



# Biology and Treatment of Epstein-Barr Virus–Associated Non-Hodgkin Lymphomas

Helen E. Heslop

**Epstein-Barr virus (EBV) is associated with several different types of aggressive non-Hodgkin lymphoma (NHL). Individuals with primary or secondary immunodeficiency are susceptible to developing B cell lymphoproliferation due to outgrowth of EBV-infected B cells that express type III latency characterized by expression of all nine latent-cycle EBV antigens. These cells would normally be susceptible to control by EBV-specific T cells, and strategies to restore EBV-**

**specific immune responses may be effective therapeutically. EBV-associated lymphomas occurring in individuals who do not have a known immunodeficiency include NK and T malignancies with cytotoxic phenotypes, sporadic cases of B-NHL and lymphomatoid granulomatosis. These malignancies respond poorly to standard chemoradiotherapy, and immunotherapeutic or pharmacologic strategies targeting EBV are being explored.**

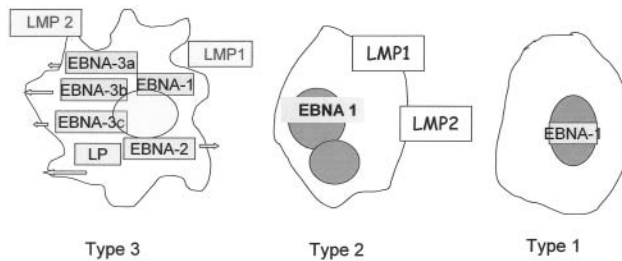
Latent Epstein-Barr virus (EBV) infection is associated with a heterogeneous group of non-Hodgkin lymphoma (NHL), including Burkitt's lymphoma, NK-T lymphomas and lymphoproliferative disease (LPD).<sup>1-3</sup> Most EBV-associated NHLs are aggressive tumors characterized by rapid growth and necrosis, and the NK and T lymphomas in particular are also associated with hemophagocytosis. The role of EBV in contributing to lymphomagenesis is well established in the EBV lymphoproliferative diseases that arise in immunosuppressed individuals. However, it is less well defined in other EBV-associated lymphomas whether EBV is a contributing factor to oncogenesis or a passenger virus. EBV is a latent gamma herpes virus that infects more than 90% of the world population. During primary infection in the oropharynx, EBV establishes lifelong latency in B cells that are preferentially infected through the CD21 receptor and subsequently programmed to the B memory cell compartment. The virus persists as an episome in infected B cells, establishing a latent infection where only a limited array of subdominant EBV antigens are expressed. The number of latently infected B cells within an individual remains stable over years unless the individual receives immunosuppressive therapy.<sup>4</sup> Healthy individuals mount a vigorous humoral and cellular immune response to primary EBV infection. Although antibodies to the viral mem-

brane proteins neutralize virus infectivity, the cellular immune response, consisting of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, is essential for controlling both primary infection and latent EBV-infected cells, and studies using tetramer technology have shown that high frequencies of EBV-specific CD8<sup>+</sup> T cells persist long term.<sup>5</sup> EBV-specific CD4<sup>+</sup> T cells may also play an important role in the cellular immune response to EBV. The EBV-specific immune response will therefore recognize and eliminate any latently infected B cells in the memory compartment that reactivate to become a proliferating lymphoblast expressing latent cycle antigens.

EBV was the first human virus implicated in oncogenesis. Since the original description in 1964, it has been associated with a heterogeneous group of malignant diseases. All EBV-positive malignancies are associated with the virus's latent cycle, and three distinct types of EBV latency have been characterized (**Figure 1**). Latency type III is expressed in lymphoblastoid cell lines (LCL), which can be readily produced by infecting B cells in vitro with EBV, and is characterized by expression of the entire array of nine EBV latency proteins: EBNA1, 2, 3A, 3B, 3C, LP, BARF0, and the two viral membrane proteins LMP1 and LMP2. These cells would normally be highly susceptible to killing by EBV-specific T cells, and this pattern of EBV gene expression is only seen in the EBV-associated lymphoproliferative diseases (EBV-LPD) that occur in individuals with congenital immunodeficiency or acquired immunodeficiency such as human immunodeficiency virus (HIV) infection or who are receiving intensive immunosuppression after solid organ or hematopoietic stem cell transplantation (HSCT). Latency type II NHL, where a more restricted array of proteins including EBNA-1, BARF0, LMP1, and LMP2 are expressed, is observed in EBV-positive Hodgkin disease, some types of T and NK-cell lymphomas, and some cases of B-cell lymphoma. In latency type I, found in EBV-positive Burkitt lymphoma, only EBNA-1 and BARF0 are expressed. Latently infected B

