

Review

Epstein-Barr virus and the origin of Hodgkin lymphoma

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Abstract

Although Epstein-Barr virus (EBV) is present in the malignant Hodgkin/Reed-Sternberg (HRS) cells of a proportion of cases of classical Hodgkin lymphoma (cHL), how the virus contributes to the pathogenesis of this disease remains poorly defined. It is clear from the studies of other EBV-associated cancers that the virus is usually not sufficient for tumor development and that other oncogenic co-factors are required. This article reviews what is known about the contribution of EBV to the pathogenesis of cHL and focuses on emerging evidence implicating chronic inflammation as a potential oncogenic co-factor in this malignancy.

Key words Epstein-Barr virus, Hodgkin lymphoma, chronic inflammation

Hodgkin lymphoma (HL) is an unusual malignancy that is characterized by the presence of a minority of malignant Hodgkin/Reed Sternberg (HRS) cells surrounded by a non-neoplastic inflammatory infiltrate. There are two distinct forms of HL, known as classical Hodgkin lymphoma (cHL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), that are separated on the basis of morphologic, immunophenotypic, and clinical differences. Despite earlier reports to the contrary, recent studies suggest that a small proportion of NLPHL cases harbor Epstein-Barr virus (EBV)^[1,2]. However, this review focuses on cHL where the link with EBV is most clearly established.

Origin of HRS Cells in cHL

The identification of clonally rearranged and somatically mutated immunoglobulin genes in single isolated HRS cells provides evidence that these cells are malignant and derived from B cells that have undergone a germinal center (GC) reaction^[3]. However, HRS cells lack a functional B-cell receptor (BCR). In some cases, this lack of BCR expression is the result of somatic mutations that destroy the coding capacity of originally functional immunoglobulin genes (so called "crippling" mutations), whereas in others, it can be caused by the loss of the immunoglobulin-specific transcription factors POU class 2 associating factor 1 (POU2AF1/BOB1), POU class 2 homeobox 2 (POU2F2/OCT2), and Spi-1 proto-oncogene

(SPI1/PU.1) or mutations in the immunoglobulin gene promoter^[4-11]. Because apoptosis is the normal fate of GC B cells lacking a functional BCR, the survival of the BCR-negative HRS cell precursor must depend upon the acquisition of novel anti-apoptotic functions.

In addition to the loss of a functional BCR, HRS cells also display a characteristic loss of B-cell lineage gene expression, including the down-regulation of components of the BCR signaling machinery^[12]. This phenotype has been attributed in part to the overexpression in HRS cells of transcription factors such as inhibitor of DNA-binding 2 (ID2), which has been shown to negatively regulate B-cell-specific transcription factors, including transcription factor 3 (TCF3/E2A) and paired box 5 (PAX5)^[13,14].

EBV and cHL

EBV was first implicated in the pathogenesis of cHL when it was shown that patients had raised antibody titers to EBV antigens and that these preceded the development of cHL by several years^[15,16]. Subsequently, EBV DNA and RNA were detected in HRS cells^[17,18]. The viral episomes present in HRS cells were also shown to be monoclonal, suggesting that infection of the tumor progenitor occurred prior to its clonal expansion^[19].

Epidemiologic studies suggest there are three forms of cHL: pediatric HL (EBV-positive, mixed cellularity type), HL of young adults (EBV-negative, nodular sclerosis type), and HL of older adults (EBV-positive, mixed cellularity type)^[20,21]. The development of EBV-positive cHL in children is thought to be a consequence of a rare, abnormal response to early primary infection, whereas EBV-positive cHL in older adults has been attributed to a decline in EBV-specific immunity associated with advancing age^[22,23]. Although senescence of EBV immunity is also suspected in a related tumor, known as "EBV-positive diffuse large B-cell lymphoma (DLBCL) of the elderly," the defects in EBV-specific immunity in these patients have yet to be defined^[24,25].

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Contribution of EBV to the Survival of HRS Cell Precursors

As described by Rowe *et al.*^[26], EBV contributes to the pathogenesis of Burkitt's lymphoma (BL) by providing the anti-apoptotic signals necessary to override c-myc-induced cell death. In the case of cHL, anti-apoptotic stimuli are also required, but this time to overcome the cell death that would otherwise occur in the absence of a functional BCR. Two pieces of evidence support a role for EBV in providing this anti-apoptotic function. First, the so-called "crippling" mutations in immunoglobulin genes described above are almost exclusively found in EBV-positive cases, and second, EBV has been shown to immortalize BCR-negative GC B cells *in vitro*^[27-30]. To begin to understand how EBV contributes to this anti-apoptotic phenotype, we need to revisit the functions of some of the latent EBV genes expressed in HRS cells.

