Malignancies Associated With Epstein-Barr Virus: Pathobiology, Clinical Features, and Evolving Treatments

Natalia Neparidze, MD, and Jill Lacy, MD

Abstract: Epstein-Barr virus (EBV) is associated with a wide variety of B-cell–derived lymphoid neoplasms, including Burkitt lymphoma, lymphomas arising in immunocompromised patients (post-transplant and HIV-associated lymphomas), and Hodgkin lymphoma. In addition, EBV has been linked to some T-cell lymphomas (angioimmunoblastic T-cell lymphoma, extranodal nasal-type natural killer/T-cell lymphoma, and other rare histotypes), nasopharyngeal cancer, and a subset of gastric cancers. Advances in our understanding of the pathobiology of EBV oncogenesis, including the transforming and immunogenic properties of the virus and the role of immune dysregulation, have provided the rationale for new treatment strategies. Emerging EBV-specific therapeutic approaches include activation of lytic viral infection combined with antiviral drugs, inhibition of EBV-induced oncogenic cellular signaling pathways, adoptive EBV-specific T-cell therapies, and EBV vaccines. This review summarizes the pathobiology, clinical features, and treatment of EBV-associated malignancies, including new and evolving therapies focused on exploiting the pathobiology of EBV.

Dr Neparidze is an assistant professor of medicine and Dr Lacy is an associate professor of medicine in the Section of Medical Oncology at the Yale University School of Medicine in West Haven, Connecticut.

Address correspondence to:
Natalia Neparidze, MD
Assistant Professor of Medicine
Section of Medical Oncology
Yale University School of Medicine
VA Connecticut Healthcare Center
950 Campbell Avenue (III-d)
West Haven, CT 06516
Phone: 203-937-3421
Fax: 203-937-3803
E-mail: natalia.neparidze@yale.edu

Keywords
Burkitt lymphoma, Epstein-Barr virus, gastric cancer, Hodgkin lymphoma, nasopharyngeal, post-transplant lymphoproliferative disorder

Introduction

Epstein-Barr virus (EBV), a ubiquitous B-lymphotropic herpesvirus, was the first virus directly linked to cancer in humans. Since its discovery and association with Burkitt lymphoma (BL) 50 years ago, EBV has been associated with a heterogeneous group of lymphomas and epithelial tumors (Table 1). It infects B and T lymphocytes, follicular dendritic cells, smooth muscle cells, squamous epithelium of the oropharynx and nasopharynx, and glandular epithelium of the thyroid, stomach, and salivary glands. B lymphocytes are the major cellular reservoir for EBV persistence, and the majority of EBV-associated malignancies derive from EBV-infected B cells. EBV encodes an array of products that mimic or activate antiapoptotic molecules, cytokines, and signal transducers, thereby promoting EBV infection, immortalization, and transformation.
Significant progress has been made in the treatment of EBV-associated malignancies, with decreased mortality and morbidity, but multiple challenges remain. The scope of ongoing and future research includes the drug discovery of inhibitors of viral targets and EBV-activated cellular targets, the disruption of latent infection, and the development of immunotherapeutic agents, including adoptive cellular therapies and vaccines.

**Pathobiology of the Epstein-Barr Virus**

EBV is a gamma herpesvirus that infects more than 95% of the world’s population by adulthood. Primary infection results in transient viremia followed by a rapid immune response. The virus achieves lifelong persistence in its human host by balancing its ability to evade the immune system via latent infection of B lymphocytes and its ability to replicate and shed from the oral mucosa. EBV infection of B lymphocytes leads to 2 processes—the production of latently infected memory B cells that persist long term, and the differentiation toward plasma cells permissive of the replication of infectious virions. These outcomes support latent viral persistence and viral propagation. In vitro, EBV transforms human B cells into continuously proliferating immortalized lymphoblastoid cells, and this process has been used to elucidate the transforming properties of the virus and its gene products.

The hallmarks of latent infection in EBV-associated malignancies are the maintenance of a stable number of extrachromosomal episomal EBV genomes and the highly restricted expression of viral genes (reviewed by Kieff and Rickinson). Latent viral genes encode 6 nuclear proteins (EBNA1, -2, -3A, -3B, -3C, and -LP); 3 cytoplasmic latent membrane proteins (LMP1, -2A, and -2B); and the nontranslated EBV-encoded RNAs (EBERs 1 and 2) and BamH1 A rightward transcripts (BARTs) (Table 2). Only EBNA1, -2, -3A, and -3C and LMP1 are essential for the transformation of B cells. EBNA1 is a DNA-binding phosphoprotein required for the replication and maintenance of EBV episomal genome. EBNA2 and the EBNA3 proteins mediate the transcriptional activation of cellular and viral genes. LMP1 is the major transforming protein of EBV and a classic oncogene in vitro. LMP1 functions as a constitutively activated member of the tumor necrosis factor receptor superfamily, mimicking CD40 signaling. The pleiotropic oncogenic properties of LMP1 are mediated via activation of several cell signaling pathways, including nuclear factor (NF)κB, with concomitant upregulation of BCL2. LMP2A mimics constitutive B-cell receptor (BCR) activation by interacting with BCR signaling molecules, conferring BCR-like prosurvival properties. Given the absence of viral lytic gene expression, including EBV DNA polymerase, latently infected cells are not susceptible to classic antiviral nucleoside analogues (eg, ganciclovir and acyclovir), and thus these agents do not have direct antitumor effects in EBV-associated malignancies.

Three distinct patterns of latent viral protein expression are identified in EBV-associated malignancies (see Table 2). BL is characterized by expression restricted to EBNA1 (latency I), whereas expression of all 6 EBNAs and LMP1, -2A, and -2B (latency III) characterizes lymphomas arising in the setting of immunosuppression. In latency II, expression is limited to EBNA1 and the LMPs, a pattern seen in Hodgkin lymphoma (HL) and nasopharyngeal carcinoma (NPC). Although specific latency patterns are linked to specific EBV-related malignancies, there is heterogeneity of expression among tumors of the same histologic type, and even among cells...
within a given tumor. The immune response to latent EBV infection correlates with the pattern of latent viral expression, an observation that has important implications for immunotherapeutic approaches to specific EBV-associated malignancies.

The pathogenetic role of EBV in oncogenesis is an area of ongoing investigation. EBV activates multiple cellular signal transduction pathways to deregulate cell growth and promote survival. A key pathway affected by the virus is NFκB, which upregulates BCL2 and promotes cell survival; the JAK-STAT, PI3 kinase (PI3K)/Akt/mTOR, JNK/AP1, and MAP kinase pathways are also affected. Immune dysregulation plays a central pathogenetic role in EBV-driven B-cell lymphomas arising in the setting of immunodeficiency. Our understanding of the pathogenesis of these malignancies has provided the rationale for the development of novel treatment strategies focused on aspects of EBV pathobiology, including inhibition of signal transduction pathways, modulation of antiviral immune mechanisms, and activation of lytic/suicidal infection in malignant cells (Table 3).

Intriguing geographic variability has been noted in the incidence of some EBV-related tumors. NPC and BL are endemic in southern China and equatorial Africa, respectively, but uncommon elsewhere in the world. Extranodal nasal-type natural killer (NK)/T-cell lymphomas show a geographic predilection for regions of Asia, South America, and Central America and are rarely seen in the United States and Europe. These geographic associations are not fully explained. Preliminary studies implicate environmental cofactors over genetic predisposition or oncogenic viral strains.

### Diagnosis of Epstein-Barr Virus–Associated Malignancies

The diagnosis of EBV-associated malignancies relies on the detection of viral DNA and/or its gene products in neoplastic cells. The detection of EBV-encoded EBER transcripts by in situ hybridization is considered the gold standard for localizing latent EBV in tissue samples, as EBER transcripts are universally expressed in all EBV-associated tumors. The immunohistochemical detection of LMP1 expression in tumor cells can be diagnostic, although LMP1 is not universally expressed in all EBV-associated malignancies (see Table 2).

The level of EBV DNA measured in whole blood, peripheral blood mononuclear cells, plasma, or serum by quantitative polymerase chain reaction (PCR), referred to as the EBV DNA load, has been extensively evaluated as a potential surrogate marker for EBV-positive malignancies, although its routine clinical use remains investigational. The EBV DNA load has been useful in predicting the development of post-transplant lymphoproliferative disorder (PTLD) and in monitoring response to treatment. In patients at high risk for PTLD, elevation of the EBV DNA load is a sensitive aid to early diagnosis, particularly when used in conjunction with studies of impaired EBV-specific T-cell recovery. In EBV-associated HL and extranodal nasal-type NK/T-cell lymphoma, a plasma EBV DNA load at diagnosis is an indicator of disease activity and is associated with an unfavorable prognosis. The detection and quantification of EBV DNA in cerebrospinal fluid by PCR is considered diagnostic of primary central nervous system lymphoma (PCNSL) in the absence of biopsy. In NPC, the EBV DNA load in

---

**Table 2. Epstein-Barr Virus Latent Genes: Function and Patterns of Expression in Epstein-Barr Virus–Associated Malignancies**

