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# Epstein-Barr virus: Current questions and challenges

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ABSTRACT

Epstein-Barr virus (EBV) infects most people worldwide and persists for life due to complicated interplay between lytic infection and multiple types of latent infections. While usually asymptomatic, EBV is a causative agent in several types of cancer and has a strong association with multiple sclerosis. Exactly how EBV promotes these diseases and why they are rare consequences of infection are incompletely understood. Here I will discuss current ideas on disease induction by EBV, including the importance of lytic protein expression in the context of latent infection as well as the possible importance of specific EBV variants in disease induction.

## 1. Introduction

Epstein-Barr virus (EBV) is a common human herpesvirus, which belongs to the gamma-family of viruses along with one other human virus, Kaposi's sarcoma associated herpesvirus (KHSV). Unlike alpha- and beta-herpesviruses, these viruses share the ability to induce cancer. Indeed, EBV was discovered due to its presence in Burkitt's lymphoma (BL) and has since been shown to be a major factor in the development of these tumours. EBV has also been shown to be a causative factor in the induction of nasopharyngeal carcinoma (NPC), gastric carcinoma (GC) (accounting for 9% of these cancers), Hodgkin's lymphoma, some T-cell lymphomas and post-transplant lymphoproliferative disease (PTLD) [1].

Spread mainly through saliva, initial EBV infections can lead to mononucleosis and always results in a life-long infection. This persistence involves alternation between latent and lytic (or productive) modes of infection. Latency occurs predominantly in B-lymphocytes, whereas lytic infection can occur in B cells or epithelial cells. Lytic infection involves the ordered expression of approximately 80 EBV proteins, amplification of the viral genomes in linear form and production of virions. Lytic infection begins with the expression of the lytic switch protein, BZLF1, a transcriptional activator that turns on the expression of EBV immediate early and early genes.

Latent infection is complicated in that it can take several forms, most of which induce cell proliferation and immortalization (see Fig. 1). In these cells, the EBV genome is replicated and maintained as a circular episome at a constant copy number. Initially, three latency forms (latency I, II and III) were defined based on the pattern of EBV proteins expressed in infections seen *in vitro* and in EBV-induced tumours [2]. Latency I, in which EBNA1 is the only viral protein expressed, is seen in BL. NPC, GC, Hodgkin's disease and T-cell lymphomas typically display latency II, in which EBNA1 and three latent membrane proteins (LMP1, 2A and 2B) are expressed. *In vitro* infection of primary resting B cells by EBV results in immortalized lymphoblastoid cell lines (LCLs) in latency III, in which all six Epstein-Barr nuclear antigens (EBNA1, EBNA2, EBNA3a, EBNA3b, EBNA3c and EBNA-LP) and the three LMPs are expressed. Latency III infection also occurs in post-transplant lymphoproliferative disease (PTLD) and AIDS-associated diffuse large B-cell lymphoma.

Subsequent analyses of EBV-infected B cells in healthy individuals indicated that all of these latency forms exist in the normal course of EBV infection, although latency III occurs very transiently due to immune pressure [3–5]. In addition, a prevalent form of latency, termed latency 0, was detected in resting memory B cells, in which none of the latency proteins were expressed. Yet another latency form was identified from *in vitro* studies of initial B-cell transformation by EBV. These revealed that, in the first few divisions before establishment of latency III, the cells express all of the EBNAs in the absence of the LMPs, a form of latency referred to as latency IIB (Fig. 1) [6–8]. All latency forms also involve the expression of high levels of short noncoding RNAs (EBERs) as well as clusters of miRNAs. Therefore, EBV latency involves a complicated interplay of different expression profiles that maybe more fluid than originally defined.

### 1.1. *Rethinking the dogma of EBV latent and lytic infection*

#### 1.1.1. *Lytic proteins contribute to B cell transformation*

Lytic infection typically involves expression of the immediate early transcriptional activators BZLF1 and BRLF1, followed by early protein

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expression, amplification of the viral DNA, late gene expression and virion production. However, it has become increasingly clear that, in some circumstance, subsets of lytic proteins can be expressed in the absence of EBV DNA amplification and virion production (reviewed in Refs. [6,9]). One instance when this occurs is upon initial infection of resting primary B cells prior to establishment of the latent infection; referred to as the pre-latent phase (Fig. 1). Initial infection has been found to involve a burst of expression of a subset of lytic proteins, prior to the first cell division, which wanes as the EBNA latency proteins and noncoding RNAs begin to accumulate. Since true lytic infection results in cell cycle arrest and eventually in cell death, lytic protein expression was unexpected and suggests additional roles for these proteins.

One lytic protein expressed immediately upon B cell infection is the AP-1-like transcriptional activator, BZLF1 [10,11]. EBV lacking BZLF1 was shown to have reduced ability to induce initial B cell proliferation, supporting a positive role for BZLF1 in this process. Normally, expression of BZLF1 starts a cascade of EBV lytic gene expression leading to lytic infection. However, in this case, lytic infection does not occur, as judged by the lack of EBV genome amplification and lack of structural protein expression. It is speculated that BZLF1 may be activating the expression of some cellular genes, as opposed to its usual EBV target genes.

In addition, the two EBV homologues of the cellular, anti-apoptotic Bcl2, protein (BHRF1 and BALF1) were found to be expressed very early in initial infection of primary B cells, and were turned off once latent infection was established [12,13]. Deletion of both of these genes abrogated the ability of EBV to induce proliferation of the cells and resulted in cell death by apoptosis. Therefore, The Bcl2 mimics of EBV play a critical role in enabling cell survival early in the transformation process [12].

B cell transformation also depends on the expression of EBV lytic proteins involved in immune modulation. EBV BCRF1, a late lytic protein and IL-10 homologue, and BNLF2a, an early lytic protein that inhibits the transporter associated with antigen processing (TAP), are both expressed immediately upon infection of primary B cells [14]. Expression of these two proteins was shown to be critical for evading immune responses against EBV in the earliest stages of infection [14]. BCRF1 interferes with cytokine responses, CD4$^{+}$ T-cell activity and NK cell mediated killing of the infected cells, while BNLF2a inhibits antigen presentation and recognition of the infected cells by CD8-T cells. Therefore, it is clear that some EBV lytic proteins are expressed outside of lytic infection and contribute to other stages of EBV infection by enabling cell survival and proliferation. How expression of these particular EBV proteins are regulated in pre-latent phase expression remains to be determined.

### *1.1.2. Latent-lytic interplay in B cells and epithelial cells*

Numerous studies have been conducted on cells infected by B95.8 EBV, isolated from an American infectious mononucleosis patient, and Akata EBV, isolated from a Japanese patient with BL. These studies led to a general picture of EBV infection in which EBV infects primary B cells much more efficiently than epithelial cells and remains latent in transformed B cells with little spontaneous reactivation. This dogma was thrown into question with the characterization of M81 EBV, isolated from an NPC patient in China. M81 was found to infect epithelial cells much more efficiently than B95.8 but to have a reduced tropism for B cells [15]. M81 transformed B cells with similar efficiency as B98.8 but the transformed cells (from several different donors) had a much higher frequency of spontaneously lytic reactivation than cells transformed by either B95.8 or Akata [15]. To determine if these very different properties of M81 might be due to geographic or disease-associated sequence variation in this particular isolate, the same group isolated EBV-transformed LCLs from 22 cancer-free Western patients (transplant recipients with high viral load) [16]. Two-thirds of these LCLs expressed lytic proteins (to varying degrees) and contained linear EBV DNA genomes typical of lytically replicating EBV. Three of these EBV isolates had lytic infection levels close to that of M81, despite their close genetic relationship to B95.8. Other studies had noted that an alternative strain of EBV (called B-type or Type 2) common in sub-Saharan Africa, supported a high frequency of lytic infection in LCLs [17,18]. Together these studies suggest that there is considerable variation in the degree to which different EBV isolates undergo lytic infection in B cells, and that EBV B cell infections are not usually tightly latent as initially thought.

There is also contradictory evidence concerning the infection of

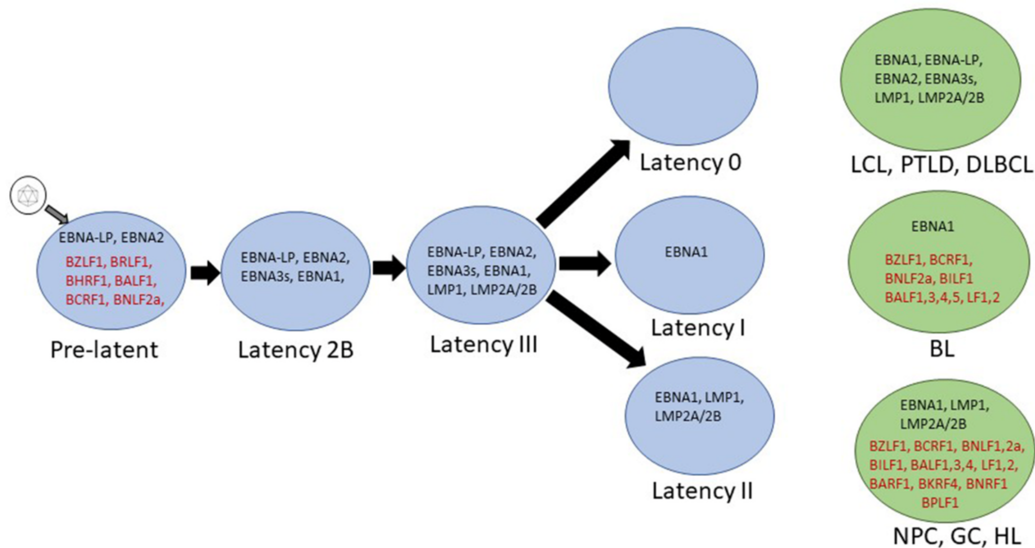


**Fig. 1.** EBV protein expression profiles in latent infections. The stages of infection of naïve, resting B cells leading to their transformation and subsequent latency forms in healthy individuals are shown (blue cells). EBV-induced cancer cells are shown in green (where LCL is lymphoblastoid cell line, PTLD is post-transplant lymphoproliferative disease, DLBCL is AIDS-associated diffuse large B-cell lymphoma). EBV latency proteins (black) and lytic proteins (red) that are known to be expressed in each cell type are indicated. Hodgkin's lymphoma (HL) is grouped with NPC and GC since it is a latency II infection, however the profiles of lytic proteins expressed in these cells have not ben determined.

epithelial cells by EBV. While the oral epithelium is the prominent site of lytic EBV infection, undifferentiated NPC and ~9% of gastric carcinomas consist of cells with latency II EBV infections, showing that latent infection of epithelial cells is possible. Understanding EBV epithelial cell infection and associated cancers has been hampered by difficulties in growing EBV-positive epithelial cells. While undifferentiated NPC cells grow aggressively in people, it has proven to be extremely difficult to get these cells to grow and maintain the EBV infection in culture. The one widely studied EBV-positive NPC cell line (C666-1) has been shown to be defective for lytic infection [19]. However, the more recently generated EBV-positive NPC cell lines (C17 and NPC43) are both highly lytic, and must be maintained in an inhibitor of Rho-associated coiled-coil containing kinases (ROCK) in order to maintain stemness and inhibit lytic reactivation [20,21]. Thus, the undifferentiated nature of the NPC cells seems to be critical to maintain EBV latent infection, and lytic reactivation is associated with cell differentiation. Similar conclusions were reached using latently EBV-infected, telomerase-immortalized normal oral keratinocyte (NOKs) cells, in which lytic reactivation occurred only in more differentiated cells [22,23]. These findings are in agreement with observations that differentiated oral epithelium is a major site for EBV lytic infection [24,25], but also raise the possibility that latent EBV infections (outside of the known EBV-induced cancers) might occur in undifferentiated epithelial cells.

### 1.2. *How does Epstein-Barr virus induce cancer?*

Epstein-Barr virus establishes life-time infections in ~90% of the world population. While usually asymptomatic, it is causatively associated with several types of cancer even in people with intact immune systems. How EBV induces these cancers and why they only occur in a small subset of infected individuals is still not understood. Since EBV cancers are considered to be latent infections, most studies have focussed on the roles of the latency proteins and noncoding RNAs consistently expressed in latent infection. These have identified important contributions of EBNA2, as a transcriptional activator, and LMP1 as a CD40 constitutively-active mimic [26]. EBNA1, which is essential for the replication and maintenance of EBV episomes, has also been found to have roles in destabilizing p53 and disrupting PML nuclear bodies, which might contribute to oncogenesis, particularly in the absence of EBNA2 and LMP1 expression in latency I infection [27–29]. However, it is becoming increasingly clear that lytic infection and lytic protein expression in the absence of lytic infection are major factors in cancer induction by EBV and merit further investigation.

Cancer induction by EBV resembles that by human papillomavirus (HPV), in that both induce cell proliferation as part of their latent infection, and are very common infections that only cause cancer in a small subset of infections. For HPV, it is known that there are specific variants (high-risk strains) that promote cancer, while most variants do not. Whether or not EBV-induced cancers are caused by specific EBV variants is presently unclear but is an active are of investigation.

### 1.3. *Importance of lytic infection*

While EBV tumours are comprised of cells in latent infection, several observations point to the importance of lytic infection as a cancer determinant. Multiple studies have indicated that high viral load (due to high lytic infection) is an important factor in EBV-induced cancers. For example, a study of children in Uganda found that those who developed BL had elevated antibody titres against EBV viral capsid antigen (VCA), a measure of lytic infection, prior to developing this cancer [30,31]. Similarly, several studies have found that people who develop NPC have significantly higher levels of antibodies against EBV lytic proteins (including VCA) that precede the clinical onset of NPC by a few years, and hence can be used as a screening tool for those at high risk of developing NPC [32–35]. High lytic infection may promote oncogenesis by increasing the chances of mutations in the viral genome and also by increasing the likelihood of infection of nasopharyngeal cells.

The importance of EBV lytic infection for cancer induction is also supported by the finding that an EBV variant with increased EBV lytic infection (due to changes in the *BZLF1* promoter) is over-represented in EBV-associated NPC, GC, BL and AIDS-related lymphoma as compared to its frequency in nonmalignant tissues [36–38]. This change in the BZLF1 promoter (Zp-V3) has been shown to increase lytic infection and B-cell transforming activities in cord blood-humanized mouse studies [39]. The importance of lytic infection for tumour induction has also been tested by generating a version of EBV with a BZLF1 knockout and assessing its ability to infect and induce disease in a humanized mouse model [40]. While the BZLF1 knockout virus supported persistent latent infection similar to the BZLF1-positive control virus, it resulted in a significantly lower frequency of diffuse large B cell lymphomas as compared to the control virus. These findings indicate that either lytic infection itself or expression of BZLF1 or BZLF1-controlled genes play a role in the development of EBV-induce cancers.

#### 1.3.1. *Role of lytic proteins in EBV-induced cancers*

In addition to the role of high lytic infection in the development of EBV-induced cancers, there is increasing evidence that expression of specific EBV lytic proteins in the context of latent infection promotes oncogenesis. Many studies have examined EBV-induced tumours for the presence of lytic proteins [41–45]. BZLF1 has been widely examined and been found to be expressed in BL, NPC and GC samples [46–50]. However, expression of some other EBV lytic proteins, including those needed for lytic DNA replication, was lacking, indicating that these cells were not undergoing lytic infection. This suggests that BZLF1 has a more direct role in promoting oncogenesis, possibly due to its effect on cellular gene expression. For example, BZLF1 has been shown to activate the expression of cellular genes for interleukin (IL)-8 and IL-10, and to downregulate MHC class II genes and apoptotic pathway genes [51]. Together these effects would enhance the growth and survival of the cells and promote immune evasion.

Several other lytic proteins have also been consistently detected in EBV tumours. The Bcl-2 homologue BALF1, that is expressed in pre-latent infection of B cells, is also expressed in BL cell lines and NPC and GC samples, suggesting that it contributes to cell survival in these cancers [50,52]. BARF1, a secreted protein that stimulates the proliferation of epithelial cells, is also expressed in NPC and GC samples [50,53–55]. In addition, these carcinomas have a highly activated lytic gene cluster encoding BALF3, BALF4, BALF5, BILF1, LF1, LF2 and BNLF2a [43,45,50,56]. A similar cluster of activated genes was found in BL, although BALF5 transcripts were not detected [57,58]. The functions of these proteins include those expected to impact tumourigenesis. BILF1 has transforming activity as it mimics a constitutively active, G protein-coupled receptor [59,60]. Additionally, BILF1 and BNLF2a work together to inhibit processing and presentation of $CD8^{+}$ T cell epitopes [61]. LF2 also interferes with immune responses, in this case by antagonizing type I interferon signaling [62].

Analysis of all transcripts in eight EBV-positive gastric carcinoma samples showed that 18 EBV lytic genes were consistently expressed [50]. In addition to the EBV proteins described above, these cells expressed EBV transcripts encoding proteins BNRF1, BKRF4 and BPLF1, all of which have functions that would be expected to contribute to oncogenesis. BNRF1 promotes chromosome instability by inducing centrosome amplification [63]. Similarly, BKRF4 binds histones and interferes with double-stranded DNA break repair, which would be expected to contribute to genomic instability [64]. BKRF4 also appears to be expressed at low levels in EBV-positive GC and NPC cell lines, suggesting a role in latent infection of epithelial cells [43,64,65]. BPLF1 is a deubiquitylating enzyme that also interferes with DNA repair through effects on PCNA and Pol eta [66,67]. In addition, it has been shown to contribute to B-cell transformation in humanized mice and to contribute to innate immune evasion by interfering with toll-like receptor signaling [68,69].

Surprisingly, some EBV genes that are consistently expressed in GCs (eg. BPLF1 and BALF4 encoding glycoprotein B) are late proteins. Late genes normally require EBV lytic DNA replication prior to their expression, however, the lack of several EBV early proteins needed for replication indicates that these late genes must be turned on in the tumour environment without the requirement for DNA replication. Indeed, the transcription of BPLF1 has been shown to be regulated in a manner distinct from most late genes [70], which may enable its expression in the absence of lytic infection. This selective expression of lytic genes in the absence of lytic infection has been referred to as abortive lytic infection. Interestingly, a similar scenario has been reported for KSHV, the other human gamma-herpesvirus, in which a subset of lytic genes are expressed in KSHV-associated cancers [71,72]. Understanding how specific EBV lytic genes become derepressed in the context of cancer cells will be an important topic for future studies.

#### 1.3.2. *Epstein-Barr virus variants*

As an increasing number of EBV isolates are studied, it is clear that different EBV isolates vary in their abilities to infect lymphocytes and epithelial cells as well as in their degree of spontaneous reactivation. This raises the possibility that only specific EBV variants are oncogenic, akin to the situation with HPV-induced cancers. Two EBV strains, type 1 and type 2 (also called type A and type B) were defined many years ago based on major differences in the EBNA2 gene and associated changes in the EBNA3 genes [73,74]. Type 1 is prevalent worldwide, while type 2 is only prevalent in sub-Saharan Africa. These stains differ in their ability to induce growth transformation, with type 1 transforming B cells into LCLs more efficiently than type 2 [75,76]. However, with more complete sequencing of multiple type 1 and type 2 isolates, additional variations between and within these strains is evident, although the functional implications of these variations are just beginning to be investigated [74].

Many labs have been addressing the question of whether specific EBV variants are more oncogenic than others. One reason why this might be is if a particular variant is more prone to reactivate to the lytic cycle, increasing the viral load, which, as discussed above, would be expected to increase the likelihood of an EBV-induced cancer. Another factor may be the efficiency with which an EBV variant infects a particular cell type. For example the NPC-associated M81 EBV infects epithelial cells more efficiently than B95-8, suggesting that EBV variants with high tropism for epithelial cells confer increased risk of carcinoma [15]. Additionally, variations in EBV might affect the ability of EBV proteins to bind and regulate cellular proteins. In HPV, high-risk strains express proteins with amino acid changes that increase their interactions with cellular tumour suppressors [77–80]. Similarly, amino acid changes in EBV proteins expressed in EBV-induced tumours could have a major impact on oncogenicity. Assessment of this possibility requires complete sequences of large numbers of EBV isolates worldwide, in order to understand the natural prevalence of each variant in different parts of the world, as well as its frequency in EBV-induced cancers. