Epstein-Barr Virus and Hodgkin's Disease

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Approximately 40% to 50% of cases of Hodgkin's disease occurring in Western populations are associated with the Epstein-Barr virus (EBV). In these cases, EBV is found in the neoplastic elements, the Reed-Sternberg and Hodgkin's cells. EBV is probably not present in all cases, but neither have any other viruses been found in the cases that are EBV-negative. EBV may play a role in the pathogenesis of Hodgkin's disease by the activation of anti-apoptotic factors in a premalignant germinal center B-lymphocyte. Regardless of their role in etiology or pathogenesis, EBV-latent antigens may represent a target for possible immune therapy.

Introduction

Hodgkin's disease was first described, albeit imperfectly, in 1832. Its neoplastic versus inflammatory nature was debated for many years, but today there is ample evidence that it is a monoclonal neoplasm. Even after Hodgkin's disease had been presumed to be neoplastic, an association with a viral infection was postulated. The basis of this association lay in interesting epidemiologic data that demonstrated a bimodal age-incidence in Western populations and in histologic features such as the Reed-Sternberg and other Hodgkin's cells exhibiting viral inclusion-like nucleoli.

The Epstein-Barr virus (EBV) is a γ herpesvirus that was first discovered in cell cultures of African Burkitt's lymphoma in 1964 [1]. Tantalizing epidemiologic studies performed in the 1970s and 1980s demonstrated that patients with Hodgkin's disease differed from control subjects in that they had elevated titers against several EBV antigens. In one study, it was shown that elevated titers to EBV viral capsid antigen (VCA) and early antigen (EA) predated the clinical development of Hodgkin's disease and that elevated titers to the Epstein-Barr nuclear antigen (EBNA) complex predicted subsequent development of Hodgkin's disease [2]. Using models from other viruses such as polio, investigators hypothesized that EBV might be of pathogenetic or even etiologic significance in at least a subset of cases, particularly those with a peak incidence in middle adulthood. The last decade has provided convincing molecular data, confirming a role for EBV in at least a significant subset of cases of Hodgkin's disease.

Epstein-Barr Virus: A Brief Review

Epstein-Barr is a large, double-stranded DNA virus, about 172 kb in length. There are two main strains, type A and type B (or type 1 and type 2), which differ in the sequences of several genes and have different epidemiologic distribution and in vitro properties [3]. EBV is endemic worldwide, with infection in greater than 90% of adults in all infected populations. In developing countries, infection occurs at an early age and is asymptomatic, whereas in developed countries, infection occurs later and presents as acute infectious mononucleosis in a subset of cases. The virus initially occurs as a lytic infection of oral epithelial cells and B lymphocytes (entering the latter via the cluster designation [CD]21 receptor) which rapidly converts to a latent infection. The EBV-infected B-cells are driven to proliferate, until controlled by CD8-positive cytotoxic T-cells. Although a high burden of EBV-positive B-cells is present initially, fewer EBV-infected B-cells are present after the development of effective cellular immunity, on the order of one in a thousand to one in a million infected B-cells. A lesser number of infected T-cells is also noted.

In a lytic infection, EBV has a linear configuration and expresses most of its approximately 100 genes, including viral capsid and envelope antigens. In a latent infection, EBV is a circular episome and expresses only a few genes. These include nuclear proteins such as EBNA1, EBNA2, EBNA3A-C, and leader protein, and membrane proteins such as latent membrane protein (LMP)1, LMP2A, and LMP2B. These proteins are responsible for maintenance of the viral infection, driving cell proliferation and transformation, and expression of a variety of host activation, adhesion, and anti-apoptosis molecules. All of the latent proteins generate a cellular immune response, with the exception of EBNA1, which is not processed for presentation in mixed histocompatibility (MHC) class I molecules because of its gly/ala repeat, which inhibits its binding to transporter-associated proteins. It is thought that early in a latent infection, all the latent genes are expressed (latency pattern 3), whereas after the development of competent cellular immunity, only EBNA1 is expressed by most

Table 1. Expression of Major EBV-latent Proteins and EBER

	Latency pattern 1	Latency pattern 2	Latency pattern 3
EBNA1	+	+	+
EBNA2	-	-	+
EBNA3A-C	-	-	+
LMP1	-	+	+
LMP2A-B	-	+	+
EBER1-2	+	+	+
Example:	Burkitt's lymphoma	Hodgkin's disease	Posttransplant lymphoproliferation

EBER—Epstein-Barr–encoded RNA.

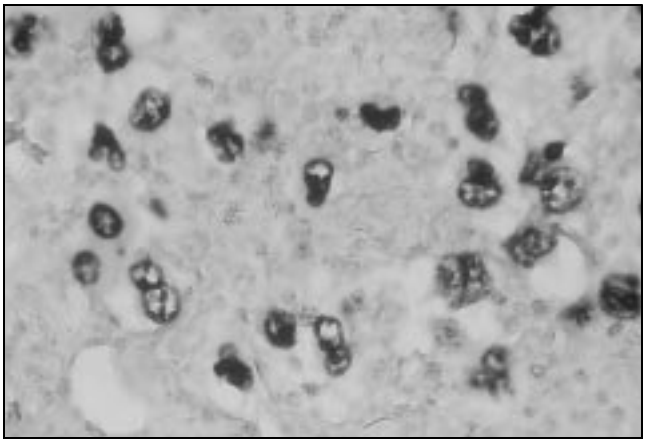

Figure 1. Depiction of EBER in situ hybridization in an EBV-positive case of Hodgkin's disease. The nuclei of the Reed-Sternberg and Hodgkin's cells are labeled.

EBV-infected B-cells (latency pattern 1), thereby avoiding immune surveillance (Table 1). In addition to expression of the latent proteins, production of a large number of copies of Epstein-Barr–encoded RNA (EBER)1 and EBER2 takes place. Although their function is not yet clear, their presence in high copy numbers makes them an excellent target for detection, for example by in situ hybridization studies.

EBV and Hodgkin's Disease: The Data

Data from a myriad of studies have demonstrated that EBV genomes can be detected in about 40% to 50% of cases of Hodgkin's disease in Western countries [4••,5–7]. In the EBV-positive cases, the EBV genome is present in all or virtually all of the Reed-Sternberg cells and variants (Fig. 1), as well as in rare EBV-positive reactive B- and T-lymphocytes. With rare exceptions, in EBV-positive cases, EBV is present in the neoplastic population at all involved sites and at relapse [8]. Southern blot studies have demonstrated that EBV is present in a monoclonal population of cells at greater than 50 copies per cell [9,10]. Immunohistochemical and other studies have demonstrated the expression of EBNA1, LMP1, LMP2A, and LMP2B, consistent with viral latency pattern 2 [11–13]. A subset of cases may also express transcripts that are consistent with an abortive lytic infection, and actual viral replication may be present in rare cases [14,15]. EBV genomes may be detected in the serum of patients with EBV-associated Hodgkin's disease [16].

EBV positivity in Hodgkin's disease correlates with histologic subtype [6, 7,17]. Cases of mixed cellularity and lymphocyte depletion are EBV-positive in about 75% of cases, whereas cases of nodular sclerosis are EBV-positive in less than 20% of cases. Cases of nodular lymphocyte predominance in Hodgkin's disease are virtually never EBV-associated [18]. No correlation exists between EBV positivity and immunophenotype of the neoplastic cells. No striking associations have been made with various oncogenes, with the exception that EBV negativity may be correlated with mutations of the *p53* gene in Reed-Sternberg cells [19]. EBV-positive Reed-Sternberg cells are associated with higher levels of human leukocyte antigen (HLA)–class I molecules than of EBV-negative Reed-Sternberg cells [20].

EBV-associated cases are more common in male than in female patients and are less common in patients between the ages of 15 and 50 years [21]. The highest group expression of positivity is in children under the age of 10 years. Higher rates of EBV positivity are seen in Asians (about 65%) and in South Americans and Africans (90% to 100%) [4••,22,23]. Within a given region, a higher rate of positivity is seen in Hispanics compared with whites [10]. Hodgkin's disease patients with prior infection with HIV have a virtually 100% association with EBV [24,25]. Patients with a history of acute infectious mononucleosis are more likely than are control subjects to develop Hodgkin's disease, but, surprisingly, these patients are not more likely to develop EBV-positive versus EBV-negative Hodgkin's disease [26]. Similarly, patients with higher titers of EBV antibodies are not more likely to develop EBV-positive versus EBV-negative Hodgkin's disease. No correlation exists between EBV positivity and various HLA types that are known to present specific EBV-latent protein antigens [27,28].

In stage I Hodgkin's disease patients, EBV positivity has been associated with presentation in neck lymph nodes [29]. However, no correlation exists between EBV positivity and overall disease stage. Patients with EBV-positive Hodgkin's disease have a slightly higher complete remission rate after chemotherapy than do patients with EBV tumors [30]. In addition, patients with EBV-positive tumors have a significantly better 2-year failure-free survival rate when compared with patients with EBV-negative tumors. Although 2- and 5-year overall survival rates have been reported to be better for patients with EBV-positive Hodgkin's disease, the differences thus far are not statistically significant.

It was initially thought that mutations of specific genes within the EBV genome might confer greater pathogenicity to certain strains, and might be correlated with more aggressive disease. For example, in one study the presence of a specific deletion in the 3'-terminal region of the *LMP1* gene had been correlated with Hodgkin's disease with greater numbers and atypia of Reed-Sternberg cells, greater degrees of tissue necrosis, and a poorer prognosis [31]. Some in vitro evidence suggests that deletions in this region, which is the site of interaction with the transcription factor nuclear factor κB (NFκB), may make *LMP1* more tumorigenic [32]. However, further study of this mutation suggests that these variations are population-related, and no convincing relationship to disease incidence or aggressiveness has been shown for this deletion, with the possible exception of the association between Hodgkin's disease and HIV infection [33]. Similar results have been found for mutations within the *EBNA1* gene in Hodgkin's disease. Some variants of the *EBNA1* gene have been suggested as relevant to the ability of EBV to persist in various cell types and with greater likelihood to lead to oncogenesis for certain neoplasms. However, we found a similar distribution of *EBNA1* variants in reactive versus Hodgkin's disease tissues [34]. Finally, it has been suggested that mutations in the *EBNA3B* gene (which codes for a protein that elicits a strong HLA-A11 cytotoxic T-lymphocyte [CTL] response) may lead to EBV strains not recognized by CTLs that may be preferentially involved in oncogenesis. However, we found similar types of mutations in the *EBNA3A* gene at similar frequencies in reactive versus Hodgkin's disease tissues; furthermore, these mutations did not show a tendency to occur in HLA-A11–positive individuals [35].

EBV-negative Hodgkin's Disease

At least 50% of cases of Hodgkin's disease have not been shown to be associated with EBV. Several explanations are possible. First, it is possible that EBV entered the preneoplastic cells, did its damage, and subsequently became invisible to detection (the "hit-and-run" hypothesis). One could envision either a complete loss of viral episomes or integration of small (but key) viral fragments into the host genome prior to loss of the majority of the genome. There is some precedent for this, in that loss of viral episomes has been shown in a Burkitt's lymphoma cell line in culture [36]. In another lymphoma cell line, integration of EBV into the host genome led to enhanced chromosomal instability, with subsequent partial deletion of the integrated EBV genome [37]. Interestingly, the LMP and EBV early untranslated RNA (*EBER*) genes, two of the most analyzed genes for EBV detection, were both deleted. In addition, one group of investigators studied a series of sporadic cases of apparently EBV-negative Burkitt's lymphoma using multiple probes from different regions of the EBV genome, and they were able to demonstrate evidence of integrated, defective viral genome in some cases [38]. Nevertheless, intensive study of EBV cases of Hodgkin's disease by multiple probes, including probes that span most of the EBV genome, have not disclosed any evidence of a defective EBV genome [39••]. In addition, cases of Hodgkin's disease occurring in seronegative individuals are well-documented [28].

If EBV is not present in a significant subset of cases, could it be that another virus takes its place in the pathogenesis of Hodgkin's disease in the EBV-negative cases? If this is true, the virus is probably not a known one. Serologic studies have shown no evidence of herpes simplex virus, varicella-zoster virus, cytomegalovirus, rubella virus, measles virus, parainfluenza virus, or human herpesvirus (HHV)-7 or HHV-8 [40]. Although evidence from some serologic and polymerase chain reaction studies are supportive of a role for HHV-6, in situ hybridization studies have been negative. In addition, molecular studies have shown no evidence for adenovirus type 5 or 12, lymphotropic papovavirus, the polyoma viruses JC and BK, or simian virus (SV)40, HHV-7, or HHV-8 [39,41]. We have spent a good deal of time in the laboratory using representational difference analysis in an attempt to identify a novel virus, to no avail. This technique, used successfully to identify HHV-8 in Kaposi's sarcoma, involves the making of a simple representation of the genes in involved versus uninvolved tissues and, through an iterative process involving ligation, hybridization, and polymerase chain reaction, identifying unique DNA sequences in the involved tissue (Weiss, Unpublished observation, 1996). Another group has employed different molecular strategies using degenerate primers derived from conserved areas of viral genomes, with similar negative results [39••].