<table>
<thead>
<tr>
<th>EBV Gene</th>
<th>Function</th>
<th>Latency I BL</th>
<th>Latency II HL, NPC, Nasal T/NK-Cell Lymphoma</th>
<th>Latency III PTLD, PCNSL, HIV-Associated DLBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBNA1</td>
<td>Episomal EBV genome maintenance</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EBNA2</td>
<td>Activation of EBV and cellular gene expression</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EBNA3A, -3B, -3C</td>
<td>Modulation of EBV and cellular gene expression</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EBNALP</td>
<td>Coactivation with EBNA2</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>LMP1</td>
<td>Mimics CD40 signaling</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LMP2A</td>
<td>Mimics BCR signaling</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EBERs</td>
<td>Noncoding RNAs</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BARTs</td>
<td>Precursors for miRNAs</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

BCR, B-cell receptor; BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; HL, Hodgkin lymphoma; miRNA, micro-RNA; NK, natural killer; NPC, nasopharyngeal carcinoma; PCNSL, primary central nervous system lymphoma; PTLD, post-transplant lymphoproliferative disorder.

*The symbols in the columns represent that latent viral protein expression is present (+), absent (-), or potentially present or absent (±).*
plasma is useful as a diagnostic and prognostic tool and in monitoring response to treatment.15,16

Epstein-Barr Virus–Associated Lymphoproliferative Disorders

The EBV-associated B- and T-cell lymphoproliferative disorders are a heterogeneous group of malignancies but share the feature of harboring latent EBV within tumor cells. Immunodeficiency states, such as HIV infection, congenital immunodeficiencies, post-transplant immunosuppression, and chronic active EBV infection, increase the risk for EBV-associated lymphoma.

These lymphomas display different patterns of latent viral gene expression, with the potential for distinct novel treatment approaches, such as T-cell immunotherapy targeting EBV.17 However, at present, the standard treatment of EBV-associated lymphomas is usually identical to that of their EBV-negative counterparts, with the exception of PTLD.

Burkitt Lymphoma

The isolation of virus particles from BL cell lines in 1964 led to the discovery of EBV and its association with BL. BL is a highly aggressive non-Hodgkin lymphoma (NHL) characterized by a diffuse infiltrate of monomorphic, medium-size B cells in a “starry sky” pattern, imparted by
numerous benign macrophages, and by an extremely high proliferative index, with a Ki-67 approaching 100%. BL is divided into 3 subtypes: endemic (eBL), sporadic (sBL), and HIV-associated. eBL presents as tumors affecting the jaw and facial bones in young children in equatorial Africa, whereas sBL occurs worldwide and involves the gut, upper respiratory tract, or Waldeyer ring. HIV-associated BL characteristically involves lymph nodes and bone marrow. BL is not universally associated with EBV. Although more than 90% of cases of eBL are EBV-positive, only 5% to 20% of cases of sBL and 40% of cases of HIV-associated BL are EBV-positive.

EBV gene expression in BL cells from primary tissue is highly restricted, with expression limited to EBNA1 (latency I). The absence of consistent expression of the immunogenic EBNA and LMP proteins facilitates evasion from cytotoxic T-lymphocyte (CTL)-mediated immunosurveillance and contributes to BL pathogenesis. Moreover, the use of adoptive cellular therapies in BL is limited by the insensitivity of EBNA1-restricted BL cells to CTL-mediated cytotoxicity.

Because EBV is not essential in the pathogenesis of BL, the mechanisms by which EBV contributes to the development of BL remain uncertain. In eBL, it is believed that hyperstimulation of B cells and suppression of T-cell activity by chronic malarial infection is permissive for the reactivation of EBV in infected B cells, leading to a dramatic expansion of EBV-infected B-cell populations. In both EBV-positive and EBV-negative BL, constitutive activation of the c-MYC oncogene through its translocation into one of the immunoglobulin loci is the critical oncogenic event. Whether there is a causal relationship between EBV and the translocation of MYC is unknown.

The optimal treatment of BL has yet to be defined, and patients with BL should be enrolled in clinical trials whenever possible. Outside a clinical trial, treatment consists of intense combination chemotherapy regimens with CNS prophylaxis, such as fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD); cyclophosphamide, vincristine, doxorubicin, and high-dose methotrexate (CODOX-M) with ifosfamide, etoposide, and high-dose cytarabine (IVAC); or the Cancer and Leukemia Group B (CALGB) 9251 protocol, usually in combination with rituximab (Rituxan, Genentech/Biogen Idec). The less intensive 9251 protocol, usually in combination with rituximab (IVAC); or the Cancer and Leukemia Group B (CALGB) 9251 protocol, usually in combination with rituximab (Rituxan, Genentech/Biogen Idec). This finding provides the rationale for the development of inhibitors of LMP2A-induced cellular targets, such as the PI3K/Akt/mTOR pathway. In a model of EBV-associated BL in Tg6/λ-MYC transgenic mice, the mTOR inhibitor rapamycin reversed splenomegaly and decreased tumor growth and metastasis in bone marrow. An alternative approach is the induction of lytic EBV infection, leading to cell lysis, coupled with anti-EBV agents. Thus, bortezomib (Velcade, Millennium Pharmaceuticals) may activate EBV lytic gene expression in BL cell lines in the context of endoplasmic reticulum stress, with C/EBPβ playing a role in this process, and lytic cytotoxicity induced by lactone parthenolide in combination with ganciclovir has shown promise as a virus-targeted therapy in BL in studies in vitro. Recently, high-throughput screen technologies have identified small molecular inhibitors of EBNA1, and further development of EBNA1 inhibitors may provide a treatment specific for EBV latent infection.

