Epstein-Barr Virus-Associated Classical Hodgkin Lymphoma and Its Therapeutic Strategies

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Abstract
Over the past few decades, our understanding of the epidemiology and immunopathogenesis of Hodgkin lymphoma (HL) has made enormous advances. Consequently, the treatment of HL has changed significantly, rendering this disease of the most curable human cancers. To date, about 80% of patients achieve long-term disease-free survival. However, therapeutic challenges still remain, particularly regarding the salvage strategies for relapsed and refractory disease, which need further identification of better prognostic markers and novel therapeutic schemes. Although the precise molecular mechanism by which Epstein-Barr virus (EBV) contributes to the generation of malignant cells present in HL still remains unknown, current increasing data on the role of EBV in the pathobiology of HL have encouraged people to start developing novel and specific therapeutic strategies for EBV-associated HL. This review will provide an overview of therapeutic approaches for acute EBV infection and the classical form of HL (cHL), especially focusing on EBV-associated HL cases.

Key Words: Classical Hodgkin lymphoma, EBV, Hodgkin and Reed-Sternberg cells

INTRODUCTION

Lymphoma is a type of cancer involving cells of the immune system, and has two major categories: Hodgkin lymphoma (HL) and all other lymphomas (non-HL). Although the two types may display the same symptoms, they are readily distinguishable via microscopic examination, and differ in the way they develop, spread and are treated. HL is a unique clinicopathological disorder characterized by the rare presence of malignant cells (usually accounting for 0.1% to 10% of the cells in the total tumor mass) in a background of a non-neoplastic cellular microenvironment comprising T- and B-lymphocytes and other cell types (Weiss et al., 1999). In the United States, about 8,500 new cases of HL were expected to be diagnosed in 2010, and the overall incidence is increasing each year (Maggioncalda et al., 2011).

Although the pathogenesis of HL is still largely unknown, the association of HL with Epstein-Barr virus (EBV) infection has been demonstrated in many reports. For examples, HL patients show elevated antibody titers to EBV antigens (Levine et al., 1971), and the risk of developing HL is increased up to three-fold in population that have experienced infectious mononucleosis caused by primary EBV infection (Gutensohn and Cole, 1980), and EBV DNA/RNA can be detected in HL tissues (Brink et al., 1997), suggesting that as a primary event in the development of this disease, EBV infection may play a very crucial role in the pathogenesis of HL.

According to a population-based cohort study, the frequency of EBV associated HL among total HL cases varies from 30 to 50% (in certain cases even higher; >90% in developing and underdeveloped countries, and >95% in HIV associated cases), and most of them appear in classical Hodgkin lymphoma (cHL), the major type of HL (Herbst et al., 1991; Pallesen et al., 1991; Ambinder et al., 1993; Leoncini et al., 1996). Since EBV is an oncogenic virus that is able to subvert cellular processes supporting growth and survival, the approach to unravel the function of latent EBV genes expressed in cHL tissues will provide strategies for more novel and targeted therapies for cHL patients. Thus, this review, based on considerable progress recently made regarding the pathways related to the generation of cHL over the past years, presents a summary of EBV antiviral drugs, the main characteristics of the disease entities, the data on the possible role of EBV in the pathogenesis of EBV-positive HL, and current therapeutic options including immunotherapy.
Table 1. Antiviral therapeutic candidates targeting EBV replication

<table>
<thead>
<tr>
<th>Target Structure</th>
<th>Drug (prodrug)</th>
<th>Mode of action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV DNA Pol</td>
<td>Acyclic nucleoside or nucleotide analogue</td>
<td>Competitive substrates or DNA chain terminator</td>
<td>(Purifoy et al., 1993)</td>
</tr>
<tr>
<td></td>
<td>Acyclovir (valaciclovir)</td>
<td></td>
<td>(Jung and Dorr, 1999)</td>
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<td></td>
<td>Ganciclovir (valganclovir)</td>
<td></td>
<td>(Boyd et al., 1986)</td>
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<td></td>
<td>Peniclovir (famiclovir)</td>
<td></td>
<td>(Friedrichs et al., 2004)</td>
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<tr>
<td></td>
<td>Adefovir</td>
<td></td>
<td>(Friedrichs et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>Cidofovir</td>
<td></td>
<td>(Datta and Hood, 1981)</td>
</tr>
<tr>
<td></td>
<td>Foscarnet, a phosphonoformic acid</td>
<td>Pyrophosphate-binding site</td>
<td>(Yajima et al., 1976)</td>
</tr>
<tr>
<td></td>
<td>Phosphonoacetic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrophosphate analogue</td>
<td>4-oxo-dihydroquinolines</td>
<td>V823 of EBV DNA Pol</td>
<td>(Hartline et al., 2005)</td>
</tr>
<tr>
<td>Molecules except for EBV DNA Pol</td>
<td>Maribavir</td>
<td>EBV ED-A</td>
<td>(Gershburg et al., 2004)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(Zacny et al., 1999)</td>
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<td></td>
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<td></td>
<td>(Gershburg and Pagano, 2002)</td>
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<td>(Kira et al., 2000)</td>
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<tr>
<td></td>
<td></td>
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<td>(Gershburg et al., 2004)</td>
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</table>

|                |                                    | Competitive substrates or DNA chain terminator       | (Purifoy et al., 1993) |

**EBV INFECTION AND ANTIVIRAL DRUGS**

EBV, a human γ herpes virus, is widely spread in human populations, and more than 95% of adults are EBV seropositive (Young and Rickinson, 2004). Primary EBV infection is usually asymptomatic in childhood, but the infection in adolescence frequently results in infectious mononucleosis (IM), and is associated with a variety of human malignancies. In addition to Burkitt’s lymphoma, nasopharyngeal carcinoma, post-transplant lymphoproliferative disease, and HL, EBV is also linked to some of the malignancies (Westphal et al., 2000; Daigle et al., 2011).

**EBV LATENCY AND HL**

During primary infection, EBV is transmitted by acutely infecting B cells in the oropharyngeal epithelium. This process results in a localized replicative infection through lytic cycles, some of which undergo latency by permanent infection of circulating B cells. Most EBV associated malignancies including HL are characterized by such viral latency, in which viral genomes are replicated by host DNA polymerase only when cells divide, and propagate into progeny cells indefinitely. Therefore, the antiviral drugs aimed at viral replication processes listed in Table 1 would not affect EBV in the latent phase, as demonstrated by their lack of effect on latently EBV-infected cell lines or tumors (Pagano, 1995). This fact subsequently emphasizes the needs of special strategies for EBV-associated tumors distinct from those for the acute infection.

In latent infection, viral gene expression is tightly regulated with expression of only a limited number of EBV latency genes. Thus, only 12 genes of EBV can be expressed in different combinations during latent viral infection whereas about 70 major open reading frames are expressed during the lytic cycle (Kieff, 1996). The EBV latency genes are expressed in 4 programs: latency 0, I, II and III (Shah and Young, 2009). In latency III, all of the 12 latency genes are expressed, including 6 nuclear proteins (EBNA 1, 2, 3A, 3B, 3C, 4A), 3 membrane proteins (LMP-1, LMP-2A and LMP-2B), BART, and 2 small RNAs (EBER 1 and 2). In latency II, the viral genes for EBNA-1, the three membrane proteins, and the EBERs are expressed while in latency state 0/I, none or only EBNA-1 is expressed. B-lymphocytes in latency III are proliferating but expressing latency genes, whereas in latency 0/I, latency gene expression is limited. In latency 0, only latency genes 2A and 2B are expressed, and B-lymphocytes in latency 0/I are resting cells. Among the 4 programs,
HL has been reported to be closely associated with latency II, expressing only 6 viral genes: EBNA1, EBER1 and 2, LMP1, and LMP2A and 2B (Murray et al., 1992; Deacon et al., 1993; Grasser et al., 1994; Chiang et al., 1996).

According to its histopathological and clinical characteristics, HL is categorized into 2 types: cHL, and the nodular lymphocyte predominance HL (NLPHL). About 95% of all HL cases represent cHL, while the rest are NLPHL (Niedobitek et al., 1997). As described, one of the most unique features of HL is the rarity of malignant cells in the tumor mass. The two types of HL have their own unique malignant cells, designated as mononuclear Hodgkin’s cells and multinucleated Reed-Sternberg (HRS) cells in cHL whereas they are called LP (lymphocyte predominant) cells in NLPHL, respectively (Poppema et al., 2008). Unlike the LP cells of NLPHL, EBV DNA genomes are frequently detected in HRS cells of cHL patients, in which the viruses display the type II form of latency expressing all of the type II-related 6 viral genes (Weiss et al., 1989; Weiss et al., 1999; Thomas et al., 2004; Kuppers, 2009). Since EBV is an oncogenic virus, this high association rate with EBV in HRS cells strongly suggests a possible role of EBV latent gene expression in the generation of the malignancies. Since the latent viral antigens are abundantly present in EBV-associated HRS cells, but not in the non-EBV-associated ones, providing extra targets for immunotherapy, the latent viral genes expressed in HRS cells and the related pathways have gained attention as additional therapeutic options in the case of EBV-associated cHL.