As described by Young *et al.*^[31] in this issue, switching between different forms of latency in the B-cell system may allow the virus to regulate its own life cycle and redirect the fate of the infected B cells towards long-term persistence in the memory pool. Some evidence suggests that the EBV-infected GC B cells of asymptomatic virus carriers express several virus genes designed to drive their transit through a GC reaction and subsequent differentiation into memory B cells^[32-34]. Critical to this process are the two viral latent membrane proteins, latent membrane protein-1 (LMP1) and LMP2A, which are commonly expressed in EBV-positive HRS cells^[35,36]. LMP1 has been shown to replace survival and differentiation signals that are similar to those provided by an activated CD40 receptor^[37,38]. When expressed in the proposed precursor cells of cHL, LMP1 contributes up to 25% of the transcriptional changes found in cHL^[39]. LMP1 may contribute to the survival of apoptosis prone GC B cells by activating several survival pathways, including the nuclear factor-kappa B (NF- κ B), Janus activated kinase/signal transducers and activators of transcription (JAK/STAT), and phosphatidylinositol 3-kinase (PI3K)/AKT pathways, signaling pathways that have been found to be constitutively active in cHL^[40-46]. LMP2A, on the other hand, mimics an activated BCR signal and is able to drive B cell survival in the absence of a functional BCR^[47-52]. In transgenic mice, LMP2A expression interferes with normal B-cell development and contributes to cell survival involving activation of the RAS/PI3K/AKT pathway^[53,54].

Loss of BCR Functions During the Evolution of EBV-positive cHL

Although EBV can provide anti-apoptotic functions that contribute to the survival of BCR-negative HRS cell progenitors, it is not clear how the loss of a functional BCR is involved in the pathogenesis of EBV-associated cHL. To answer this question, we need to return to another aspect of EBV biology, namely the regulation of the viral replicative cycle.

In addition to existing in various latent states, EBV can also induce its replicative cycle in B cells, a process that eventually leads to the production of new viral particles or virions. The switch from latency to the replicative cycle is triggered by two

distinct mechanisms—activation of BCR signaling and plasma cell differentiation, and the switch is regulated in part by the two latent membrane proteins. By providing a BCR-like signal, LMP2A induces entry into the viral replicative cycle^[55,56] (**Figure 1**). On the other hand, LMP1 prevents entry into the replicative cycle by suppressing plasma cell differentiation^[57,58].

Because virus release results in cell death, eventual completion of the EBV replicative cycle is most likely incompatible with tumor development^[59]. In the context of the development of cHL, the loss of a functional BCR could be important because it would be expected to prevent BCR-mediated entry into the replicative cycle and thus cell death. Although LMP2A can activate EBV replication even in the absence of a functional BCR, we have shown that LMP2A cannot induce the replicative cycle when essential components of the BCR signaling machinery are missing^[60]. Thus, the loss of BCR as well as of BCR signaling components combine to prevent both BCR- and LMP2A-induced virus replication and might explain why cells lacking BCR signaling functions are positively selected during the development of EBV-associated cHL (**Figure 1**). However, HRS cells retain LMP2A expression, suggesting that this virus protein has BCR-independent functions that are important for tumor development and/or maintenance.

Human Immunodeficiency Virus (HIV), Chronic Immune Stimulation, and cHL

Compared to the general population, the incidence of cHL is 5–15 times higher among people with HIV and acquired immune deficiency syndrome (AIDS), and most cases of cHL in HIV-positive patients are EBV-positive and of mixed cellularity type^[61-64]. However, in contrast to other forms of HIV-associated lymphomas, the incidence of cHL is the highest when CD4⁺ T-cell counts are only modestly reduced^[63]. Furthermore, the incidence of cHL in HIV-positive patients has not fallen in the post-highly active anti-retroviral therapy (HAART) era^[65-67]; indeed, some studies suggest HL risk may be increased in the first few months following immune reconstitution on HAART^[64,68]. Two interpretations might explain these data. First, at very low CD4⁺ T-cell counts, the morphologic presentation of cHL may shift to an appearance more similar to non-Hodgkin lymphoma (in which there are fewer CD4⁺ T cells in the tumor microenvironment), resulting in a diagnostic misclassification. A second, more likely explanation is that CD4⁺ T cells may be required for the development of cHL. Indeed, the inflammatory infiltrate of cHL is known to be rich in CD4⁺ T cells that can promote HRS survival through direct and cytokine-mediated interactions^[69]. EBV genes, particularly *LMP1*, have been shown to contribute to this microenvironment by producing cytokines and chemokines that recruit and modify the chronic inflammatory cells, including CD4⁺ T cells^[69-71].

There is emerging evidence that other aspects of HIV infection, in addition to the reduced EBV-specific immunity, might contribute to lymphomagenesis. Increased EBV loads are usually observed only during the early stages of HIV infection when there is little or no T-cell impairment and are attributed to a generalized activation by HIV of the B-cell system^[72]. The increased risk of BL that occurs

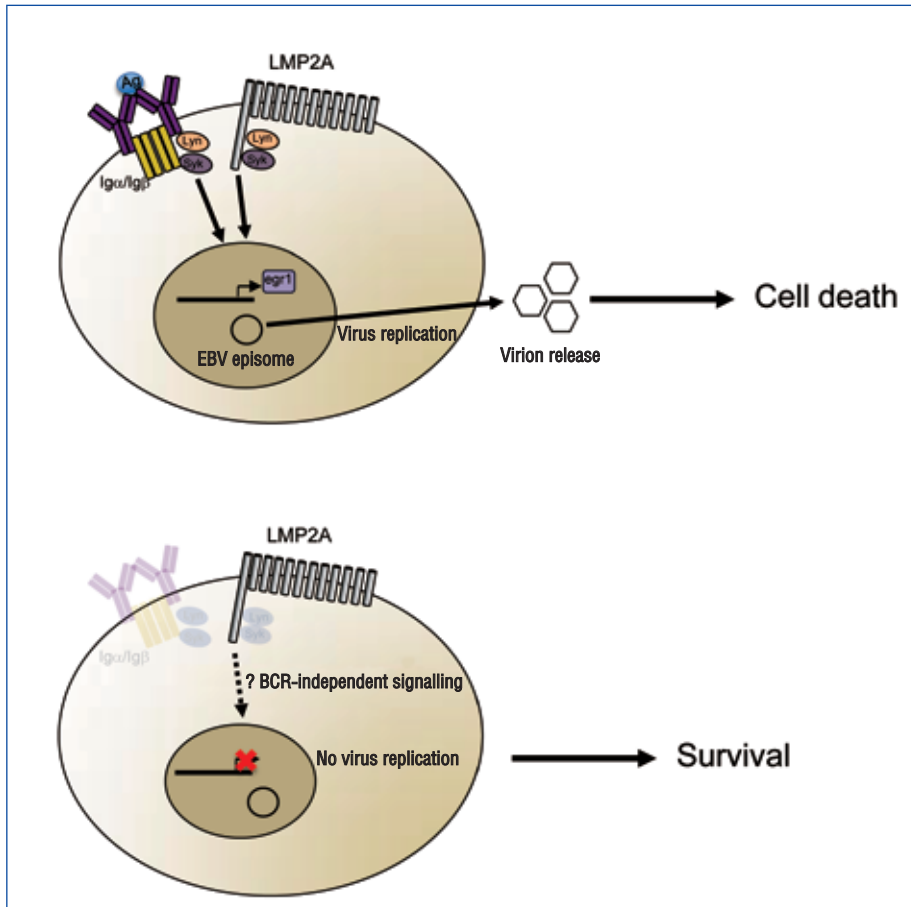


Figure 1. Loss of viral replication functions may promote the development of Epstein-Barr virus (EBV)-positive classical Hodgkin lymphoma (cHL). Upper panel: in normal B cells, either B-cell receptor (BCR)- or latent membrane protein 2A (LMP2A)-mediated signaling can induce the EBV lytic cycle, ultimately leading to the release of infectious virions and eventual cell death. Lower panel: Hodgkin/Reed-Sternberg (HRS) cells lack not only a functional BCR but also essential components of the BCR signaling machinery such as Lyn and Syk and the transcription factor Egr1. Thus, BCR-negative germinal center (GC) B cells that also lack essential BCR signaling components might be positively selected during the development of cHL because they are protected from entry into the EBV replicative cycle and the ensuing cell death. LMP2A expression is retained in EBV-positive HRS cells, but it is not known how LMP2A signals in the absence of BCR signaling components.

in the early stages of HIV infection is thought to be a consequence of the expansion of the pool of EBV-infected B-cell precursors that results from this chronic B-cell stimulation (reviewed in Ref. [73]). This could eventually lead to the development of BL if one or more of these infected GC precursors acquires the necessary genetic alterations characteristic of BL (reviewed in Ref. [73]). It remains to be established if hyper-stimulation of the B-cell system also contributes in a similar fashion to the pathogenesis of cHL.