Correspondence: Helen E. Heslop, MD, Baylor College of Medicine, Center for Cell and Gene Therapy, 6621 Fannin St., MC 3-3320, Houston TX 77030-2303; Phone: (832) 824-4662, Fax: (832) 825-4668, hheslop@bcm.tmc.edu

Acknowledgments: This work was supported by National Institutes of Health grants PO1 CA94237 and CA61384, the General Clinical Research Center at Baylor College of Medicine (RR00188), a Specialized Center of Research Award from the Leukemia Lymphoma Society, and a Doris Duke Distinguished Clinical Scientist Award



**Figure 1. Types of Epstein-Barr virus latency.**

Abbreviations: EBV, Epstein-Barr virus; LP, leader protein; LMP, latent membrane protein; EBNA, EBV nuclear antigen

cells also transcribe two short EBV-encoded non-polyadenylated RNAs, EBER1 and EBER2.

EBV-associated NHL can be divided in two main categories: those arising in patients with immunodeficiency and those arising in individuals without a documented immunodeficiency (Table 1). EBV lymphomas arising in patients with immunodeficiency are B cell malignancies that usually express type 3 latency, rendering them theoretically susceptible to strategies that restore the immune response to EBV. EBV-associated lymphomas in patients without an underlying immunodeficiency usually manifest type 1 or 2 latency and may have additional genetic

lesions. These include Burkitt's lymphoma, some other B cell malignancies, and NK and T lymphomas. The neoplastic cells of extranodal EBV-positive T and NK cell lymphomas have a cytotoxic phenotype, and it has been postulated that the pathogenesis of EBV-positive T and NK cell NHL may relate to cytotoxic T lymphocytes (CTLs) or NK cells becoming infected by EBV during killing of an EBV-infected target cell.<sup>6</sup>

## EBV-associated Lymphomas in Immunodeficient Patients

### Primary immunodeficiency

Several primary immunodeficiencies associated with defective T cell function, such as Wiskott Aldrich syndrome and severe combined immunodeficiency (SCID), are associated with an increased risk of developing EBV-associated lymphoma. A particular predisposition is seen in patients with X-linked lymphoproliferative (XLP) disease who have a defect in a gene known as SH2D1A. This gene encodes a small SH2 binding protein and is involved in regulation of the intense CD8 T cell cytotoxicity stimulated by acute EBV infection. Individuals with XLP have a selective immunodeficiency to EBV, which results in either severe infectious mononucleosis, which is often fatal, or development of lymphoma on initial exposure to EBV.

**Table 1. Epstein-Barr virus (EBV)-associated non-Hodgkin lymphoma (NHL).**

Type of NHL	Target Cell	EBV Frequency	Latency
<b>Immunosuppressed Patients</b>			
NHL associated with primary immunodeficiency	B cell	> 95%	III
Post transplant lymphoma			
Hematopoietic stem cell transplant	B cell	> 95%	III
Solid organ transplant	B cell	> 95% in first year post-transplant, 70%-80% after 1 year	Usually III but late lymphomas may be II or I
HIV-associated lymphomas			
Primary CNS lymphoma	B cell	> 95%	III
Primary effusion lymphoma	B cell	> 90%	I
Diffuse large cell lymphoma	B cell	30%-60%	II or I
Burkitt's lymphoma	B cell	30%-60%	I
Methotrexate-induced lymphomas	B cell	> 95%	III
<b>Immunocompetent Patients</b>			
Extranodal NK-T cell lymphoma	NK cell with some T cell-associated antigens	> 95%	II
Aggressive NK leukemia lymphoma	NK cell with some T cell-associated antigens	30%-60%	II or I
SCAEBV	T and NK and B cells	> 95%	II
Lymphatoid granulomatosis	B cell	80%-95%	II
Diffuse large B cell lymphoma and CD30 <sup>+</sup> Ki-1 <sup>+</sup> anaplastic large cell lymphoma	B cell	10%-35%	II
T cell rich B cell lymphoma	B cell	20%	II
Angioimmunoblastic lymphoma	B cells and T cells	> 80%	II

Abbreviations: CNS, central nervous system; HIV, human immunodeficiency virus; SCAEBV, severe chronic active EBV infection

