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Epstein–Barr virus associated lymphoproliferations in the AIDS setting

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Abstract

Epstein–Barr virus (EBV) is a ubiquitous gammaherpesvirus that is associated with a variety of malignancies. *In vivo* infection of B lymphocytes is initially associated with the broad expression of immunodominant viral latency genes and proliferation of infected cells. Ultimately, a viral reservoir is established in resting B cells with restricted expression of viral latency genes and no expression of immunodominant viral genes. Among the tumours associated with EBV that are relevant to a consideration of EBV in HIV-associated malignancies are posttransplant lymphoproliferative disease, Burkitt's lymphoma (BL) and Hodgkin's disease (HD). BL carries whereas EBV in only a minority of cases whereas HD in patients infected with HIV is virtually always EBV-associated. EBV-directed T cell therapies have proven effective in posttransplant lymphomas in bone marrow transplantation patients. In patients with HIV infection, primary central nervous system (CNS) and immunoblastic lymphomas show similarities with post-transplant lymphoproliferative disease. EBV detection studies in cerebrospinal fluid are useful diagnostically in primary CNS lymphoma. T cell therapies may be useful in the treatment of EBV-associated lymphomas. Thus, a better understanding of the relationship between EBV and these tumours will not only help to clarify their pathogenesis, but may facilitate the development of new diagnostic and therapeutic strategies.

Keywords: Epstein–Barr; Lymphoma; AIDS; Brain lymphoma; Virus; HIV

1. Introduction

Epstein–Barr virus (EBV) has been associated with a variety of malignancies including B and T cell lymphomas, Hodgkin's disease (HD), nasopharyngeal and gastric carcinomas, and smooth muscle tumours [1]. Some of these malignancies occur with increased frequency in immunocompromised patients while others have no apparent association with immunocompromise. In others, notably Burkitt's lymphoma (BL), the tumour in which EBV was first discovered, the role of immunocompromise in pathogenesis remains indeterminate.

The association of EBV with malignancy is of interest not only in terms of its pathogenesis, but is also of diagnostic importance and is becoming important in terms of new approaches to treatment. Aspects of the biology of the virus that are relevant to tumorigenesis, the nature of the association with the various tumours and diagnostic and possible therapeutic implications will be considered in this article.

2. Aspects of viral biology

The viral genome encodes approximately 80 open reading frames and is approximately 171 kb in length [2]. The viral life cycle includes lytic and latent states. In lytic or productive infection, virions are produced. Viral enzymes involved in nucleotide metabolism and virus capsid structural proteins are expressed [3]. Virions are not produced in latent infection. Host cell enzymes replicate viral DNA. However, latency should not be equated with genetic silence. On the contrary, the growth properties of the infected cells are profoundly influenced by viral latency. Indeed, viral-driven proliferation during latent infection is a key aspect of the EBV life cycle and facilitates viral spread throughout the B cell compartment following primary infection. A signal difference between the gammaherpesviruses (EBV and Kaposi's sarcoma herpes virus (KSHV)) and the alpha and beta human herpesviruses (e.g. herpes simplex and cytomegalovirus, respectively) is that diseases are associated with latent infection by the gammaherpesvirus, whereas no diseases have been associated with latent infection by the alpha or betaherpesvirus.

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EBV infection is widespread in all adult human populations irrespective of race, ethnicity or geography [1]. Infectious mononucleosis is commonly associated with primary infection in adolescents and in adults, whereas infection in childhood is usually asymptomatic. Although infection is common, EBV-associated malignancies are rare. Similarly, other human tumour viruses such as hepatitis B, papillomavirus and HTLVI are associated with tumours in only a small fraction of those infected.

2.1. Infection and latency in B cells

Transmission through saliva leads to infection of B cells in the oral mucosa [4]. Infected B cells are driven to proliferate by the expression of viral proteins and the pool of infected cells expands [5]. Early in infection, before the cellular immune response several lymphocytes may harbour virus [6–8]. Ultimately, these proliferating B cells are targeted for destruction by virus-specific cytotoxic T-lymphocytes [5,9]. However, a subset of infected lymphocytes persists indefinitely. The frequency of these cells in adults is approximately 1 infected cell/100 000 peripheral blood mononuclear cells with a wide variation among normals [5,10,11]. In contrast to the proliferating cells that initially expand the latently-infected pool, these cells that persist are not cycling [12]. They also are not targeted for immune destruction, perhaps because viral antigen expression is so limited.