**THERAPEUTIC STRATEGIES AGAINST cHL**

Based on differences in histological features and cellular composition, cHL is further divided into 4 morphological subtypes (Pileri et al., 2002; Eberle et al., 2009): nodular sclerosis (NS) which accounts for the majority of cases, mixed cellularity (MC), lymphocyte-rich (LR), and lymphocyte-depleted (LD) HL. LRHL and LDHL each comprises less than 5% of all cHL cases, and the majority is the NSHL subtype (Table 2). The detection rate of EBV in cHL varies depending on multifactorial etiological factors such as country, sex, ethnicity, and the age of patients. For example, EBV-positive cHL is less common in developed populations compared to underdeveloped countries (20-50% in North American and European countries vs. 60-100% in Peru and Kenya) (Weiss et al., 1991; Herbst et al., 1992; Hummel et al., 1992; Chang et al., 1993; Weinreb et al., 1996). Among the subtypes of cHL, EBV genomes or proteins more commonly appear in the MC subtype with an increased incidence but less frequently in the other subtypes (Pallesen et al., 1991; Murray et al., 1992; Pinkus et al., 1994). Despite the complexity in its histological and cellular characteristics, the primary therapeutic strategies against the all subtypes in cHL cases are similar because the immunophenotypic and general features of the malignant cells of cHL, the HRS cells, are surprisingly alike irrespective of the subtype or the presence of EBV.

With conventional chemotherapy, more than 80% of patients suffering from cHL are cured, which is otherwise a fatal disease with 90% of untreated patients dying within 2 to 3 years. However, up to 30% of patients with advanced HL will progress or relapse even after the therapeutic treatment (Connors et al., 2001); thus the development of new and more potent regimens with improved outcomes has become more necessary. In this respect, in addition to the general targets of HRS cells, the presence of EBV latent genes that are expressed in malignant cells provides an excellent opportunity for targeted therapy.

**Chemotherapy for early-stage cHL**

Regardless of its type, data of patients with early-stage HL show that present therapies result in high expectations of cure for HL patients, and more than 80% of patients have had their cancer successfully eradicated. Currently, treatment options of HL are tailored to type, stage, patient age, and an assessment of the risk of resistance (Diehl et al., 2003; Klimm et al., 2005). The induction chemotherapy regimens given as the initial treatment for HL are shown in Table 3. Although ABVD is the gold standard for all early HL cases, there are several data suggesting improved outcomes using the aggressive escalated BEACOPP regimen in advanced-stage HL (Carbone et al.,

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**Table 2. Epidemiology of Hodgkin lymphoma and their EBV association**

<table>
<thead>
<tr>
<th>REAL/WHO classification (% cases/total HL)</th>
<th>Subtypes</th>
<th>Malignant cell</th>
<th>% cases of subtypes</th>
<th>Association level with EBV*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>cHL (95%)</td>
<td>NSHL</td>
<td>HRS cell</td>
<td>60-80% of cHL</td>
<td>Positive or negative</td>
<td>(Jarrett et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>MCHL</td>
<td></td>
<td>15-30% of cHL</td>
<td>+++ (up to 96%)</td>
<td>(Flavell et al., 2000)</td>
</tr>
<tr>
<td></td>
<td>LDHL</td>
<td></td>
<td>&lt;5% of cHL</td>
<td>++</td>
<td>(Herbst et al., 1992)</td>
</tr>
<tr>
<td></td>
<td>LRHL</td>
<td></td>
<td>&lt;5% of cHL</td>
<td>+/- (less than 10%)</td>
<td>(Spitz et al., 1986)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Herbst et al., 1992)</td>
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<td>(Cleary et al., 1994)</td>
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<td>(Slack et al., 2009)</td>
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<td></td>
<td></td>
<td></td>
<td>(Shimabukuro-Vormhagen et al., 2005)</td>
</tr>
<tr>
<td>NLPHEL (5%)</td>
<td>LP cell</td>
<td></td>
<td></td>
<td>Negative</td>
<td>(Chan, 1999)</td>
</tr>
</tbody>
</table>


*varies depending on genetic and environmental factors of the affected patients.
Although it has been reported that chemotherapy alone may be more efficient for controlling some disease cases (Meyer et al., 2004), combined-modality therapy is currently the most common treatment of HL, which consists of an abbreviated course of chemotherapy and involved field radiation.