### **Post-transplant**

Post-transplant lymphoproliferative disease (LPD) is a serious, life-threatening disease and encompasses a heterogeneous group of lymphoproliferative disorders ranging from reactive, polyclonal hyperplasias to aggressive NHLs. In solid organ transplant (SOT) recipients, the development of EBV-PTLD is a result of an impaired EBV-specific cellular immune response caused by immunosuppression administered to prevent graft rejection. The incidence is dependent on the type of solid organ graft and the degree of immunosuppression. The incidence is highest in EBV-seronegative children who do not have a pre-existing immune response to EBV and receive an EBV-seropositive graft.<sup>3</sup> After HSCT, the risk factors include the degree of mismatch between donor and recipient, manipulation of the graft to deplete T cells, and the degree of immunosuppression used to prevent graft-versus-host disease (GVHD).<sup>3</sup> Almost all cases of PTLD after HSCT and the majority of cases after SOT occur during the first year post-transplant when the recipient is most severely immunocompromised. LPDs which occur later after SOT are more heterogeneous and may be EBV negative with additional genetic changes.

### **HIV infection**

Patients with HIV are susceptible to three types of NHL that have different frequencies of EBV infection. Patients with HIV have a high frequency of EBV-infected B cells, independent of the development of lymphoma that may be due in part to a defect in T-cell immunity to EBV. The risk of developing an AIDS-related lymphoma correlates with the decrease in either the number or function of EBV-specific cytotoxic lymphocytes with a concomitant increase in EBV viral load.<sup>7</sup> It has been proposed that immunosuppression and EBV infection favor the expansion of B cell clones, increasing the risk that clones that have undergone alterations in oncogenes or tumor suppressor genes will proliferate. The pathogenetic role of EBV is most clearly defined in primary central nervous system lymphoma (PCNSL), where virtually all cases are EBV positive. EBV may also be involved in primary effusion lymphoma, which is characterized by its predilection for body cavities such as the peritoneal, pleural, and pericardial spaces; however, infection with human herpesvirus-8 is thought to be of primary importance in these lymphomas. The association of EBV with other lymphomas, including systemic HIV-related Burkitt's lymphoma and HIV-related diffuse large cell lymphoma, ranges from 30% to 60%. In biopsies of EBV-associated HIV-NHL, there is considerable variation in the number of EBV-positive cells, and the pattern of EBV latent gene expression varies among tumor types as in immunocompetent individuals.

### **Methotrexate-induced lymphomas**

Patients with rheumatoid arthritis or polymyositis treated with methotrexate (MTX) develop EBV-positive lymphomas more frequently than patients treated with alternative

agents that should be equally or more immunosuppressive. A recent study suggests that this predisposition may be due the ability of MTX to induce EBV replication while at the same time producing immunosuppression.<sup>8</sup> Such lymphomas may respond to withdrawal of MTX but often need systemic therapy. EBV-associated lymphomas may also arise in patients receiving immunosuppressive chemotherapy. It is important to consider this possibility as reversal of immunosuppression may treat the disease.

## **EBV-Associated Lymphomas in Immunocompetent Patients**

### **Burkitt's lymphoma**

Burkitt's lymphoma is a high-grade malignant small non-cleaved cell lymphoma that may occur in the endemic form found in Africa or the sporadic form found elsewhere. Over 95% of endemic Burkitt's lymphomas are associated with EBV, but the association with sporadic cases is less frequent and in the United States only 20% of Burkitt's lymphoma are EBV positive. However, almost all cases of both endemic and sporadic Burkitt's lymphoma are characterized by translocations between chromosome 8 and one of the immunoglobulin genes on chromosomes 2, 14, or 22, resulting in deregulation of the *c-myc* gene on chromosome 8, suggesting that this genetic change is critical for oncogenesis. Burkitt's lymphoma cells evade the immune system by expressing type I latency and also by down-regulating the expression of cell adhesion molecules and major histocompatibility class (MHC) class I molecules.

### **Lymphomatoid granulomatosis**

Lymphomatoid granulomatosis (LYG) is a rare angiocentric-destructive process characterized by an EBV-positive B cell proliferation associated with a reactive T cell proliferation. It is likely due to a defective immune response to EBV. LYG is graded I-II, showing rare to moderate large EBV-positive B cells (usually polyclonal or oligoclonal), and grade III, showing numerous large EBV-positive B cells (usually monoclonal), likely reflecting progressive transformation. It presents in extranodal sites, and lung involvement is common. In most cases of LYG there is evidence of immune deficiency with low CD8 and CD4 counts and anergy, although EBV viral load is relatively low.

### **Other EBV-positive B-NHL**

EBV-positive B cell lymphomas occurring in the immunocompetent host include some cases of diffuse large B cell lymphoma and CD30<sup>+</sup> Ki-1<sup>+</sup> anaplastic large cell lymphoma of B cell type. Between 10% and 35% of these tumors are EBV positive expressing a type 2 latency pattern with a higher frequency seen in oral cavity lymphomas.<sup>9,10</sup> T cell-rich-B cell NHL can be difficult to distinguish from Hodgkin disease, but when strict morphological and immunophenotypic criteria are applied, approximately 20% of these tumors are found to be LMP1 positive.<sup>11</sup>

### **Angioimmunoblastic lymphoma**

Angioimmunoblastic lymphoma is characterized by oligoclonal proliferations of T and B cells, and EBV is detected in many cases in either T or B cells. The clinical presentation usually includes systemic disease with B symptoms, while pathology reveals a polymorphous infiltrate involving lymph nodes, with a prominent proliferation of endothelial and dendritic cells. Some patients develop a secondary EBV-positive large B-cell lymphoma.

### **Extranodal NK/T cell lymphoma, nasal type**

Extranodal natural killer/T-cell lymphoma, nasal-type, or nasal NK/T-cell lymphoma, was formerly called angiocentric lymphoma. It is the most common cause of the syndrome known as "lethal midline granuloma." It is an extranodal lymphoma, usually with an immature NK-cell phenotype and positive for Epstein-Barr virus (EBV), with a broad morphologic spectrum, frequent necrosis, and angioinvasion.<sup>12</sup> It is often associated with hemophagocytosis. This lymphoma most commonly presents in the midfacial region, but may also occur in other extranodal sites. It is designated NK/T because of uncertainty regarding its cellular lineage. Although originally thought to be T cells, the malignant cells express CD2 and CD56 but lack surface CD3 and T cell receptor gene rearrangements.<sup>12</sup> Thus, these tumors are probably of NK cell origin.

### **Aggressive NK LGL leukemia/lymphoma**

This syndrome presents with lymphadenopathy, hepatosplenomegaly, and the presence of atypical lymphocytes in blood and marrow. The phenotype is similar to that of the NK-T lymphomas described above, and the disease is aggressive with a poor prognosis.<sup>12</sup> Infection with EBV has been implicated in more than 50% of the cases of NK LGL leukemia reported in Japan.

### **Severe chronic EBV infection**

Severe chronic active EBV infection (SCAEBV) is a rare entity characterized by an abnormal immune response to EBV resulting in high viral load, an abnormal pattern of antibody response, and predisposition to the development of hemophagocytic syndromes, organ dysfunction, and lymphoma. A fulminant form of T cell lymphoma has been described following acute EBV infection and is most common in Southeast Asia.<sup>13,14</sup> The clinical illness is characterized by fever, hepatosplenomegaly, and pancytopenia; hemophagocytosis is often seen. There is a high mortality rate, and molecular analysis has demonstrated clonality of the EBV-infected T cells.<sup>13</sup> Less fulminant forms may target T or NK cells, and the NK variant is associated with hypersensitivity to mosquito bites.<sup>14</sup> The majority of patients have evidence of monoclonal EBV in the target cells, although the patients with T cell SCAEBV are more likely to develop overt lymphoma.<sup>14</sup> B cell lymphoma may also develop in patients with SCAEBV.

### **Treatment**

Most EBV-associated tumors respond poorly to intensive chemotherapy regimens or have a significant relapse rate, and the presence of the EBV genome within these tumors raises the possibility of developing strategies directed at viral targets.<sup>15</sup> Approaches under evaluation include immunotherapy approaches, interferon, and small molecules targeting aspects of virus biology.<sup>15</sup>

### **Cellular therapy to reconstitute immune response to EBV**

As EBNA-1 is not well processed by the class I processing machinery, lymphomas expressing type I latency have not been considered a good target for immunotherapy approaches. Immunotherapy approaches targeting EBV antigens, however, do have potential for treating type II and type III latency EBV lymphomas. The main application at present has been in type III latency lymphomas in immunosuppressed patients following solid organ or hematopoietic stem cell transplant. In primary immunodeficiency the usual recommended therapy is allogeneic HSCT to restore a normal donor-derived immune response.