The proliferating lymphocytes present early after infection have many similarities to B lymphocytes immortalised by EBV *in vitro*. An episomal genome expresses six nuclear proteins, three membrane proteins and two small polymerase III transcripts (the EBERs 1 and 2) [3]. Genetic studies show that immortalisation requires the coordinated expression of five viral genes [3]. Among these, Epstein–Barr virus nuclear antigen 1 (EBNA1) is required for replication of the viral episome [13]. EBNA2 is a transcriptional activator of specific viral and cellular genes particularly in the Notch pathway, and the latency membrane protein-1 (LMP1) interacts with tumour necrosis factor receptor (TNFR)-associated factors (TRAFs) that lead to activation of nuclear factor-kappa β (NF-kB) and modulation of a variety of apoptotic and growth pathways [14,15]. In murine cell lines, LMP1 leads to transformation (loss of contact inhibition, anchorage independence), and tumorigenicity in nude mice [16]. These cells are readily targeted by immune surveillance systems that target cells expressing immunodominant proteins, particularly EBNA3A, 3B and 3C.

Not required for immortalisation but worthy of mention are LMP2A which inhibits lytic cycle activation by blocking normal B cell transduction mechanisms and the EBERs 1 and 2 whose functions are unknown, but which have emerged as important markers of EBV latent infection by virtue of their abundance (estimated at 10 million copies per cell) [17–19].

2.2. Lytic infection

The lytic cycle involves the expression of a cascade of immediate early, early and late proteins ultimately leading to synthesis of linear double-stranded DNA genomes, which are packaged in an icosahedral capsid and then released. Replication of the viral DNA requires a viral DNA polymerase that is susceptible to specific inhibition by a variety of antiviral agents including ganciclovir and acyclovir [20]. Lytic infection is detected only rarely *in vivo* or *in vitro*. Early after primary infection, lytic viral gene expression can be detected in peripheral blood mononuclear cells as assessed by reverse transcriptase-polymerase chain reaction (RT-PCR) [5]. However, lytic transcripts are rare thereafter and are either not detected or are detected at low levels [11]. Lytic EBV infection is detected in oral hairy leucoplakia, a benign epithelial lesion that affects the lateral margins of the tongue in patients with HIV, but is not otherwise known to be associated with disease [21].

2.3. Spontaneous outgrowth in tissue culture

When peripheral blood mononuclear cells are placed in tissue culture, some of the resting EBV-infected cells undergo lytic replication. The virions released infect other lymphocytes. When measures have been taken to inhibit T cell function such as the addition of cyclosporine or tacrolimus, these lymphocytes yield an immortalised EBV cell line (so-called 'spontaneous outgrowth') [1]. Antiviral agents that inhibit the viral DNA polymerase can block the first step of the process, but have no impact on the process once infectious virions have been synthesised. Thus, EBV-immortalised B cell lines can grow in the presence of acyclovir or ganciclovir.

2.4. Patterns of latency and tumours

As noted above, following primary infection there is an EBV-driven proliferation of latently-infected B cells. Later on these cells disappear and infection is maintained in a reservoir of resting latently-infected B cells. The patterns of viral gene expression in the two settings are very different. Virus-driven proliferation is associated with broad latency antigen expression, whereas resting B cells have a highly restricted pattern of viral gene expression that facilitates their escape from immunosurveillance. These different patterns correspond to patterns of viral gene expression found in tumours.

B cell lines expressing the full spectrum of latency antigens resemble some of the tumours arising in

immunocompromised patients, particularly posttransplant lymphoproliferative disease. The resting B cells harbouring EBV with a very restricted pattern of antigen expression are similar with regard to antigen expression in endemic BL [22,23]. In other tumours, intermediate patterns of viral gene expression have been recognised. In HD, the LMPs are expressed, but not the immunodominant EBNAs [24,25]. In primary central nervous system (CNS) lymphoma in AIDS patients, an association between LMP1 and BCL2 expression has also been recognised [26]. EBNA2 is also detected in a subset of AIDS primary CNS lymphomas [27].

In neoplasia, where a broad spectrum of viral latency genes are expressed, there may not be a requirement for further genetic or epigenetic changes to establish malignancy. EBV-immortalised B cells will grow as tumours in SCID mice. However, in neoplasia associated with more restricted patterns of viral antigen expression such as BL, the viral genes expressed are not sufficient for growth transformation, and other genetic changes such as the *myc* translocation are needed. In the context of this discussion of viral latency in tumours, it might be noted that whereas diseases associated with lytic herpesvirus infections, such as zoster or cytomegalovirus retinitis, these may be controlled by agents that inhibit the viral DNA polymerase required for lytic replication whereas neoplasia is unaffected by these antiviral agents.

2.5. Immune response

During acute infection, EBV-specific and non-specific cellular responses are generated. Memory T cells specific for EBV persist at a frequency of approximately 1 in 10^3 circulating T cells. The immunodominant EBNAs (EBNA3A, 3B and 3C) are the most frequently targeted latency antigens [28]. CD8(+) T cells, although they target EBNA1, are ineffective in the surveillance of EBNA1-expressing cells because a *cis*-acting glycine-alanine repetitive sequence inhibits antigen processing and major histocompatibility complex (MHC) class I presentation [29]. It is worth noting that the response to the immunodominant antigens is so strong that tumours expressing these antigens are only seen in protected locations such as the CNS or profoundly immunosuppressed patients.

3. Lymphoma

EBV is associated with a variety of lymphoid neoplasms including approximately 40–67% of non-Hodgkin's lymphomas (NHL) and virtually all HD arising in HIV-infected patients [30]. Consideration of post-transplant lymphoma, BL and HD all help to put in perspective EBV lymphomas arising in HIV-infected patients.

3.1. Posttransplant lymphoproliferative disease

EBV-associated B cell lymphoproliferative disorders occur with increased frequency in solid organ and bone marrow transplant recipients [31,32]. From benign hyperplasia, to monoclonal polymorphic proliferations, to monomorphic histologies there is a spectrum of histological appearances. The incidence of these disorders varies with the character of the immunosuppression used and the organ transplanted [33–36]. Heart–lung transplant recipients and liver transplant recipients have much higher incidences than renal transplant recipients [37].

In the development of primary CNS lymphoma, EBV and immunosuppression were recognised as the important factors in organ transplant recipients. These tumours accounted for approximately 1/3 of post-transplant lymphomas in the precyclosporine era. Curiously, with the introduction of cyclosporine the overall incidence of post-transplant lymphoma increased, but primary CNS lymphomas almost disappeared from organ transplant recipients — an observation that remains unexplained.

Post-transplant lymphoproliferative disease frequently arises in the donor organ in solid organ transplant recipients and when arising elsewhere is commonly extranodal. Although there is considerable intra- and intertumour heterogeneity with more restricted patterns of antigen expression common, these tumours will often express all latency antigens and thus may be regarded as similar to cell lines immortalised by EBV [38–40]. Quantitative PCR is also emerging as a useful tool in the study of EBV-associated malignancies. In post-transplant lymphoma, several investigators have reported that the number of EBV genomes in peripheral blood mononuclear cells is increased, although there is striking variability in absolute values across studies [41–50]. Our investigations have shown that this increase in copy number is accounted for by increased numbers of infected cells rather than an increase in the viral copy number per infected cell [11].

In serum, EBV DNA is not detected in healthy seropositives. It has been detected in the serum of patients with infectious mononucleosis and posttransplant lymphoproliferative disease [51–53]. In patients with tumours, this viral DNA in the serum appears to correspond to DNA released from dying cells rather than virion DNA.

Adoptive cellular therapies have led to some dramatic successes. Lymphoproliferative disease following T-cell depleted allogeneic bone marrow transplantation has been treated very successfully with donor lymphocyte infusion [54]. The interpretation that EBV-reactive T cells play a critical role is consistent with the high frequency of EBV-specific T cells in peripheral blood. However, most convincing was the demonstration that

infusions of EBV-specific cytotoxic T cells could be used to treat or prevent the development of posttransplant lymphoproliferative disease [55].

3.2. Burkitt's lymphoma

The association of EBV with BL remains perplexing. The viral genes that drive lymphoid proliferation in posttransplant lymphoproliferative disease (or in immortalised lymphocytes) are not expressed. The incidence of the tumour is highest in areas of Africa with holoendemic malaria. In these areas, the tumour is uniformly associated with the virus, whereas in other parts of the world, the incidence of the tumour and its association with EBV are variable [56–58]. A constant feature of this lymphoma with or without EBV are the chromosomal translocations that juxtapose the *c-myc* gene with an immunoglobulin locus, usually the heavy chain locus on chromosome 14. However, endemic and non-endemic tumours show subtle differences in the distribution of chromosomal breakpoints.

How important EBV is for the maintenance of the transformed phenotype in BL is not clear. However, studies of a Burkitt's cell line which spontaneously loses episomes makes clear that viral episomes contribute to the maintenance of the transformed phenotype [59]. A role for EBV in sporadic BL has also been entertained following the observation that integrated fragments of viral genomes have been detected in some cases [60].

In contrast to posttransplant lymphoproliferative disease, BL is resistant to immune killing. It expresses only EBNA1 which, as noted above, is not recognised by cytotoxic T lymphocytes. In addition, essential elements of the class I pathway, including the peptide transporters TAP1 and TAP2 are downregulated and in some instances, even the class I molecule is not expressed [61–63]. These observations suggest the possibility that immunocompromise plays no role in the pathogenesis of BL.

3.3. Hodgkin's disease

Cohort studies of patients with serologically documented infectious mononucleosis consistently show a 2- to 4-fold excess of HD [64,65]. Furthermore, serological studies show higher mean antibody titres to viral antigens in patients with HD than in controls. The serological differences are most marked several years before the diagnosis of HD [66].

When EBV is present in the Reed-Sternberg cells of HD, the pattern of viral gene expression is very consistent. LMP1 and LMP2 are expressed, but the immunodominant EBNAs are not. Indeed the level of expression of the LMPs is higher than in any other setting. In EBV-negative HD, MHC class I molecules are not expressed [24,67]. However, in EBV-positive HD they are — perhaps because expression of class I molecules is activated by LMP1. This suggests that HD might be an appropriate target for immune manipulations, such as have been used in posttransplant lymphoproliferative disease, if the therapy could be focused on the subdominant LMP1 or 2 antigens [68]. Although it should be noted that local production of cytokines and chemokines may inhibit the cytotoxic effector cell function [69–71].

3.4. HIV-associated lymphomas

The association of EBV with tumour appears to be similar in various HIV risk groups (gay men, intravenous (i.v.) drug users, patients with haemophilia, etc.). However, the association differs by histology. Immunoblastic lymphomas and extranodal lymphomas, particularly primary CNS lymphomas are virtually always EBV-associated [72–74]. Similarly, anaplastic large cell lymphomas in AIDS patients are usually EBV-positive [75].

Lymphadenopathy syndrome has been identified as a possible precursor lesion in EBV-positive AIDS lymphoma. However, although high EBV loads have been detected in some instances, it is not believed that EBV plays a role in either the pathogenesis of HIV-associated lymphadenopathy, nor that EBV-carrying nodes from patients with HIV lymphadenopathy syndrome are high risk lesions for the development of EBV-positive lymphoma [76].

Two strains of EBV are recognised that differ principally in the sequence of EBNA2, EBNA3A, 3B and 3C. *In vitro*, strain 1 virus is more efficient at immortalisation, although both strains are capable of immortalisation. Most malignancies including those arising in organ transplant recipients carry strain 1 virus [77]. The exception are HIV-related malignancies, particularly HD where strain 2 virus has been reported to be more common [78]. There remains uncertainty, however, as to whether these differences reflect biologically important strain differences with regard to pathogenesis, or merely that strain 2 EBV is more common in the gay population for unknown reasons [79].

Primary CNS lymphoma was common among patients with AIDS, particularly before the introduction of highly active antiretroviral therapy (HAART). Some autopsy series have reported incidences in excess of 10%. Among persons with AIDS, the incidence of primary CNS lymphoma was 3600-fold greater than in the general population [80]. With the advent of HAART, the incidence of primary CNS lymphoma in AIDS patients appears to be falling [81].