### Treatment strategies for the patients with relapsed cHL

Although most HL patients attain a remission after induction chemotherapy, relapse rates range from 10-15% in the early stage (Specht et al., 1998) to 30-40% in the advanced stage (Oza et al., 1993). For patients with relapsed HL, high dose chemotherapy plus autologous hematopoietic stem cell transplantation (HDC/HSCT) has become recognized as the most effective treatment. However, the fact that more than 15% of the relapsed patients are still dying of progressive lymphoma after HDC/HSCT treatment stresses an obvious need for more successful therapeutic strategies.

Currently, one of the most promising types of treatment for HL is immunotherapy targeting the malignant HRS cells of cHL. Since HRS cells display virtually the same immunopathological characteristics among different HL subtypes, drug development in HL has been based on the general understanding of cellular pathways altered in HRS cells and of interactions between HRS cells and the tumor microenvironment (summarized in Fig. 1).

**HRS cells:** The most unique characteristic of HL compared to other tumors is the presence of a small number of malignant HRS cells in a background of non-neoplastic reactive lymphoid cell population. Although the origin of the HRS cells had been an issue of intense debate due to no obvious normal cellular counterpart to the phenotype of HRS cells, the presence of non-functional somatic mutations in rearranged immunoglobulin genes of HRS cells confirmed that they originate from pre-apoptotic B cells that lost the capacity to express a high-affinity B-cell receptor, which were then rescued from apoptosis by transforming events (Kuppers et al., 1994; Kuppers, 2003). To date, many cases of aberrant activation of signaling pathways and transcription factors involved in the rescue of HRS cells from apoptosis have been identified, knowledge from which has been applied to the development of novel therapeutic agents for relapsed HL after primary treatment.

Studies on signaling pathways of HRS cells have implicated deregulated activation of a variety of intercellular proteins (NF-κB, Jak/STATs, Akt/mTOR, Notch-1, and ERK) as well as surface receptors (CD30 and CD40) (Clodi and Younes, 1997; Fiumara et al., 2001; Younes et al., 2003; Zheng et al., 2003).

<table>
<thead>
<tr>
<th>Therapy regimen</th>
<th>Combination of agents</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOPP</td>
<td>Mechlorethamine, Vincristine, Procarbazine, Prednisone</td>
<td>Developed in 1964.</td>
<td>(Longo et al., 1986)</td>
</tr>
<tr>
<td>ABVD</td>
<td>Adriamycin (doxorubicin), Bleomycin, Vinblastine, Dacarbazine</td>
<td>Developed in the early 1970’s as an alternative to MOPP.</td>
<td>(Boleti and Mead, 2007)</td>
</tr>
<tr>
<td>Stanford V</td>
<td>Doxorubicin, Vinblastine, Mustard, Bleomycin, Vincristine, Etoposide, Prednisone</td>
<td>Developed in 1985 at Stanford University.</td>
<td>(Hoppe et al., 1985; Hoppe et al., 1989)</td>
</tr>
<tr>
<td>BEACOPP</td>
<td>Bleomycin, Etoposide, Doxorubicin, Cyclophosphamide, Vincristine, Procarbazine, Prednisone</td>
<td>Developed in 1997 for advanced-stage HL</td>
<td>(Diehl et al., 1997)</td>
</tr>
<tr>
<td>Escalated BEACOPP</td>
<td>Higher doses of etoposide and doxorubicin and cyclophosphamide and the addition of granulocyte colony-stimulating factor for neutrophil support</td>
<td>Modified to improve treatment results with unfavorable and advanced-stage HL.</td>
<td>(Diehl et al., 1998a; Diehl et al., 1998b; Engert et al., 2009)</td>
</tr>
</tbody>
</table>
which are highly associated with pro-survival signals. Each of these proteins can be a therapeutic target of selective small molecule inhibitors. The AKT/mTOR pathway is an intracellular signaling pathway associated with the apoptotic process. In many malignant cells, this pathway is overactivated, and upon blockage of the pathway, cell cycle arrest and apoptosis of the cells subsequently take place. A preclinical study demonstrated that inhibition of mTOR, the mammalian target of rapamycin, caused apoptosis in HL cells. In addition, everolimus, a rapamycin-derived mTOR inhibitor, verified antitumor activity in relapsed HL patients in the phase I clinical study (Jundt et al., 2005; Yee et al., 2006), suggesting mTOR inhibitors as excellent therapeutic candidates for HL. Since NF-κB plays a central role in the regulation of various gene expression involved in cell survival, apoptosis, carcinogenesis, and inflammation, this molecule has been regarded as a very potential therapeutic target for many cancers (Baud and Karin, 2009). Bortezomib, a tripeptide with pyrazinoic acid, phenylalanine, and leucine with boronic acid, is a proteasome inhibitor that hinders activation of NF-κB by inhibiting cytoplasmic IκBα degradation (O’Connor, 2005). In preclinical studies, it inhibited proliferation of HL cell lines, and induced cell cycle arrest as well as apoptosis (Adams, 2003; Zheng et al., 2004). Not only the intracellular proteins described above but also surface receptor molecules can be specific targets for HL therapy. HRS cells characteristically express high levels of CD30 and CD40, both of which play a role in the increased NF-κB activity observed in HL as a member of the TNF receptor family. Immunotherapeutic agents targeting CD30 and CD40 are currently being evaluated for potential use in HL. Since they are surface receptor antigens that contain extracellular moieties, various monoclonal antibodies (mAbs) and mAb-drug conjugates are easily accessible to their target molecules.

Initially, unconjugated mAbs targeting CD30 (MDX-060 and SGN-30) were evaluated in relapsed HL clinical cases. The antibodies failed to show efficient antitumor activity (Ansell et al., 2007; Forero-Torres et al., 2009; Blum et al., 2010), but a next-generation drug-antibody conjugate, SGN-35 (brentuximab vedotin), improved the modest clinical activity of the unconjugated mAbs. This potent immunotoxin contains the anti-tubulin agent monomethyl auristatin E attached to SGN-30 (Oflozoglu et al., 2008). The mAb-drug conjugate is rapidly internalized on CD30 binding. Recently, the U.S. Food and Drug Administration granted fast-track designation for SGN-35 for the treatment of HL. In case of anti-CD40 mAbs, HCD122 and SGN-40 had been evaluated in patients with CD40-expressing lymphoid malignancies (Law et al., 2005; Luqman et al., 2008; Robak, 2008; Younes, 2009).

In addition to the drugs acting on specific target molecules, anticancer drugs that broadly inhibit the signaling pathways activated in HRS cells are often used for patients with refractory HL. These wide-ranging inhibitors, such as systemic chemotherapeutic drugs, histone deacetylase (HDAC) inhibitors, and proteasome inhibitors, can modulate several unrelated signaling molecules (Bolden et al., 2006; Brogdon et al., 2007; Gedya et al., 2008; Tarasenko et al., 2008). Most systemic chemotherapeutic drugs with antitumor activity are DNA replication inhibitors. For patients with relapsed HL, systemic chemotherapeutic drugs, such as gemcitabine (a nucleoside analog), vinblastine (an anti-microtubule drug), fludarabine (a purine analogue), and melphalan (an alkylating agent) have been used as a single agent or in combination regimens (Friedberg et al., 2003; Straus et al., 2011).

HDACs in cooperation with histone acetyltransferases (HATs) play an important role in epigenetic regulation of broad gene expression via post-transcriptional modification of his-

Table 4. Functions of six EBV genes expressed in HRS cells

<table>
<thead>
<tr>
<th>EBV genes expressed in HRS cells</th>
<th>Functions (target cellular proteins)</th>
<th>Therapeutic strategies</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBER 1 and 2</td>
<td>Cell growth (IGF-1, IL-6, IL-9)</td>
<td>Nt</td>
<td>(Ho et al., 1999; Komano et al., 1999; Kitagawa et al., 2000; Yamamoto et al., 2000; Iwakiri et al., 2003; Iwakiri et al., 2005; Samanta et al., 2006; Samanta et al., 2008; Iwakiri et al., 2009; Iwakiri and Takada, 2010)</td>
</tr>
<tr>
<td>EBER 1 and 2</td>
<td>Modulation of immune response (IL-10, TLR3, RIG-1)</td>
<td>Enhanced proteosomal degradation (enhancement of immunogenicity)</td>
<td>(Tellam et al., 2001)</td>
</tr>
<tr>
<td>EBNA1</td>
<td>Inhibition of apoptosis (survivin)</td>
<td>Enhanced proteosomal degradation (enhancement of immunogenicity, and rescue from oncogenic phenotypes)</td>
<td>(Duraiswamy et al., 2003; Tellam et al., 2003; Taylor et al., 2004; Smith et al., 2006; Pan et al., 2009; Lutzky et al., 2010; Chia et al., 2011)</td>
</tr>
<tr>
<td>LMP1</td>
<td>Inhibition of apoptosis (NF-κB, PI3K/Akt/mTOR, Jak/STAT1)</td>
<td>Adaptive immunotherapy (transfer of LMP1-specific CTLs, or primed DCs)</td>
<td>(Taylor et al., 2004; Smith et al., 2006; Pan et al., 2009; Shah et al., 2009; Lutzky et al., 2010; Chia et al., 2011)</td>
</tr>
<tr>
<td>LMP2A</td>
<td>Prosurvival signal (Ras/PI3K/Akt, Notch and β-catenin/Wnt)</td>
<td>Immunization with LMP2A gene or polyepitope expression vectors</td>
<td>(Shah et al., 2009)</td>
</tr>
<tr>
<td>LMP2B</td>
<td>Modulation of innate immune response</td>
<td>Adoptive immunotherapy (transfer of LMP2A-specific CTLs, or primed DCs)</td>
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</tbody>
</table>