### **Cellular immunotherapy post-HSCT**

EBV lymphoma arising after allogeneic HSCT is an excellent model for evaluating EBV-specific CTLs, as the tumor cells express all nine latent-cycle EBV antigens (including the immunodominant EBNA3 antigens), and most donors are seropositive. Unmanipulated donor lymphocytes have therefore been used to treat EBV-PTLD arising after HSCT. The response rate to this therapy varies in different reports, likely reflecting differences in patient populations or better outcome with early diagnosis and treatment. Furthermore, there is a significant risk of GVHD due to alloreactive cells in the product.<sup>3,16</sup> An alternate approach is to generate EBV-specific CTLs using lymphoblastoid cell lines, which can be generated by infecting normal peripheral blood B cells with EBV, as antigen-presenting cells. EBV-specific T cell lines have been used as prophylaxis for EBV-induced lymphoma in patients who received a T cell-depleted HSCT or who were transplanted for an EBV-associated malignancy.<sup>3,17,18</sup> In some patients the CTLs were genetically modified with a retroviral vector encoding the neomycin resistance gene to enable tracking of infused cells, and marked CTLs have been detected for up to 7 years. This strategy also resulted in reconstitution of antiviral immunity,<sup>17</sup> and none of the patients treated prophylactically developed EBV lymphoma compared with an incidence of 11% in patients receiving the same transplant regimen who did not receive prophylactic CTLs.<sup>18</sup> A second study evaluating the use of prophylactic CTLs confirmed the value of this approach to reconstitute T cell immunity with infusion of EBV-specific CTLs, resulting in reduction of the viral load in five of six patients.<sup>19</sup>

This strategy has also been evaluated in 6 patients with established EBV lymphoma. Complete sustained re-

sponses were seen in 5 patients, accompanied by accumulation of gene-marked CTLs at sites of disease in 2 patients who had follow-up biopsies.<sup>18</sup> In 1 patient with extremely bulky disease significant inflammation was seen at sites of disease after CTL administration, illustrating the benefits of treating patients with early rather than advanced disease.<sup>18</sup> The patient who failed treatment was found to have a mutation resulting in deletion of the two immunodominant HLA-11 restricted epitopes in EBNA 3B recognized by the donor CTL line.<sup>20</sup>

### **Therapy post-solid organ transplant**

LPD arising in solid organ transplants will often regress if immunosuppression can be reduced, providing a rationale for evaluating whether adoptively transferred EBV-specific CTLs can reconstitute EBV-specific immune responses. Generation of EBV-specific CTL in this patient population presents different challenges compared with HSCT recipients, as the SOT donor is not HLA-matched and usually deceased. Several groups have evaluated autologous EBV CTLs as prophylaxis in solid organ transplant recipients and shown transient increases in numbers of CTL precursor cells and decrease in EBV viral load.<sup>21,22</sup> Khanna et al<sup>23</sup> evaluated autologous EBV-specific CTL as therapy in a renal transplant recipient with EBV-LPD and observed significant regression following two infusions of CTLs. However, new lymphoma lesions developed 10 weeks after the second CTL infusion.<sup>23</sup> In a second report from this group a cardiac transplant patient who developed multiple subcutaneous nodules required six doses of CTLs to achieve remission, and response was coincident with reconstitution of T cell reactivity to latent epitopes.<sup>24</sup> This experience contrasts with that of post-HSCT transplant where the infused CTLs persisted long term and there were no recurrences in patients successfully treated for PTLD. This suggests that transferred CTL may not function long term in solid organ recipients who receive continuous immunosuppressive therapy. Multiple infusions or infusions of CTLs in conjunction with rituximab may be required.

Another obstacle to autologous CTL for prophylaxis or therapy is the time required for generating autologous EBV-specific CTLs. One solution is to develop a bank of allogeneic EBV-specific CTLs so an “off the shelf” product is immediately available. Several reports describe regression of PTLD after administration of partially matched allogeneic EBV-specific CTLs.<sup>25,26</sup> While these results are encouraging, the patients who responded also had their immunosuppressive treatment reduced. As a result, it is difficult to definitively ascribe benefit to the allogeneic CTLs, especially as neither group showed persistence of the allogeneic cells.

### **Immunotherapy for HIV**

In HIV-infected patients, the development of EBV-associated HIV-NHL is preceded by a loss of functional EBV-specific CTLs,<sup>7</sup> suggesting that strategies to boost the en-

dogenous EBV-specific T cell response might prevent lymphomas. In contrast to other HIV-related malignancies, the incidence of HIV-NHL has not declined after the introduction of highly active antiretroviral therapy (HAART). One explanation is that once EBV-specific CD4<sup>+</sup> T cells are depleted from the patient’s immunological repertoire, a full and sustained cellular immune response to EBV cannot be restored.<sup>27</sup> There is one report of a patient with an EBV-positive B cell lymphoma that was refractory to chemotherapy, but regressed after treatment with EBV-specific CTLs generated from archived autologous peripheral blood mononuclear cells (PBMCs).<sup>28</sup> However, obtaining PBMCs from HIV-infected patients early in the clinical course (when their EBV-specific cellular immunity is still intact) for later generation of EBV-specific T cells would be logistically demanding. Furthermore, if the HIV infection of the patient is not controlled with HAART therapy the protection offered by infused EBV-specific T cells is likely to be short-lived.