Primary CNS lymphoma has often been a diagnostic challenge. However, several studies have shown that EBV can be fairly consistently detected in the cerebrospinal fluid of these patients and only rarely in HIV

patients without primary CNS [82–84]. In one instance EBV detection was documented to precede lymphoma diagnosis by 17 months [85,86]. In combination with radiological studies, EBV PCR may make brain biopsy unnecessary in many cases.

Curiously enough, although EBV was discovered in African BL and is present in nearly 100% of endemic BL, AIDS-associated BL is the histological type least frequently associated with EBV (20–30%). Furthermore, in areas of Africa where BBL is endemic, there has been little or no increase in the incidence of this lymphoma in HIV-infected populations [86].

Other AIDS lymphomas, particularly BL, or large cell lymphomas occurring in nodal sites in individuals with earlier stage AIDS are only occasionally EBV-associated [72].

Primary effusion lymphomas, in which the tumour cells are found in serosal cavities without the formation of tumour masses, consistently show dual infection with EBV and HHV8, the Kaposi's sarcoma-associated herpesvirus [87,88]. The pattern of EBV gene expression is highly restricted and resembles that of BL. Neither the immunodominant EBNAs nor the subdominant LMPs is expressed [89,90].

Virtually all cases of HD in HIV-infected patients are EBV-associated [91–93]. RS cells of HIV-HD display a post germinal centre phenotype. Although the RS cells express CD40, in contrast to HD with this phenotype in the general population, they are not surrounded by CD40 ligand-positive (CD40L+) reactive T lymphocytes. It has been suggested that the role of CD40/CD40L interactions in the general population may be supplanted by LMP1 which is functionally homologous to CD40 [86,94].

The *LMP1* gene shows some strain variability. There have been several reports suggesting that virus with a 30 bp carboxyterminal deletion is more common in HIV-associated HD than in other HD [95–97]. The significance is uncertain, but this deletion variant is suggested to have a longer intracellular half-life and to be less immunogenic than full length LMP1.

4. Conclusion and future directions

The presence of EBV in a subset of HIV-associated lymphomas helps to explain their pathogenesis. In at least some of these cases, simple immunosuppression allowing growth of tumours that would under other circumstances be eradicated by immune surveillance must play an important role. In other instances, such as BL, the observations in patients with HIV infection merely serve to highlight how little we understand of the pathogenesis of these tumours in any setting.

On the diagnostic front, the consistent association of EBV with primary CNS lymphoma and the ability to detect viral DNA in the cerebrospinal fluid are already having a major impact on the frequency with which diagnostic biopsies are carried out. It may be that there will also be a role for quantitative PCR in diagnosing systemic lymphoma or in monitoring response to therapy, although these applications have yet to be systematically addressed.

With regard to treatment and perhaps prevention, the presence of EBV in tumour tissues offers a multitude of targets for intervention. In bone marrow transplant recipients, adoptive cellular immunotherapy has proven dramatically effective in preventing or treating post-transplant lymphoproliferative disease. Such an approach might also be relevant to the treatment of HIV-infected patients. One of the major questions to be addressed is the appropriate source of cells for re-infusion. These might be autologous or allogeneic cells expanded *in vitro* — but either source carries with it serious limitations. Alternatively, in the era of HAART and recovering $CD4^+$ T cell counts, there may be a role for vaccination to enhance waning responses that might protect against the development of lymphoma or might directly suppress extant lymphoma.

Other EBV-specific treatment options also loom on the horizon. It has been suggested that hydroxyurea cures cells of viral episomes [98]. A very different strategy involves upregulation of EBV genes such as *thymidine kinase* that are not expressed in latency, but which, if expressed would allow phosphorylation of ganciclovir and direct killing of tumour cells with ganciclovir [99]. Finally, rituximab has emerged as effective therapy in some cases of posttransplant lymphoma [11,100]. Insofar as it rapidly destroys circulating B cells, it might be employed prophylactically to removed the viral transformation target if an appropriately high risk group of individuals could be identified.

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