Hodgkin Lymphoma

The identification of EBV DNA, EBER RNA, and LMP1 in Reed-Sternberg (RS) cells in a subset of HL has confirmed the link between EBV and HL. Furthermore, epidemiologic and case-control studies have shown an increased risk for EBV-positive but not EBV-negative HL in individuals with a history of infectious mononucleosis or an altered serologic response to EBV latent antigens, supporting a causal association between EBV and HL. The prevalence of EBV varies widely among the pathologic subtypes of HL. EBV is present in 70% of cases of mixed-cellularity HL, 95% of cases of lymphocyte-depleted HL, and 10% to 40% of cases of nodular sclerosing HL, whereas nodular lymphocyte-predominant HL is generally EBV-negative. HL arising in the setting of immunodeficiency (eg, HIV infection, iatrogenic immunodeficiency) is usually EBV-positive.

EBV gene expression in RS cells is restricted to EBNA1, LMP1, LMP2A and -2B, and EBERs (type II latency). The EBV genomes found in RS cells are clonal, indicating that EBV infection precedes clonal expansion and implicating an etiologic role of the virus. However, the precise role of EBV in HL pathogenesis is uncertain. Expression of LMP1 and LMP2A may prevent apoptosis through the induction of antiapoptotic proteins. RS cells (both EBV-positive and EBV-negative) produce immunosuppressive cytokines such as interleukin 10, interleukin 13, and transforming growth factor-β. EBV infection of primary RS cells and RS cell–derived cell lines has been
shown to increase expression of the CCL20 chemokines, which in turn increases the migration of CD4+/FOXP3+ regulatory T cells (Tregs). This observation identifies a mechanism by which EBV-infected RS cells can recruit Tregs to the HL microenvironment and prevent immune responses against the virus-infected RS cells.\(^{39,51}\)

Standard treatment of EBV-positive HL is not different from that for EBV-negative HL of the same stage, histology, and prognosis\(^{52}\), and is guided by clinical stage and risk stratification. Systemic chemotherapy with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD), followed by involved-field radiotherapy when indicated, is considered the gold standard. The regimen of bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPP) is an alternative option for patients with high-risk, advanced-stage disease.

The success of adoptive immunotherapy with ex vivo expanded allogeneic or autologous EBV-specific CTLs in PTLD (described below) has led to the application of this strategy in HL. Limited experience with EBV-specific CTLs in patients with recurrent, refractory, EBV-positive HL has shown promise.\(^{53-55}\) However, this strategy has not been widely adopted because of the complexity of the technique. In addition, the immune microenvironment of the tumor might impede the efficacy of CTLs in HL.

The incidence of classic HL is increased in settings of impaired immunity, including after transplant. Post-transplant HL is invariably EBV-positive and should fulfill the diagnostic criteria for classic HL. The majority of patients are men, and all have received post-transplant immunosuppression.\(^{56-59}\) The time from transplant to the onset of the disease ranges from a few months to several years and is generally longer than that for non-Hodgkin PTLDs.\(^{39,68}\) In 50% of cases, the disease presents as extranodal masses in liver or lung, and other extranodal sites can be involved.

The optimal treatment of post-transplant HL is not well defined. The clinical course is aggressive, and the outcome is poor. The majority of patients are initially managed by reduction or withdrawal of immunosuppression. The use of chemotherapy may be limited because of comorbidities, and the response rate is lower than in classic HL. Rituximab is highly effective in non-Hodgkin PTLD,\(^{61}\) and some patients with post-transplant HL respond to rituximab.

**Post-transplant Lymphoproliferative Disorder**

It is widely recognized that the incidence of lymphoproliferative disorders is increased in transplant recipients of both solid organ and hematopoietic stem cell allografts. The vast majority of these PTLDs are associated with EBV. The process likely begins with dysregulated EBV-driven B-cell proliferation due to impaired EBV-specific T-cell–mediated immune surveillance of infected recipient or donor B cells. This leads to a dramatic expansion of the EBV-infected B-cell population, the acquisition of mutations, and ultimately, malignant transformation.\(^{52}\)

Approximately 95% of all PTLDs are associated with EBV, as shown by EBER expression in tissue-infiltrating lymphocytes and/or immunoblasts. There are 4 major World Health Organization (WHO) categories of PTLD: early lesions, polymorphic PTLD, monomorphic PTLD, and classic HL-type PTLD. In practice, a clear separation between the WHO categories of PTLD is not always possible. Early lesions, polymorphic PTLD, and monomorphic PTLD probably represent a pathologic spectrum. Early lesions are polyclonal/oligoclonal, whereas polymorphic PTLD and monomorphic PTLD are usually monoclonal by immunoglobulin gene rearrangement and EBV episomal testing, although the latter is not routinely performed in clinical practice.\(^{53}\) The diagnosis of PTLD is based upon an evaluation of histologic, immunophenotypic, virologic, and genetic studies interpreted in the context of the clinical scenario.\(^{63-65}\)

PTLD-like tumors occasionally occur in patients without transplants who are immunosuppressed for other reasons, such as patients with rheumatoid arthritis who are on methotrexate therapy. As in PTLD, these tumors are often EBV-positive and respond favorably to immune reconstitution.

The incidence of PTLD is greatest within the first year after transplant. Major factors related to the risk for its development are the degree of T-cell–specific immunosuppression and the EBV seronegative status of the recipient. Specific risk factors for PTLD after hematopoietic stem cell transplant (HSCT) include T-cell depletion of the allograft, T-cell–depleting conditioning regimens, the use of antithymocyte globulin, acute and chronic graft-versus-host disease (GVHD), second allogeneic HSCT, and age older than 50 years.\(^{56}\)

Management strategies that may reduce the incidence of overt PTLD include limiting exposure to aggressive immunosuppressive regimens by judicious tapering to maintenance target levels and the use of anti-EBV prophylaxis (eg, ganciclovir) to prevent EBV reactivation.\(^{57,69}\) Preemptive treatment of the reactivation of EBV infection, as determined by monitoring the EBV load in peripheral blood, with rituximab or with reduced immunosuppression can prevent PTLD and clear EBV from the peripheral blood.\(^{7}\) Thus, many transplant centers routinely incorporate post-transplant surveillance of the EBV DNA load and preemptive treatment strategies into their transplant protocols.

PTLD is a life-threatening complication of allogeneic transplantation. Early in its course, PTLD may cause...
minimal or no symptoms. When it is symptomatic, the manifestations are variable and include constitutional symptoms (eg, fever, weight loss, fatigue), lymphadenopathy, and dysfunction of affected organs (eg, severe hepatitis, pneumonia, colitis, nephritis). Involvement of extranodal sites is common, including the CNS. In 25% of patients, the allograft itself is infiltrated with PTLD, which can cause allograft failure.70-71 Laboratory studies often demonstrate an elevated lactate dehydrogenase level and monoclonal protein in serum or urine.

Treatment for PTLD depends on the subtype of PTLD, type of allograft, need for rapid cytoreduction, and treatment-associated toxicities. Treatment entails a reduction of immunosuppression to permit restoration of the EBV-specific CTL response, unless graft rejection precludes this intervention. Other options include rituximab, cytotoxic chemotherapy, and radiation. Single-agent rituximab is highly effective and is considered the first-line treatment at many transplant centers.61 Because of the heterogeneity of PTLD and the unique features of each case, approaches to both initial treatment and salvage therapy must be individualized. In general, in the absence of fulminant disease, treatment proceeds in a stepwise fashion, with the most intensive therapies reserved for patients with pathologically and clinically aggressive or recurrent disease. Notably, EBV-negative PTLD occurs later (usually 2 years) after transplant and does not respond as well to the withdrawal of immunosuppression.72

CNS involvement is a particularly poor prognostic feature of PTLD.73-74 Treatment strategies include the use of antiviral agents, immunotherapy, radiation therapy, and chemotherapy, but outcomes remain dismal. Although the use of intense chemotherapy poses unique risks to transplant recipients, high-dose methotrexate can be efficacious and tolerable in patients with CNS PTLD.75-78

Adoptive transfer of EBV-specific CTLs is a highly effective investigational approach for the prevention or treatment of PTLD. PTLDs are characterized by the expression of all of the immunodominant EBV latency proteins (latency III), and thus, in contrast to BL (latency I), they are amenable to T-cell–based cellular therapies. This strategy typically uses EBV-infected lymphoblastic cell lines to repetitively stimulate donor-derived T cells (or autologous T cells in the setting of a solid organ transplant), followed by ex vivo expansion over several weeks and finally transfer to the affected patient.79-81 In contrast to unmanipulated donor lymphocyte infusions, EBV-specific CTLs can reconstitute an in vivo immune response without inducing GVHD. Prophylactic infusions of EBV-specific CTLs prevent PTLD in virtually all patients, and the clinical outcomes of patients with overt PTLD are favorable, with the majority of patients achieving durable remissions.79-82,83 However, the widespread use of adoptive cellular therapy for PTLD and other EBV-associated malignancies is limited by the need for specialized facilities and the length of time required to prepare the EBV-specific CTLs (8-12 weeks). Recent efforts have focused on developing technologies that will decrease the time required to produce EBV-specific CTLs and thus broaden the applicability of this approach, such as rapid ex vivo culture, rapid isolation of EBV peptide–selected CTLs, and the use of banked, “third party” human leukocyte antigen–typed EBV-specific T-cell lines.84-87

HIV-Associated Non-Hodgkin Lymphomas

HIV infection is associated with a dramatic increase in the risk of developing NHL; 40% of these cases are associated with EBV. The risk of HIV-associated NHL, also known as AIDS-related NHL, is related to the degree of immune dysfunction and is greatest in patients with low CD4-positive cell counts (<100/μL) and high HIV loads. Although the incidence of HIV-associated NHL has decreased with the widespread use of highly active antiretroviral therapy (ART), these diseases continue to make up a substantial portion of NHLs in the United States (6% of diffuse large cell B-cell lymphomas [DLBCLs], 20% of BLs, and 27% of PCNSLs).

NHLs arising in the setting of HIV are generally diffuse aggressive or highly aggressive subtypes. In contrast to PTLD, which includes polyclonal lesions, HIV-associated NHLs are always monoclonal. The most common forms are DLBCL with immunoblastic or centroblast histology, BL, and PCNSL. Less common lymphomas, encountered almost exclusively in HIV-infected patients, include plasmablastic lymphoma of the oral cavity type and human herpesvirus 8/Kaposi sarcoma herpesvirus–positive primary effusion lymphoma (PEL) and its solid variant. Although 40% of HIV-associated lymphomas are EBV-positive, the incidence varies with the histologic subtype and site of disease. EBV is present in nearly 100% of PCNSLs, PELs, and plasmablastic lymphomas; in 70% of DLBCLs (100% of immunoblastic and 40% of centroblastic DLBCLs); and in just 30% of BLs. EBV-positive HIV-associated NHLs typically exhibit plasmacytoid-plasmablastic differentiation as a unifying histopathologic feature.88

EBV-associated lymphomagenesis in HIV infection is attributed to the transforming properties of EBV in conjunction with impaired immunosurveillance of EBV. In contrast to PTLD, EBV-positive HIV-associated NHLs are always monoclonal, implicating an important pathogenetic role of superimposed genetic events. Altered EBV antibody patterns and decreased EBV-specific T-cell responses are shown to precede the onset of EBV-positive HIV-associated NHL.89-91 Although the EBV DNA load in peripheral blood mononuclear cells is not predictive of lymphoma occurrence in patients with HIV infection.92 In
healthy individuals, CD27-positive memory B cells are the main carriers of EBV infection. Despite the loss of memory B cells in HIV, elevated EBV loads are observed, suggesting that populations of B cells other than memory B cells are implicated in EBV persistence and lymphomagenesis.99

As with NHL in HIV-negative patients, the choice of chemotherapy regimen, need for CNS prophylaxis, and role of radiotherapy are dictated by the pathologic subtype, stage, and institutional preference. The inclusion of ART in management improves the response rate and survival and decreases opportunistic infections in patients with HIV-associated NHL undergoing chemotherapy. In contrast to PTLD, HIV-associated NHLs are inadequately treated with immune reconstitution alone (ie, the initiation of ART). Special considerations in treatment include the increased risk for infection. Patients should receive growth factor support and Pneumocystis prophylaxis with consideration of enteric antibiotic, antiherpetic, and/or antifungal prophylaxis. The inclusion of rituximab improves remission rates in CD20-positive HIV-associated NHLs, although it may be associated with an increased risk for infectious deaths in patients who have severe lymphopenia (CD4-positive cell count <50/μL).94 Pooled analysis from 19 prospective trials revealed that the inclusion of rituximab, the use of a dose-intensive regimen such as doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone (ACVBP) or an infusional regimen such as etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (EPOCH), and concurrent ART are all associated with improved overall survival in HIV-associated NHL.95 Recent evidence suggests that incorporating high-dose methotrexate into initial therapy results in lower rates of CNS relapse in patients with high-risk DLBCL.96

The presence of EBV has been exploited to develop novel therapies for HIV-associated NHLs. The strategy of pharmacologic induction of lytic infection is thought to induce cytotoxicity and render tumor cells susceptible to antiherpes nucleoside analogues (eg, ganciclovir), which require phosphorylation by the lytic-specific EBV thymidine kinase.97,98 Several agents, including short-chain fatty acids and other histone deacetylase inhibitors, bortezomib, and chemotherapeutic agents, disrupt EBV latency and sensitize EBV-transformed B cells to nucleoside antiviral agents in vitro.36,97,99,100 This strategy has shown promise in a pilot study of arginine butyrate in combination with ganciclovir in patients with refractory EBV-positive lymphoid malignancies.100 Zidovudine (AZT), alone or with chemotherapy (eg, hydroxyurea), induces apoptosis in EBV-positive BL cell lines, possibly by inhibition of NFκB and activation of the lytic cycle.101 Thus, the combination of AZT with methotrexate or hydroxyurea, both of which penetrate the blood-brain barrier, may be of particular benefit in PCNSL, and responses in patients with PCNSL have been reported anecdotally with these combinations.102

Other novel therapeutic approaches exploit the activation of signal transduction pathways by EBV, including NFκB and PI3K/Akt/mTOR. Bortezomib and rapamycin have shown promise in preclinical and limited clinical studies for the treatment of PEL, which responds poorly to traditional chemotheraphy.103-105 Other novel agents have shown promise in PEL cell lines or murine xenograft PEL models, including interferon alfa combined with arsenic trioxide, the anti-CD30 drug conjugate brentuximab vedotin (Adcetris, Seattle Genetics), and histone deacetylase inhibitors.105-108 There have been isolated reports of success with intracavitary cidofovir and with systemic cidofovir combined with interferon alfa and ART.109,110

**Rare EBV-Associated B-Cell Lymphoproliferative Disorders**

**EBV-Positive Diffuse Large B-Cell Lymphoma of the Elderly (“Senile Type”).** Although DLBCL in immunocompetent patients is rarely EBV-positive, the uncommon EBV-positive DLBCL of the elderly (“senile type”) is an EBV1-positive variant of DLBCL that occurs in patients older than 50 years without any known immunodeficiency or prior lymphoma. It is postulated that chronic inflammation and the immune senescence of aging may be cofactors in the development of this subtype. Although rare in Western countries, DLBCL of the elderly accounts for 8% to 10% of cases of DLBCL in Asia.111

The histopathology is characterized by RS-like cells and polymorph features. Expression of CD30, EBER, and LMP1 is detectable in more than 90% of cases, and both latency II and latency III patterns of EBV gene expression have been described. Clonality of the immunoglobulin genes and EBV genome can usually be detected by molecular techniques. DLBCL of the elderly is characterized by prominent NFκB pathway activation, likely mediated by EBV.112,113 Treatment consists of the rituximab (R)-CHOP regimen, although in significant numbers of patients the disease is refractory to chemotherapy. The clinical course is aggressive, and patients have a median survival of about 2 years.114

**Lymphomatoid Granulomatosis.** Lymphomatoid granulomatosis (LYG) is a rare EBV-associated lymphoproliferative disease. Although most patients with LYG are not overtly immunocompromised, the disease is encountered in patients with genetic and iatrogenic immunodeficiency syndromes. LYG invariably involves the lungs in a nodular pattern, but it may also affect the CNS, skin, liver, and kidneys.115 Pathologically, it is characterized by an angiocentric and angiodestructive infiltrate made up of a small
The extent of the disease dictates the choice of treatment. Localized disease is managed by radiotherapy, often
with concurrent chemotherapy; disseminated disease is treated with chemotherapy (eg, CHOP, CHOP-Bleo, l-asparaginase–containing regimens).\textsuperscript{134,135} Large randomized trials comparing treatments for this disease entity are lacking. The prognosis of patients with disseminated disease is poor.

**Epstein-Barr Virus–Associated Epithelial Malignancies**

EBV is an etiologic factor in nonkeratinizing NPC and a subset of gastric carcinoma. The pathogenetic role of EBV in epithelial carcinogenesis is not completely understood. As in EBV-associated lymphomas, infection in these epithelial malignancies is latent, with expression of EBNA1, LMP2A, and LMP2B and variable expression of LMP1, as well as the nontranslated EBERs and BARTs. The LMP oncoproteins are believed to play a direct oncogenic role in EBV-associated epithelial malignancies, given their profound effects on cellular gene expression and cell growth and survival. The recent discovery of EBV-encoded micro-RNAs showed that these micro-RNAs function as post-transcriptional gene regulators and may play a role in carcinogenesis.\textsuperscript{136-138}

EBV infection alone is insufficient to transform epithelial cells, and multiple genetic and epigenetic abnormalities have been described in EBV-associated NPC and gastric cancers. In NPC, recurring chromosomal aberrations (eg, loss of 3p, 9p, and 14q; gain of 12p and 3q) and widespread hypermethylation of the genome result in inactivation of key tumor suppressor genes (eg, p16/CDKN2A, RASSF1A, E-cadherin/CDH1) and activation of key oncogenes (eg, PI3K/PIK3CA).\textsuperscript{139} Several lines of evidence in NPC suggest that preexisting genetic alterations in precursor dysplastic lesions are important in susceptibility to EBV infection and maintenance of latency, and that EBV promotes additional epigenetic change.

**Nasopharyngeal Carcinoma**

The geographic incidence of undifferentiated nonkeratinizing NPC (type III) varies widely. The incidence is highest in southern China, where NPC is the fourth most common cancer diagnosis. In contrast to keratinizing squamous cell NPC, endemic NPC is generally associated with EBV.\textsuperscript{140,141} Clonal EBV genomes have been detected in precursor dysplastic lesions of the nasopharynx and in invasive NPC, supporting a possible causal link between EBV and NPC.\textsuperscript{142,143} EBV serologies in NPC are aberrant, characterized by the presence of immunoglobulin A (IgA) antibodies directed against EBV viral capsid antigen (VCA IgA) and early antigen (EA IgA). The plasma EBV load correlates with the diagnosis and the pre- and post-treatment prognosis of NPC, and it is useful in response assessment and post-treatment surveillance.\textsuperscript{144,145} EBV serologies and EBV DNA load have been useful in screening for NPC in areas where NPC is endemic.\textsuperscript{148,149}

Currently, the standard treatment of NPC is radiotherapy for stage I disease and concurrent chemoradiotherapy with cisplatin for locally advanced disease in stages II through IVB (reviewed by Ma and colleagues).\textsuperscript{150} Although current treatments for NPC have improved the 5-year survival rate to 50%, metastatic disease for which there is no curative therapy eventually develops in 20% to 25% of patients.

As in EBV-associated lymphomas, the presence of EBV has been exploited in the development of novel treatments for recurrent and metastatic NPC. These approaches include targeting EBV-activated signal transduction pathways, lytic cycle induction, and immunomodulation with autologous EBV-specific CTLs or EBV-specific vaccines.\textsuperscript{151-157} Chemotherapy in combination with targeted therapy or immunotherapy may further improve treatment results in the future.

**Epstein-Barr Virus–Associated Gastric Cancer**

The role of EBV in gastric cancer was first suggested in studies of patients from Asia.\textsuperscript{158,159} EBV genomes were identified within the gastric carcinoma and adjacent dysplastic epithelium but were absent in surrounding lymphocytes, stromal cells, intestinal metaplasia, and normal mucosa. In addition, the detection of monoclonal EBV episomes in EBV-associated gastric cancer strongly suggested that EBV plays an etiologic role in gastric carcinogenesis. EBV-associated gastric cancer is characterized by global and nonrandom CpG island methylation in the promoter region of many cancer-related genes with associated silencing, including PTEN, p16, and E-cadherin (CpG island methylation phenotype, or CIMP).\textsuperscript{160,161} The pattern of latent EBV gene expression is more restricted than in NPC in that LMP1 is not expressed in EBV-associated gastric cancer.\textsuperscript{162} Histopathologically, there are 2 subtypes of EBV-associated gastric cancer—lymphoepithelioma-like carcinoma and conventional gastric adenocarcinoma—and they make up nearly 10% of all cases of gastric cancer.

There have been conflicting data in the literature regarding the clinicopathologic characteristics and prognosis of EBV-associated gastric cancer. Overall, it is believed that the clinical and molecular characteristics of EBV-associated gastric cancer may be quite different from those of conventional gastric adenocarcinoma.\textsuperscript{163} Further studies are needed to determine the effect of EBV on the clinical course and survival of patients with gastric cancer, and whether there are any treatment implications related to the presence of EBV.
Summary

EBV possesses potent growth-transforming properties, and its role in the pathogenesis of a range of lymphoid and epithelial malignancies is well established. Over the past 2 decades, an evolving understanding of the diversity and pathology of EBV-related malignancies has paralleled the evolution of EBV-based therapeutic approaches. Although EBV-specific therapies remain largely investigational, several strategies exploiting aspects of EBV pathology have shown promise. These strategies include inhibition of viral targets and EBV-activated cellular targets, disruption of latent infection coupled with antiviral drugs, and adoptive cellular therapies. It is anticipated that ongoing studies of the pathology of specific EBV-associated malignancies will lead to novel therapies appropriate for each disease.

Disclosures

The authors have no relevant conflicts of interest to disclose.

References