### **Immunotherapy for type II latency tumors**

Adapting immunotherapy approaches that have proved successful in type III latency EBV tumors to type II latency tumors provides a challenge, since a more restricted array of subdominant EBV antigens is expressed, and the frequency of clones recognizing the LMP1 or 2 antigens expressed on these tumors is low.<sup>29</sup> Current studies are evaluating whether CTL lines biased toward the LMP2 antigen expressed in these tumors have anti-tumor effects, and whether EBV-specific activity can be boosted by vaccination.

### **Antibodies**

The availability of rituximab, a chimeric murine/human monoclonal anti-CD20 antibody, offers another prospect for treating EBV-associated B cell LPDs. The response rate to rituximab has varied between 70% and 100%, and these differences likely reflect better outcome with early diagnosis and treatment.<sup>3,30,31</sup> Limitations of this approach are that it may select for CD20<sup>-</sup> variants, the induction of profound B-cell depletion for 6-8 months, and the fact that rituximab does not restore the cellular immune response to EBV, which may be crucial for the long-term control of EBV-mediated B cell proliferation.

### **Interferon**

Interferon- $\alpha$  has been evaluated as an immunomodulatory and antiviral agent in a wide range of EBV-associated lymphomas. Therapy with interferon- $\alpha$  and intravenous immune globulin have been used in SOT recipients and in a small number of HSCT patients with EBV-associated LPD with some responses, but have now been replaced by CD20 antibodies or adoptive immunotherapy approaches as first-line therapy.<sup>32</sup> Evaluation of interferon is continuing in some type II latency lymphomas and SCAEBV, and a recent abstract reports high response rates in LYG with du-

rable complete responses in grade I-II LYG and improvement of low CD8 counts.<sup>33</sup>

### Pharmacologic approaches

Antiviral agents such as acyclovir and ganciclovir have activity against lytic EBV disease as they are converted by the viral thymidine kinase gene, which is expressed only during the lytic form of infection, into their active, cytotoxic forms. One approach has therefore been to co-administer antiviral agents with demethylating agents such as 5-azacytidine, which will induce lytic cycle genes.<sup>34</sup> Other chemotherapy agents may also induce lytic infection, and one study has shown that the combination of gemcitabine or doxorubicin and ganciclovir was significantly more effective for the inhibition of EBV-driven lymphoproliferative disease in SCID mice than chemotherapy alone.<sup>35</sup> A phase I clinical trial has used arginine butyrate to induce viral Tk followed by ganciclovir therapy and reported complete clinical responses in 5 of 10 patients with EBV-associated malignancies.<sup>36</sup>

A second pharmacologic approach relies on the assumption that persistent expression of certain EBV-encoded gene products is required for the continued growth of EBV-associated lymphomas. Hydroxyurea can eradicate extrachromosomal DNA elements and has been shown to eliminate EBV episomes in EBV-positive Burkitt's lymphoma cell lines. Clinical experience is limited, but two reports describe activity against EBV-associated primary central nervous system lymphoma in 2 patients with HIV<sup>37</sup> and against central nervous system LPD post-HSCT in 1 patient.<sup>38</sup> Preclinical studies are also evaluating the use of peptides and proteins targeted at modifying the function of EBV proteins, for example LCL growth in vitro can be impaired by using a short peptide mimic to block the function of EBNA2.<sup>39</sup> Another approach is to target adhesion molecules that are crucial for cell-cell interactions, and the statin Simvastatin, which inhibits LFA-1, is able to induce apoptosis on EBV-transformed LCLs in vitro and delay the development of EBV lymphomas in a SCID mouse model.<sup>40</sup> EBV-associated gene products may also be inhibited by antisense therapies.

### Conclusions

Although the contribution of EBV to oncogenesis is more clear-cut in the B cell lymphoproliferative diseases arising in immunosuppressed patients than in other aggressive EBV-NHL, the presence of the virus in all of these malignancies offers the prospect for therapeutic interventions targeting virus-encoded proteins. Ultimately the combination of immunotherapy and vaccination approaches with small molecules upregulating lytic cycle antigens, or targeting viral proteins or the microenvironment, may prove to be the most efficacious therapeutic approach to these diseases.

