cell lymphoma

against EBV. Activation of EBV-infected memory B cells can lead to their differentiation into plasma cells, a process that might switch-on the lytic cycle of EBV and produce viral particles [8]. Three latency patterns are associated with different types of lymphoid proliferations (Table 1). In latency type I there is selective expression of EBNA-1 and LMP-2A, and this pattern is found typically in Burkitt lymphoma (BL). In latency type II, there is expression of EBNA-1, LMP-1, LMP-2A, and LMP-2B, and this pattern is found in classical Hodgkin lymphoma (HL), primary effusion lymphoma, angioimmunoblastic T-cell lymphoma (AITL), peripheral T-cell lymphoma (PTCL) and NK/T cell lymphoma or leukemia. In latency type III there is expression of all nine latent cycle EBV antigens, and this pattern is typically found in posttransplant lymphoproliferative disorders (PTLD) and in AIDS-related lymphoma. Because there are more viral proteins expressed in latency type II and III infections, respective EBV-associated lymphoproliferations are more immunogenic than diseases associated with a latency type I.

EBV-Related Lymphoproliferative Disorders

Burkitt Lymphoma

Burkitt lymphoma (BL) is a highly aggressive B-cell lymphoma that was initially described in children around equatorial Africa [9]. There are three clinical variants of BL: endemic, sporadic, and human immunodeficiency virus (HIV)-associated.

The EBV genome is present in almost all cases of endemic BL and there is strong epidemiological link with endemic malaria. In sporadic BL, EBV is identified in 15–30% of cases, while it is more common in HIV-associated BL.

The neoplastic cells of BL express B-cell lineage and B-cell germinal center cell markers CD10 and Bcl6. At the molecular level, the neoplastic cells undergo somatic hypermutation (SHM) and class-switch recombination (CSR) supporting the notion that they derive from germinal center cells. It is suspected that SHM and CSR predispose GC cells to chromosome translocations or mutations in non-Ig genes [10]. Virtually all BL cases carry one of three characteristic chromosomal translocations between the *MYC* gene in chromosome 8 and one of the immunoglobulin genes on chromosomes 2, 14, or 22 [1]. The role of *MYC* deregulation as the key factor in the pathogenesis of BL is compelling [11–13]. Most cases of BL show DNA breakpoints in rearranged VJ regions or in S regions of the immunoglobulin heavy chain (IgH) loci, thus it is generally accepted that the chromosomal translocations are mediated by aberrant SHM or CSR, which require the intervention of DNA-modifying enzymes known as activation-induced deaminase (AID). Most *MYC*/Ig breakpoints in EBV-positive endemic BL appear to originate from aberrant SHM. On the other hand, the translocations in sporadic cases mostly involve the Ig switch regions of the IgH locus [14]. EBV may be accounted for the difference in *MYC* breakpoints between EBV-positive BL and EBV-negative BL [15].

Genes downstream of *MYC* regulate cell cycle progression, cell growth, apoptosis, and senescence. Deregulated expression of *MYC* induces p53 response and triggers apoptosis [16, 17].

EBV has a latency type I pattern, with expression of EBNA1 and LMP2A. Since EBNA1 is the only viral gene product in all latency patterns, it is suspected that EBNA1 promotes lymphomagenesis in EBV+ processes. Although Kang et al. [18] suggested that EBNA1 is limited to maintenance of the viral genome, other researchers showed that EBNA1 may activate the catalytic subunit p91 of the NADPH oxidase2 (NOX2) at the transcriptional level. Thus EBNA1 generates reactive oxygen species that may contribute to DNA damage and genomic instability [19]. Furthermore, other researchers reported that EBNA1 binds to the deubiquitinating enzyme HAUSP/USP7 and together sequester p53, contributing to p53 degradation [20].