Modulation of EBV Gene Expression and Function by the Microenvironment

There is increasing evidence that the microenvironment of the EBV-infected B cell can regulate virus gene expression. The regulation of virus latency is of course not only important in dictating the fate of the EBV-infected B cell transiting through normal lymphoid tissues such as the tonsil but could also be a critical determinant of gene expression in tumors in which there is disruption of the normal microenvironment. For example, the cytokines interleukin-21 (IL-21) and IL-2, along with intercellular interactions such as CD40 ligation, all present in the GC of the tonsil, have been shown to down-regulate the expression of Epstein-Barr virus nuclear antigen-2 (EBNA2) and up-regulate the expression of LMP1, thus imposing a type II

expression profile similar to that observed in cHL^[74,75]. Furthermore, our recent data have identified a significant role for Notch ligation in the regulation of LMP1^[76]. Activated Notch inhibits the initiation of LMP1 expression from the conventional LMP1 promoter by EBNA2 during primary infection of resting B cells. Activated Notch also inhibits LMP2A expression during primary B-cell infection but only transiently down-regulates LMP2A in established lymphoblastoid cell lines (LCLs). This is of particular interest given that LMP2A expression is often observed in cHL. LMP2A can apparently induce its own promoter, and several reports have demonstrated that LMP2A constitutively activates the Notch pathway^[77,78].

In addition to regulating EBV expression, there is emerging evidence that the microenvironment can also modulate the function of individual virus proteins. For example, we have shown that LMP1 can induce the expression of the collagen receptor, discoidin domain receptor 1 (DDR1). This is important because collagen is a major constituent of the chronic inflammatory microenvironment of cHL^[79-82]. Ligation of DDR1 by collagen promotes the survival of lymphoma cells *in vitro*, suggesting that the excess collagen present in lymphomas could drive some oncogenic functions of LMP1^[82] (Figure 2). Changes to the microenvironment of the infected B cell might help to explain why on the one hand LMP1 expression in the asymptomatic host provides only those signals required to drive the

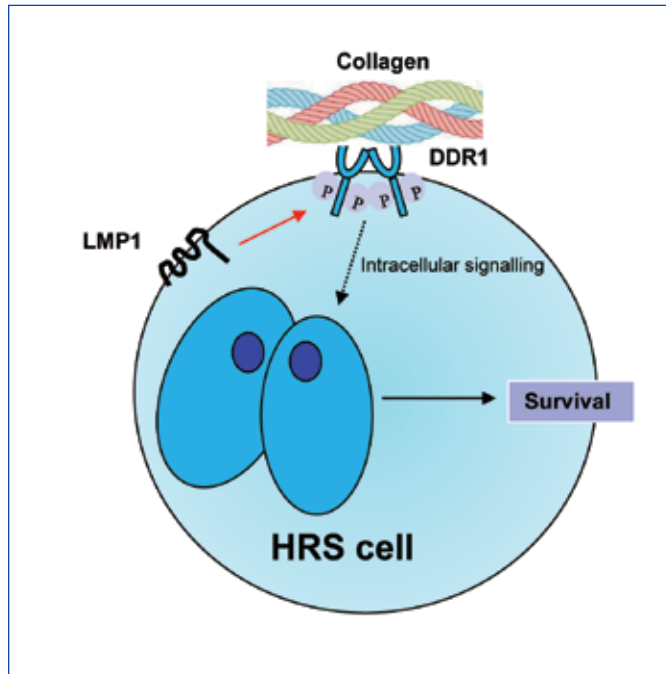


Figure 2. Potential role for collagen in activating the oncogenic functions of LMP1. LMP1 can induce expression of the collagen receptor, discoidin domain receptor 1 (DDR1), in the normal EBV-infected GC B cell (indicated by red arrow). During a chronic inflammatory response, collagen is deposited in the microenvironment of the EBV-infected cell and can activate DDR1, leading to growth-promoting signaling.

differentiation of EBV-infected GC B cells, yet on the other hand, LMP1 has potentially oncogenic functions. An important area of future research will be to unravel the complex interactions between the EBV-infected B cell and its microenvironment and to determine how disruption or modification of these interactions might be tumor-promoting.

Conclusions

EBV is present in a proportion of cHL cases and probably provides important anti-apoptotic signals that prevent cell death in

HRS progenitors lacking a functional BCR. Loss of BCR and of other key components of the BCR signaling machinery could be important for the pathogenesis of cHL because they might protect HRS progenitors from entry into the EBV replicative cycle and subsequent cell death. Chronic inflammation in the microenvironment of cHL might not only dictate the pattern of EBV gene expression but also modulate the oncogenic functions of individual EBV genes such as *LMP1*.

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