Pathogenesis of Hodgkin's Disease and Possible Role of EBV

Studies of rare cases of Hodgkin's disease occurring in the setting of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) suggest a role for EBV in the pathogenesis of Hodgkin's disease [42]. Although transformation to a high-grade non-Hodgkin's lymphoma is a much more common event, rare cases of transformation to

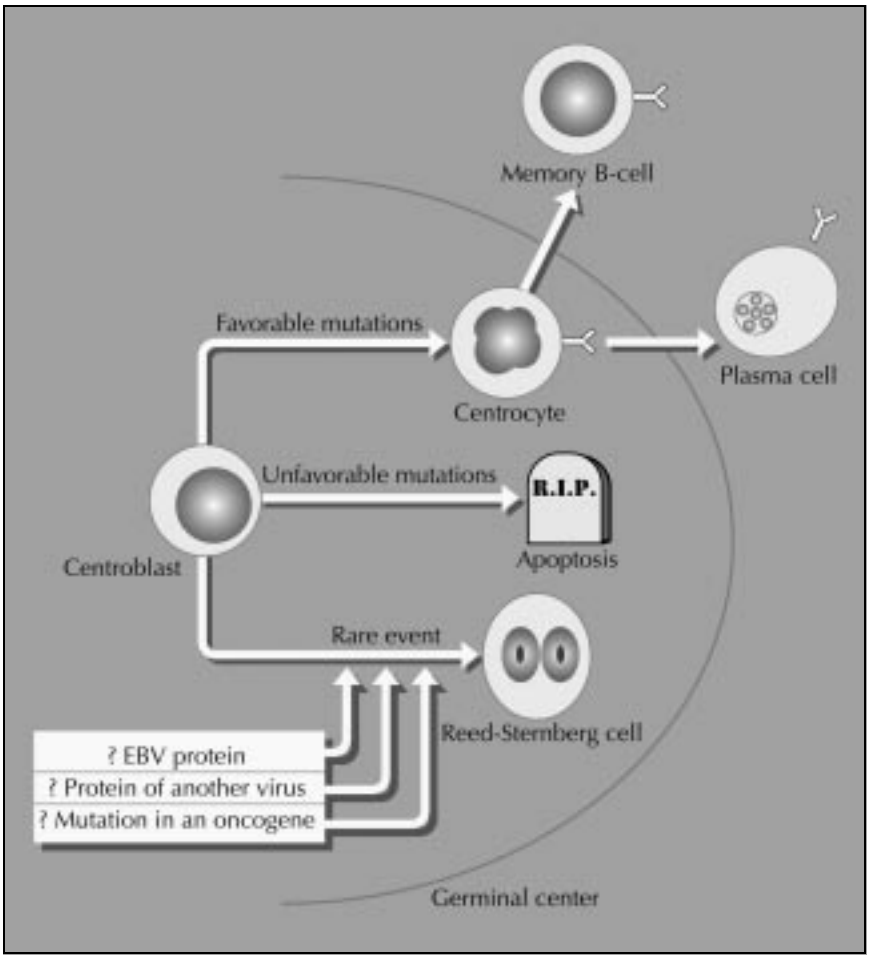

Figure 2. Depiction of possible pathogenesis of Hodgkin's disease.

Hodgkin's disease have been described. CLL/SLL is never EBV-associated, but almost all cases of Hodgkin's disease arising in the setting of chronic lymphocytic leukemia/small lymphocytic lymphoma are EBV-associated. In these cases, the Reed-Sternberg cells usually occur within a background of CLL/SLL, and the Reed-Sternberg/Hodgkin's cells often have transitional B-cell/Hodgkin's disease phenotypes, suggesting direct transformation from the low-grade lymphoproliferation. Some investigators have reported identification of rare small lymphocytes with EBV infection, suggesting that it is these cells that may transform to Hodgkin's cells.