LMP2A expression was recently confirmed in endemic BL using a sensitive RT-PCR assay [13, 21]. LMP2A contains a 119 amino terminal cytoplasmic domain that includes eight tyrosine residues, two of which form an immunoreceptor tyrosine-based activation motif (ITAM) [22]. Experimental data using EBV LCLs in vitro and LMP2A-transgenic mice indicate that the cytoplasmic tail of LMP2A mimic signals used by the B-cell receptor (BCR) and promotes B-cell development. In effect, LMP2A constitutively phosphorylates Lyn and Syk, with Lyn binding to tyrosine 112 and Syk binding to the ITAM motif of LMP2A. Additional studies using LMP2A-transgenic mice demonstrate that LMP2A constitutively phosphorylates and activates many of the proteins induced by BCR, such as Lyn, Syk, BLNK, BTK, Ras, P13K, NF- κ B, and MAP kinases [23–26]. LMP2A also increases the levels of anti-apoptotic Bcl family members and protect B cells from apoptosis [13].

Chemotherapy is the standard of care for immunocompetent patients with EBV-associated BL; however it is expected that targeting EBV therapy may contribute to better outcomes, or the use of EBV vaccines may decrease the incidence of endemic BL.

Diffuse Large B-Cell Lymphoma of the Elderly

EBV-positive diffuse large B-cell lymphoma (EBV+ DLBCL) of the elderly, also known as age-related EBV+ lymphoproliferative disorder, accounts for 8–10% of DLBCL cases [27]. Neoplastic cells are B-lymphocytes that lack germinal center cell markers CD10 and Bcl6, consistent with post GC B-cells. The neoplastic cells most often display an EBV type III latency, positive for EBV protein products including LMP1 and EBNA-2, although some cases express latency II, and lack EBNA expression [28, 29] The variable presence of EBNA2 expression is attributed to the variable degrees of immune surveillance in aging individuals.

EBV infection and waning immunity that is part of the aging process, where a decrease in T-cell response occurs, naturally appear to be the main driving mechanisms [30]. Decrease in T-cell function leads to EBV reactivation that manifests with the expression of proteins such as LMP1 that leads to upregulation of anti-apoptotic proteins Bcl-2, MCL-1, and A20 [31, 32].

The prognosis of EBV-positive DLBCL of the elderly is worse than EBV-negative DLBCL, partially compounded by a high median age of patients between 70 and 75 years, who are often unable to tolerate aggressive therapeutic regimens, thus an optimal regimen has not been established for EBV-positive DLBCL of the elderly. The development of adoptive immunotherapy with cytotoxic T cells (CTLs) directed against EBV latency antigens has the potential of improving the outcome of this group of patients.

Classical Hodgkin Lymphoma

Classical Hodgkin lymphoma (CHL) is clinically distinct from non-Hodgkin lymphoma and histologically is characterized by an exuberant inflammatory background and only rare or few neoplastic cells. The neoplastic cells are large mononuclear or multinucleated known as Hodgkin Reed-Sternberg (HRS) cells. CHL comprises four histological subtypes [33] and the prevalence of EBV varies with the histological subtype. The prevalence is highest (~75%) in mixed cellularity HL, and lowest (10~40%) in nodular sclerosis HL [34]. It is also notorious that the prevalence of EBV in CHL varies with epidemiologic factors; EBV infection is more prevalent in developing countries and affects mostly childhood, and older adult age groups (age >50 years).

The neoplastic cells of CHL are monoclonal B cells at the germinal center stage of differentiation [35–37]. Analysis of the Ig variable (IgV) gene regions show evidence of somatic hypermutation, revealing a germinal center (GC) or post-GC origin [38].

EBV is believed to play a causal role in the pathogenesis of CHL. EBV is detected in HRS cells and the virus is clonal, indicating that infection occurred prior to neoplastic transformation [39, 40]. In EBV-positive CHL, the HRS cells demonstrate latency type II pattern, and express EBNA1, LMP1 and LMP2.