Treatment strategies for the patients with EBV-associated cHL

The incidence of high relapse rates in EBV-associated HL compared to non-EBV-associated HL after primary treatment is still conflicting. However, in population-based studies have consistently shown a noticeable survival disadvantage in EBV-positive HL especially in the case of older patients (Enblad et al., 1999; Clarke et al., 2001; Stark et al., 2002), indicating the influence of EBV existence in HRS cells on clinical outcome. Furthermore, the fact that an EBV transforming protein, LMP1, is expressed almost 100% in HRS cells of HL in HIV-infected patients suggests that EBV may play as a crucial etiological agent in the generation of HRS cells, at least in certain circumstances (Thompson et al., 2004; Carbone et al., 2009).

EBV infection in HRS cells exhibits the pattern of the type II latency, expressing only a limited number of viral genes: EBERs, EBNA1, LMP1, LMP2A and 2B. Thus, the viral genes expressed during the type II latency are hypothetically the most excellent candidates for the therapeutic target against the malignant HRS cells of HL (Table 4).

**EBERs**: EBV-encoded small RNA 1 and 2 (EBER1 and EBER2) are nonpolyadenylated and noncoding RNAs of 167 and 172 nucleotides, respectively (Rosa et al., 1981). They form stem-loop double-stranded RNA (dsRNA) structures by intermolecular base-pairing, which enable them to interact with several cellular proteins (Ho et al., 1999). It has been demonstrated that EBERs play a role in EBV-mediated oncogenesis in EBV latent-infected cells. First, EBERs affect membrane signaling by initiating the production of various interleukins (ILs) that are involved in cell growth, such as IL-6, IL-9, or insulin-like growth factor-1 (Ho et al., 1999; Kitagawa et al., 2000; Iwakiri et al., 2003; Iwaki et al., 2005). They also play a role in regulation of apoptosis by binding the RNA-dependent protein kinase (PKR), subsequently inhibiting PKR-mediated apoptosis in the EBV infected cells (Komano et al., 1999; Yamamoto et al., 2000). In addition, EBERs can modulate interferon (IFN)/antiviral response. It was demonstrated that EBERs induce expression of type-I IFNs by binding to the retinoic acid-inducible gene I (RIG-I) product that is a cytosolic protein that recognizes viral dsRNA inside the cell (Samanta et al., 2006). Another evidence of a role of EBERs in innate immunity is their binding to Toll-like receptor 3 after secreted from EBV-infected cells (Iwaki et al., 2009; Iwakiri and Takada, 2010), triggering innate immunity that might explain immunopathologic diseases caused by active EBV infection. However, EBERs also induce an anti-inflammatory cytokine, IL-10, through RIG-1-mediated interferon regulatory factor (IRF) 3 (Samanta et al., 2008), suggesting that modulation of innate immune signaling by EBERs may contribute to EBV-mediated oncogenesis.

In all types of EBV-associated cancers, EBERs are abundantly expressed (Forte and Luftig, 2011). This fact makes EBERs a potentially important target for novel therapies for EBV-associated cancers including HL. However, other studies have found that EBERs are not essential for primary infection, viral replication, or B-cell immortalization (Swaminathan et al., 1991). Furthermore, deletion of EBERs showed little effect on the EBV transformation frequency of primary B cells (Gregorovic et al., 2011). Thus, to develop novel therapeutic approaches to EBV-associated cancers based on their EBER expression, clear mechanisms of EBERs should be identified by further studies.

**EBNA1**: EBNA1 is an indispensable protein for viral DNA replication and maintenance of the viral episome in infected cells (Hsieh et al., 1993). It has been shown that EBNA1 not only promotes the efficiency of immortalization of human primary B cells in vivo, but also inhibits apoptosis by up-regulating the apoptosis suppressor protein, survivin (Humme et al., 2003; Lu et al., 2011), suggesting that it may play a direct role in the pathogenesis of EBV-associated malignancies.