### References

1. Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. *Nat Rev Cancer*. 2004;4:757-768.
2. Cohen JL. Benign and malignant Epstein-Barr virus-associated B-cell lymphoproliferative diseases. *Semin Hematol*. 2003;40:116-123.
3. Gottschalk S, Rooney CM, Heslop HE. Post-transplant lymphoproliferative disorders. *Annu Rev Med*. 2005;56:29-44.
4. Thorley-Lawson DA, Gross A. Persistence of the Epstein-Barr virus and the origins of associated lymphomas. *N Engl J Med*. 2004;350:1328-1337.
5. Tan LC, Gudgeon N, Annels NE, et al. A re-evaluation of the frequency of CD8<sup>+</sup> T cells specific for EBV in healthy virus carriers. *J Immunol*. 1999;162:1827-1835.
6. Brink AA, ten Berge RL, van den Brule AJ, et al. Epstein-Barr virus is present in neoplastic cytotoxic T cells in extranodal, and predominantly in B cells in nodal T non-Hodgkin lymphomas. *J Pathol*. 2000;191:400-406.
7. van Baarle D, Hovenkamp E, Callan MF, et al. Dysfunctional Epstein-Barr virus (EBV)-specific CD8(+) T lymphocytes and increased EBV load in HIV-1 infected individuals progressing to AIDS-related non-Hodgkin lymphoma. *Blood*. 2001;98:146-155.
8. Feng WH, Cohen JL, Fischer S, et al. Reactivation of latent Epstein-Barr virus by methotrexate: a potential contributor to methotrexate-associated lymphomas. *J Natl Cancer Inst*. 2004;96:1691-1702.
9. Kuze T, Nakamura N, Hashimoto Y, Abe M, Wakasa H. Clinicopathological, immunological and genetic studies of CD30<sup>+</sup> anaplastic large cell lymphoma of B-cell type; association with Epstein-Barr virus in a Japanese population. *J Pathol*. 1996;180:236-242.
10. Solomides CC, Miller AS, Christman RA, Talwar J, Simpkins H. Lymphomas of the oral cavity: histology, immunologic type, and incidence of Epstein-Barr virus infection. *Hum Pathol*. 2002;33:153-157.
11. Axtdorff U, Porwit-Macdonald A, Sjöberg J, Grimfors G, Björkholm M. T-cell-rich B-cell lymphoma: diagnostic and therapeutic aspects. *APMIS*. 2002;110:379-390.
12. Nava VE, Jaffe ES. The pathology of NK-cell lymphomas and leukemias. *Adv Anat Pathol*. 2005;12:27-34.
13. Quintanilla-Martinez L, Kumar S, Fend F, et al. Fulminant EBV(+) T-cell lymphoproliferative disorder following acute/chronic EBV infection: a distinct clinicopathologic syndrome. *Blood*. 2000;96:443-451.
14. Kimura H, Hoshino Y, Kanegane H, et al. Clinical and virologic characteristics of chronic active Epstein-Barr virus infection. *Blood*. 2001;98:280-286.
15. Israel BF, Kenney SC. Virally targeted therapies for EBV-associated malignancies. *Oncogene*. 2003;22:5122-5130.
16. O'Reilly RJ, Small TN, Papadopoulos E, et al. Biology and adoptive cell therapy of Epstein-Barr virus-associated lymphoproliferative disorders in recipients of marrow allografts. *Immunol Rev*. 1997;157:195-216.
17. Heslop HE, Ng CYC, Li C, et al. Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. *Nature Med*. 1996;2:551-555.
18. Rooney CM, Smith CA, Ng CYC, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. *Blood*. 1998;92:1549-1555.
19. Gustafsson A, Levitsky V, Zou JZ, et al. Epstein-Barr virus (EBV) load in bone marrow transplant recipients at risk to develop posttransplant lymphoproliferative disease: prophylactic infusion of EBV-specific cytotoxic T cells. *Blood*. 2000;95:807-814.

20. Gottschalk S, Ng CYC, Smith CA, et al. An Epstein-Barr virus deletion mutant that causes fatal lymphoproliferative disease unresponsive to virus-specific T cell therapy. *Blood*. 2001;97:835-843.
21. Haque T, Amlot PL, Helling N, et al. Reconstitution of EBV-specific T cell immunity in solid organ transplant recipients. *J Immunol*. 1998;160:6204-6209.
22. Comoli P, Labirio M, Basso S, et al. Infusion of autologous Epstein-Barr virus (EBV)-specific cytotoxic T cells for prevention of EBV-related lymphoproliferative disorder in solid organ transplant recipients with evidence of active virus replication. *Blood*. 2002;99:2592-2598.
23. Khanna R, Bell S, Sherritt M, et al. Activation and adoptive transfer of Epstein-Barr virus-specific cytotoxic T cells in solid organ transplant patients with posttransplant lymphoproliferative disease. *Proc Natl Acad Sci U S A*. 1999;96:10391-10396.
24. Sherritt MA, Bharadwaj M, Burrows JM, et al. Reconstitution of the latent T-lymphocyte response to Epstein-Barr virus is coincident with long-term recovery from posttransplant lymphoma after adoptive immunotherapy. *Transplantation*. 2003;75:1556-1560.
25. Haque T, Wilkie GM, Taylor C, et al. Treatment of Epstein-Barr-virus-positive post-transplantation lymphoproliferative disease with partly HLA-matched allogeneic cytotoxic T cells. *Lancet*. 2002;360:436-442.
26. Sun Q, Burton R, Reddy V, Lucas KG. Safety of allogeneic Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes for patients with refractory EBV-related lymphoma. *Br J Haematol*. 2002;118:799-808.
27. Porcu P, Caligiuri MA. Acquired immunodeficiency syndrome-related lymphomas: future directions. *Semin Oncol*. 2000;27:454-462.
28. Wheatley HG, McKinnon K, Lilly S, Smith C, Lyerly HK. Adoptive immunotherapy using autologous EBV-specific CTL in a HIV infected patient with refractory Epstein Barr virus expressing B cell lymphoma (Abstract). *J AIDS Hum Retrovirol*. 1997;14(4):A53.
29. Bollard CM, Aguilar L, Straathof KC, et al. Cytotoxic T lymphocyte therapy for Epstein-Barr virus+ Hodgkin's disease. *J Exp Med*. 2004;200:1623-1633.
30. Milpied N, Vasseur B, Parquet N, et al. Humanized anti-CD20 monoclonal antibody (Rituximab) in post transplant B-lymphoproliferative disorder: a retrospective analysis on 32 patients. *Ann Oncol*. 2000;11 Suppl 1:113-116.
31. Kuehnle I, Huls MH, Liu Z, et al. CD20 monoclonal antibody (rituximab) for therapy of Epstein-Barr virus lymphoma after hemopoietic stem-cell transplantation. *Blood*. 2000;95:1502-1505.
32. Gross TG, Steinbuch M, DeFor T, et al. B cell lymphoproliferative disorders following hematopoietic stem cell transplantation: risk factors, treatment and outcome. *Bone Marrow Transplant*. 1999;23:251-258.
33. Dunleavy K, Janik J, Cohen J, et al. Study of the treatment and biology of lymphomatoid granulomatosis; a rare EBV lymphoproliferative disorder (abstract). *Lugano Lymphoma Conference 2005*.
34. Chan AT, Tao Q, Robertson KD, et al. Azacitidine induces demethylation of the Epstein-Barr virus genome in tumors. *J Clin Oncol*. 2004;22:1373-1381.
35. Feng WH, Hong G, Delecluse HJ, Kenney SC. Lytic induction therapy for Epstein-Barr virus-positive B-cell lymphomas. *J Virol*. 2004;78:1893-1902.
36. Faller DV, Mentzer SJ, Perrine SP. Induction of the Epstein-Barr virus thymidine kinase gene with concomitant nucleoside antivirals as a therapeutic strategy for Epstein-Barr virus-associated malignancies. *Curr Opin Oncol*. 2001;13:360-367.
37. Slobod KS, Taylor GH, Sandlund JT, et al. Epstein-Barr virus-targeted therapy for AIDS-related primary lymphoma of the central nervous system. *Lancet*. 2000;356:1493-1494.
38. Pakakasama S, Eames GM, Morriss MC, et al. Treatment of Epstein-Barr virus lymphoproliferative disease after hematopoietic stem-cell transplantation with hydroxyurea and cytotoxic T-cell lymphocytes. *Transplantation*. 2004;78:755-757.
39. Farrell CJ, Lee JM, Shin EC, et al. Inhibition of Epstein-Barr virus-induced growth proliferation by a nuclear antigen EBNA2-TAT peptide. *Proc Natl Acad Sci U S A*. 2004;101:4625-4630.
40. Katano H, Pesnicak L, Cohen JI. Simvastatin induces apoptosis of Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines and delays development of EBV lymphomas. *Proc Natl Acad Sci U S A*. 2004;101:4960-4965.