LMP1 is considered the major transforming protein of EBV. It has an integral membrane protein comprising 386 amino acids and consists of a short amino (N)-terminal cytoplasmic stretch, 6 trans-membrane (6TM) domains, and a long carboxyl (C)-terminal cytoplasmic region with no significant extracellular domain [41]. The 6TM domains regulate their own synthesis and degradation via the unfolded protein response (UPR) and autophagy. The carboxy terminal domain induces proliferation and survival of EBV-infected B cells in vitro and in vivo [42, 43]. LMP1 mimics CD40 and can substitute for the signaling of CD40 in B cells [41, 42, 44].

LMP1 activates the signaling pathways of nuclear factor- κ B (NF- κ B), activated protein 1 (AP1), and signal transducer and activator of transcription (STAT). Aberrant activation of NF- κ B plays a determinant role in cell transformation, while tumor promotion is mediated by its anti-apoptotic functions. Evidence shows that NF- κ B activation by LMP1 is critical for B cell transformation in vitro and in vivo [45, 46]. LMP1 can induce most of the phenotypic changes of neoplastic cells, including expression of surface antigens CD21, CD23, CD30, CD40, CD44, and Fas as well as cell adhesion molecules ICAM1, LFA1, and LFA3. LMP1 also upregulates expression of the anti-apoptotic proteins Bcl-2, A20, Bfl, and Mcl1 and stimulates production of cytokines interleukin (IL)-6 and IL-8 [47, 48]. HRS cells express constitutively CD30 that is a trans-membrane protein which belongs to the tumor necrosis factor (TNF) receptor family [49]. When stimulated by CD30 ligand, CD30 interacts with TNF receptor-associated factors TRAF2 and TRAF5, mediating signal transduction that leads to the activation of the NF- κ B pathway. Another potential role of LMP1 is the downregulation of CD99. Loss of CD99 has been associated with generation of B cells with CHL immunophenotype.

LMP2A mimics the BCR and competes with BCR to bind tyrosine kinases, thereby modulating the activity of these tyrosine kinases. LMP2B is not essential for EBV-induced B-cell transformation in vitro [50].

Mechanisms and cell interactions of HRS in CHL are complex. In EBV+ CHL, most of the reactive T lymphocytes have a regulatory T-cell phenotype, and LMP1 may mediate their attraction through IL-10 secretion [51]. The presence of regulatory cells around HRS cells causes a profoundly immunosuppressive microenvironment, contributing to evade or suppress immune T-cell responses. Thus, the use of EBV-specific T-cells to deliver immunostimulatory cytokines may counteract immunoregulatory T-cells and contribute to eliminate HRS cells. Similarly, HRS cells positive for LMP1 and LMP2A can be targeted with adoptive transfer of

EBV-CTL (cytotoxic T cells) [52]. Clinical data showed that LMP2-specific CTLs augmented T-cell responses, migrated to tumor deposits, and caused regression of tumors in a subset of patients with CHL [53]. LMP1 is another potential target for CHL immunotherapy. LMP1 effect or function has been targeted directly, using single-chain antibodies or antisense RNA approaches, and indirectly, by the genetic or pharmacological interception of its downstream effects on NF- κ B [54].

NK/T-Cell Lymphoproliferative Disorders

EBV-related NK/T-cell lymphoproliferative disorders (LPD) include aggressive NK-cell leukemia, EBV-positive T-cell lymphoproliferative disorders of childhood, angioimmunoblastic T-cell lymphoma (AITL), extranodal NK/T-cell lymphoma, nasal type, and peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS).

EBV can infect CD4+ and CD8+ peripheral blood T-cells as well as NK-cells in a minority of patients with infectious mononucleosis. The mechanisms of access of EBV into T cells and NK cells in vivo are speculative. It has been shown that NK cells activated by EBV acquire CD21 by synaptic transfer from CD21+ B cells, and these ectopic receptors allow EBV binding to NK-cells. It is possible that NK cells in close contact with EBV-infected B cells may acquire EBV infection directly and then expand clonally [55].

Most EBV-associated T-cell lymphomas are thought to arise from chronic active EBV infection (CAEBV). CAEBV is considered as a progressive EBV infection from infected B-cells. Evidence suggests that CAEBV of NK/T-cells develops into NK/T cell lymphoma [56].

In EBV-positive T-cell LPD of childhood, there is a monoclonal proliferation of CD4+ or CD8+ cells with viral gene expression of EBNA1, LMP1 and LMP2, consistent with a latency type II pattern. In extranodal NK/T-cell lymphoma, nasal type, disease usually presents in the nasal or upper aerodigestive tract and most patients are of Asian origin.

The pathogenesis of EBV in NK/T-cell lymphoma may be similar to that of CHL, since both have a latency type II pattern of EBV infection. In this model, LMP1 mimics and activates NF- κ B pathway, as previously discussed. Yang et al. [57] reported that increased IL-9 levels induced by the EBERS possess anti-apoptotic effects and promote T-cell proliferation and transformation. Further research is needed to determine how EBV infects NK/T cells and the pathogenic role of EBV.

Since LMP1 expression in EBV-associated NK/T cell lymphomas activates the NF- κ B pathway, targeted therapies have been applied to inhibit NF- κ B activation. Several targets that inhibit the NF- κ B pathway have been identified [58]. For example, bortezomib, a proteasome inhibitor, leads to increased levels of I- κ B kinase and inhibits activation of NF- κ B. Dehydroxymethylepoxyquinomicin, another inhibitor of NF- κ B, induces apoptosis of EBV-transformed B cells [48].

Immunodeficiency-Related Lymphoproliferative Disorders

Congenital Immunodeficiency-Related Lymphoproliferation

Congenital immunodeficiency also called primary immunodeficiency is present at the time of birth, and may occur as a result of defects in B- or T-lymphocytes, or both. X-linked lymphoproliferative disease (XLP) is an inherited syndrome characterized by extreme sensitivity to EBV infection that leads to severe infectious mononucleosis, acquired hypogammaglobulinemia, and/or malignant lymphoma [59]. The defective gene in XLP has been identified as src homology 2 domain protein 1A (SH2D1A) also known as signaling lymphocytic activation molecule (SLAM)-associated protein (SAP) gene [60]. SAP is a key regulator of normal immune function in T cells, NK cells, and in certain B-cell lines. Evidence both from knockout mice and from XLP patients show that SAP deficiency has multiple immunologic effects. These include significantly impaired Th2-like CD4+ T-cell responses, reflected as poor IL-10 production in in vitro assays of T-dependent B cell responses that associate with in vivo defective Ig class switching, affinity maturation of antibody responses, and memory B cell development [61]. Both functional cytotoxic T-cell defects and abnormal cytokine production as a result of SAP deficiency may explain the failure to control EBV infection and predisposition to B-cell lymphoma.

For some patients with primary immunodeficiency, there is promise with hematopoietic stem cell transplant (HSCT).

Lymphoproliferative Disorders Associated with Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

There is an increased frequency of EBV infection in human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS)-associated lymphomas. AIDS patients have 10–20 times more EBV-infected B-cells than healthy counterparts and the risk of NHL is 60–200 times higher than persons noninfected with HIV [1]. EBV is detected in up to 60% of all HIV-related lymphomas, including nearly 100% of primary CNS lymphomas, 80% of DLBCL with immunoblastic features, 30–50% of BL, 70% of primary effusion lymphomas, and nearly 100% of CHL [62, 63]. The increased risk for EBV-related lymphoma among HIV-infected individuals appears related to multiple factors, including duration and degree of immunosuppression, induction of cytokines leading to B-cell proliferation, and coinfection with HHV8. HIV infection can act as chronic stimuli for the B-cell system, characterized by marked hyperplasia of germinal centers, which greatly increases the chances of *MYC* translocations. HIV also induces abnormal immune response and probably increases the number of EBV-infected B-cells that are at risk of being recruited into the germinal center reaction [48]. There is constitutive expression of LMP1 that supports an EBV-driven proliferation.

The use of highly active antiretroviral therapy (HAART) has resulted in a fall in the incidence of opportunistic infections and AIDS-related malignancies, including lymphoma. However, EBV-associated lymphoma still is one of the most frequent causes of death in HIV-infected patients. Therefore, there is a need in identifying viral targets and evaluate the potential benefit of molecular-targeted chemotherapy.

Iatrogenic Immunodeficiency-Associated Lymphoproliferative Disorders

The WHO classification includes the category of “Other iatrogenic immunodeficiency-associated lymphoproliferative disorders” that are lymphoid proliferations or lymphomas that arise in patients treated with immunosuppressive drugs for autoimmune diseases or conditions other than in the transplant setting. The most common subtype of lymphoma is DLBCL. The better-known agent is methotrexate (MTX), which is commonly used in patients with rheumatoid arthritis. MTX directly reactivates EBV with subsequent release of infectious virions [64]. Since patients with rheumatoid arthritis have impaired T-cell responses to EBV products [65], therapy with MTX results in higher EBV loads in their blood and immunodeficient conditions that predispose to EBV-driven lymphoma.

Posttransplant Lymphoproliferative Disorders

Posttransplant lymphoproliferative disorders (PTLD) constitute a heterogeneous group of lymphoproliferations that occur in the setting of allogeneic transplantation of solid organs (SOT) or hematopoietic stem cells (HSCT). In patients with PTLD, the incidence of EBV ranges from 73 to 100% [5]. Morphologically, PTLD can be subdivided into monomorphic, polymorphic, plasmacytic, or HL-like variants. Most PTLD are of B-cell origin, and 10–15% are of T-cell origin [66, 67].

Patients with PTLD have impaired anti-EBV cellular immunity because of iatrogenic immunosuppression, resulting in EBV-induced transformation of B-cells. In addition, the immunomodulation used to prevent graft-versus-host disease (GVHD) remove T-cells nonspecifically from the graft and increases the risk of PLTD [68].

The pathogenic mechanisms of EBV in PTLD are presumably similar to those in CHL. Because approximately 50% cases of PTLD are derived from aberrant GC B cells that lack a functional BCR, HRS cells escape apoptosis through alternative survival signals. It is considered that LMP1 and LMP2A replace survival signals induced by activated CD40 and BCR receptors and activate NF- κ B signaling pathway, inducing proliferation of neoplastic cells. As already mentioned, the decreased cytotoxic T-cell surveillance also increases the susceptibility to EBV.

In the absence of effective T-cell surveillance, EBV+ lymphomas in immunodeficient individuals usually express a latency type III pattern. All the EBNA and LMP viral proteins are expressed together with various noncoding small RNAs (EBERs and miRNAs). EBNA3 family of proteins (EBNA3A, 3B, and 3C) are nuclear phosphoproteins that act as transcriptional regulators. Only EBNA3A and EBNA3B have been shown to be essential for B-cell transformation. A number of functional domains have been characterized for these proteins, including transactivation, repression, and nuclear localization domains, but their roles have not been elucidated. It has been noted that conserved regions of EBNA3A, 3B, 3C are capable of binding to RBP, while EBNA3A and 3C can bind to the ATPase/Helicase DP103 [69]. Krauer et al. [70] showed that EBNA-3 disrupt the DNA damage and replication at the G2/M checkpoint. EBNA3A and EBNA3C together have been found to interfere with the proapoptotic protein Bim [71]. EBNA3C appears to also have repressor functions potentially mediated via its interaction with a histone deacetylase [72]. In addition, studies have shown that EBNA3C is an immortalizing oncogene capable of cooperating with (Ha)-ras in cotransformation assays and is capable of overriding Rb-mediated pathways [73].

In latency III, EBNA2 acts as a transcription factor to induce expression of the viral LMP genes and many cell genes. EBNA2 interacts with a sequence-specific DNA-binding protein, κ -recombination-binding protein (RBP- κ) [3], to transcriptionally activate cellular genes such as CD21 and CD23 and key viral genes LMP1 and LMP2A [74, 75]. In addition, EBNA2 can modify chromatin structure through recruitment of SWI/SNF. EBNA-LP interacts with EBNA2 and is required for the efficient outgrowth of virus-transformed B cells in vitro. The transcriptional activation mediated by EBNA2/EBNA-LP is modulated by the EBNA3 family of proteins, repressing transactivation.

Therapeutic Targets of EBV

The association of EBV with various lymphoproliferative disorders suggests that EBV plays a pathogenic role. Various pathways linked with tumorigenesis are activated in these processes, and plausible mechanisms involving viral products have been identified. Thus, the identification of the molecular mechanisms associated with EBV tumor promotion and progression may contribute to identify molecular targets for immune attack, small molecules or interfering RNA. Other therapeutic options include adoptive immunotherapy, antiviral therapy, and therapies against EBV-driven signaling or transfer of antigens.

Adoptive transfer of EBV-specific cytotoxic T-cells (CTLs) has been proven effective in treating posttransplant EBV-associated lymphomas. The research in this field has moved quickly from animal experiments to the bedside [75]. EBV-specific CTL have been successfully infused in patients subjected to hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT), both with therapeutic and prophylactic purposes. In a study that included 108 HSCT recipients and 21 SOT

recipients, patients received virus-specific CTL as a preemptive approach. No events of lymphoproliferation were reported at follow-up, except for one patient who received an infusion lacking a well-defined EBV-specific component [76]. The success of CTL therapy in PTLD has not been reproduced in CHL and BL probably due to cell mechanisms that evade EBV-specific immune responses. For example, HRS cells create a microenvironment that suppresses EBV-specific T-cell responses.

EBV-related lymphoproliferations have different latency patterns that reflect the interaction between the immune response of the host and the virus. Thus, the virus expresses as many proteins as the immune system of the host allows. As a rule, the more immunosuppressed the host is, the more viral particles are produced, and thus EBV+ neoplasms express more viral antigens. Of the viral antigens, EBNA1, LMP1, LMP2 are the targets more frequently challenged using CTL therapy. In particular, cytotoxic LMP1 or EBNA1-specific CD4+ T cells, have proven effective not only in vitro against LCLs and infected NK/T cells, but also against naturally expressed targets in NK/T cell lymphoma and CAEBV [77, 78]. These therapies need further refinement in generating such in vitro and in demonstrating their efficacy in vivo.

Antiviral therapy is another therapeutic approach. A phase I/II trial of arginine butyrate and ganciclovir in 15 patients with refractory EBV+ lymphoid malignancies was well tolerated, achieving good antitumor response in ten patients [79]. Cidofovir, another antiviral drug, downregulated LMP1 expression and decrease BCL-2 levels in lymphoma cells.

Anti-CD30 monoclonal antibody, which inhibits growth of CD30-expressing tumor cells has been used in patients with refractory CHL, independent of EBV status, achieving success [80]. Another study showed that the combination of MTX and irradiation significantly induced apoptosis and growth inhibition in two EBV-expressing NK/T-cell lines, via downregulation of NF- κ B signaling. The NF- κ B inhibition highlighted an efficacious therapeutic approach for patients with nasal T/NK-cell lymphoma and other EBV-related lymphomas.

Further experience and better delivery of molecular therapies may provide safe and efficacious therapeutic benefits for EBV-related lymphoproliferative disorders, which coupled with other therapies that target simultaneously other mechanisms of oncogenesis may contribute to better management of these disorders.

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