Since EBNA1 is consistently expressed not only in chronic active EBV infection, but also in all EBV-associated malignancies (Yoshioka et al., 2003), the viral antigen has been suggested as a very potent target for immunotherapy against HL. However, it is not easy to detect EBNA1-specific cytotoxic T lymphocytes (CTLs) from blood taken during primary infection or from healthy virus carriers (Masucci et al., 1992; Rickinson et al., 1992; Steven et al., 1996), because EBNA1 is protected from processing and presentation via the MHC class I pathway due to its internal Glycine-Alanine repeat (GAR) domain (Levitskaya et al., 1995), which acts as a inhibitory signal to prevent proteasome dependent degradation of this antigen (Levitskaya et al., 1997). Nevertheless EBNA1 is a very attractive viral antigen for a therapeutic target due to its ubiquitous presence in all EBV infected cells, and novel strategies to avoid the restricted class I processing of EBNA1 have been reported by several groups. In the experiment of Tellam and his colleagues, the EBNA1 gene was covalently linked with ubiquitin, and subject to targeting to the N-end rule pathway, in which the stability level of a protein in vivo can be dramatically changed by the identity of its N-terminal residue (Varshavsky, 1996; Tobery and Siliciano, 1999; Dantuma et al., 2000). These modifications dramatically enhanced intracellular degradation of the protein and restored CD8+ T cell recognition, demonstrating that GAR-mediated proteosomal blockade on EBNA1 can be reversed (Tellam et al., 2001). Moreover, it was demonstrated that CD8 T cells from patients with HL were successfully stimulated in vitro with a construct containing a GAR-deleted EBNA1, reversing the functional T cell impairment as well as responding to tumor cells expressing EBNA1 (Smith et al., 2006).

Currently, it is not easy to discover a therapeutic drug that specifically modulates the degradation of EBNA1. However, if fine pharmacologic manipulation of the ubiquitin proteasome system that could alter the outcome of many diseases become possible, one might be able to develop highly specific drugs that target the degradation pathways of a single or a few proteins with no side effect on other proteins (Reinstein and Ciechanover, 2006).

**EBV-encoded latent membrane proteins, LMP1, LMP2A, and 2B**: LMP1 acts as a constitutively active receptor in a ligand-independent manner, mimicking CD40 (Gires et al., 1997). It activates the majority of signaling pathways that are known to be activated in HRS cells, including NF-κB, PI3K/Akt/mTOR, and Jak/STAT pathways, thereby induces various antiapoptotic proteins and cytokines (Chen et al., 2003; Lambert and Martinez, 2007; Kung et al., 2011). Since normal germinal center B cells that lack BCR in their cell surfaces are eliminated by apoptosis, the HRS precursor cell is assumed to be rescued by LMP1 that plays a critical role in the protection of B cells from apoptotic death by up-regulating several antiapoptosis genes (Asso-Bonnet et al., 1998; Lee et al., 2003).

The LMP2 gene encodes two protein isoforms, LMP2A and LMP2B. They are identical except for an additional 119 aa-
long cytoplasmic region at the amino-terminus of the LMP2A isoform (Longnecker and Kieff, 1990). LMP2A mimics BCR, thereby provides an essential prosurvival signal for B cells. In EBV-infected B cells, LMP2A functions to promote viral latency, providing signals to ensure cell survival in the absence of BCR signaling (Longnecker, 2000). It can enhance cell growth, survival, and cellular differentiation through activation of the Ras/PI3K/Akt, Notch and β-catenin/Wnt signaling pathways (Scholle et al., 2000; Morrison et al., 2003; Anderson and Longnecker, 2008). Compared to LMP2A, the role of LMP2B is relatively unknown. Recently, it was reported that LMP2B as well as LMP2A modulate signaling from receptors involved in innate immune responses (Shaah et al., 2009).

Overall, the two viral proteins, LMP1 and LMP2A, are considered the major players in the generation of EBV-associated HRS phenotypes (Bechtel et al., 2005).

Use of mAbs as immunotoxins against the upregulated surface molecules of HRS cells, such as CD30 and CD40, for targeted immunotherapy was stated in the previous section. In order to function as a potent target molecule against immunotoxins, the presence of an extracellular domain and its ease accessibility are essential, which are lacking in LMP1 as well as LMP2A (Flanagan et al., 2003). These LMPs contain relatively large cytoplasmic domains with extremely short extracellular loops connecting transmembrane segments, which are not easily accessible by antibodies (Gires et al., 1997; Panousis and Rowe, 1997; Lynch et al., 2002). Moreover, the extracellular loop regions marginally elicit antibodies in the course of natural infection and tumorogenesis (Paramita et al., 2011). Thus, despite their abundance, antibody-based targeting of LMP1 or LMP2 on EBV-positive HRS cells has low therapeutic possibilities.

Recently, it was shown that conformational peptides mimicking two adjacent loops of LMP1 induce high-affinity antibodies with antitumor activities in mice (Delbende et al., 2009). However, unlike the in vivo experimental animal settings, the induction of effective CTL responses against those proteins has showed difficulties per se due to unique characteristics of HRS cells and their microenvironment as described (Marshall et al., 2003). For example, in vitro HRS cells that present epitopes from LMP1 and LMP2A are subject to lysis by EBV-specific CTLs, but EBV-infected HRS cells survive in vivo (Sing et al., 1997; Chapman et al., 2001; Su et al., 2001). Thus, although EBV-associated HL patients initially achieve an effective anti-EBV response after the period of first EBV infection, they do not completely eradicate EBV-infected HRS cells.

In addition, despite the presence of EBV-specific CTLs in the peripheral blood, the CTLs are not found in the immediate surrounding areas of EBV-positive HRS cells (Frisan et al., 1995). Thus, for successful CTL therapy for EBV-positive HL, not only effective EBV-specific CTLs, but possibly also the modulation of the tumor microenvironment to eliminate the barriers that inhibit CTL function will be required.

Nevertheless, these LMP molecules still draw attention as major targets for therapeutic purposes. Since patients with HL display functional impairment of CTL, adoptive cellular immunotherapy based on efficient EBV-specific CTLs may provide an answer to success. Development of an epitope-based vaccination strategy to augment EBV-specific cytolytic activity of CTLs is currently paid attention to as one of the preferred approaches for the treatment of EBV-associated relapsed HL. Firstly, efficient antigen (epitope) presentation by antigen presenting cells may improve the CTL activities. The therapeutic potentials of dendritic cell (DC) vaccine transduced by a recombinant vaccinia or adeno-associated virus carrying LMP1 and LMP2 CTL epitope DNA were evaluated, and the treatment of the vaccine was effective on eliminating tumors of syngeneic animals (Taylor et al., 2004; Pan et al., 2009). And a phase II study on vaccination of autologous DCs transduced with an adenosine encoding a truncated LMP1 and full-length LMP2 is under evaluation for the safety and efficacy for patients with metastatic EBV-positive nasopharyngeal carcinoma (Lutzky et al., 2010; Chia et al., 2011). In this case, the truncated form of LMP1 was utilized because the full-length protein has both oncogenic and immunosuppressive properties (Gottschalk et al., 2003). Secondly, antigen presentation by polyepitope vaccines may enhance CTL activities. A polyepitope comprises at least one recombinant protein including multiple CTL epitopes from one or more pathogens. A recombinant poxvirus vaccine encoding a polyepitope derived from LMP1, called as an LMP1 polyepitope vaccine, displayed stronger anti-LMP1 responses (Duraiaswamy et al., 2003). In addition, an exposure to a replication-deficient adenaloviral system with polyepitopes from LMP1 or LMP2 induced effective expanding of specific T cells. HL patients with this adenaloviral construct in combination with IL-2, were sufficient to reverse the functional T cell impairment and restored cytolyltic function (Smith et al., 2006). Like EBNA1, LMP1 molecules can be efficiently presented via cotranslational ubiquitination combined with N-end rule targeting. Since this method is involved with enhanced degradation of LMP1, this strategy completely abrogates cellular signaling pathways associated with the oncogenic phenotype, and helps in enhancing immunogenicity (Tellam et al., 2003), suggesting that the proteasomal targeting strategy could be therapeutically utilized for various tumor-associated oncogenes.

CONCLUSIONS

In the early twentieth century, cHL was incurable in most cases. Although cHL is currently considered one of the most curable forms of cancer with conventional chemotherapy, therapeutic challenges still remain, especially in finding novel strategies to control patients with relapsed cHL, albeit in small numbers. Since immunopathogenic features of HRS cells are very similar in both EBV-positive cHL and EBV-negative cHL, efforts to find cellular targets for drug development to treat cHL have been mainly focused on common phenotypes of HRS cells regardless of the presence of EBV. However, the presence of EBV-latent antigens abundantly expressed in the malignant HRS cells represents an attractive therapeutic option for targeted immunotherapy. Through continued researches of existing and new treatment options for cHL, advances should continue to be made.

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