In *de novo* Hodgkin's disease, the neoplastic cells (the Reed-Sternberg and Hodgkin's cells) have been shown to be derived from germinal center B-cells [43•]. The majority of germinal center B-cells normally undergo apoptosis, unless there is contact with antigen, mediated in a specific way by membrane immunoglobulin receptors (Fig. 2). Reed-Sternberg and Hodgkin's cells have lost the capacity to produce (and therefore to express) immunoglobulin receptor, possibly because of crippling mutations outside of the coding region that affect the regulation of immunoglobulin gene expression. Reed-Sternberg and Hodgkin's cells have markedly increased numbers of somatic mutations of the immunoglobulin genes, suggesting that they have resided in the germinal centers for an abnormally long time without undergoing apoptosis. The factor responsible for the anti-apoptotic effects may be nuclear transcription factor (NF)-κB, which is known to be constituently expressed in Hodgkin's disease (Fig. 3) [44]. EBV LMP1 is known to activate NF-κB via upregulation of a number of tumor necrosis factor receptor–associated factors (TRAFs), including TRAF-1 and TRAF-2 [45]. TRAF-1 is known to be overexpressed in Hodgkin's disease [46•], and it has been shown that overexpression of TRAF-1 inhibits antigen-induced apoptosis in CD8-positive T-lymphocytes [47]. There may be a separate mechanism for the inhibition of apoptosis in EBV-negative cases, either by another protein taking the place of EBV LMP1 or by a separate pathway, perhaps related to the *p53* system [19].

EBV as a Target for Treatment in Hodgkin's Disease

Regardless of whether EBV has a role in the pathogenesis of Hodgkin's disease, the fact that EBV antigens are present on Reed-Sternberg and Hodgkin's cells in about 50% of cases raises the possibility that these antigens could be used as a target for therapy. It has been shown previously that adoptive transfer of EBV-specific CTLs can be useful for

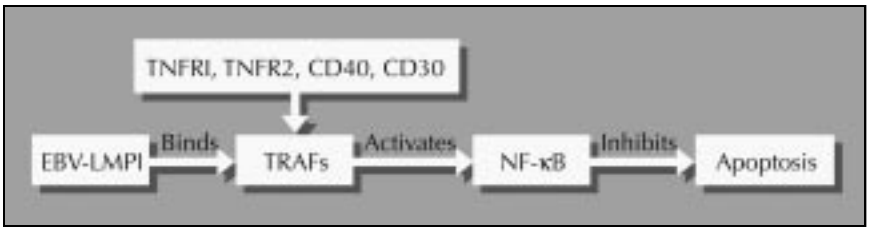

Figure 3. Depiction of tumor necrosis factor receptor superfamily signaling pathway utilized by EBV-LMP1 to inhibit apoptosis. CD—cluster designation; NF-κB—nuclear transcription factor-κB; TNFR—tumor necrosis factor receptor; TRAF—tumor necrosis factor–associated factor.

patients who have received T-cell–depleted allogeneic stem cell transplants, and is effective as prophylaxis and treatment of EBV-associated immunoblastic lymphoma [48]. EBV LMP1 and LMP2 antigens that are expressed on EBV-positive Reed-Sternberg cells can each potentially elicit a CD8-positive CTL response. It has been shown that Reed-Sternberg cells are able to process and present viral proteins, and they are efficiently lysed by specific CTLs in a class I–restricted manner [49]. In fact, a stable epitope in LMP2A has been found that is restricted to HLA-A2.1, a relatively common allele in white populations [20]. Moreover, LMP-specific clones have been generated that persist for more than 13 weeks post-infusion and retain their potent antiviral effects in vivo [50•]. It is hoped that LMP-specific clones may be useful as adjuvant therapy in Hodgkin's disease, particularly for those patients with relapsed disease.

Conclusions

In the last 10 years, we have made much progress in learning about the association of Epstein-Barr virus and Hodgkin's disease. EBV is found in the neoplastic element of Hodgkin's disease in about 40% to 50% of cases in Western populations, but it is not present in all cases. No other viruses have been identified in the EBV-negative cases. EBV may contribute to the pathogenesis of Hodgkin's disease via the interference of EBV LMP1 or another latent protein with the normal apoptotic process of a preneoplastic B-cell in the germinal center. Regardless of the role of EBV in the etiology or pathogenesis of Hodgkin's disease, it may represent a target for immune therapy.

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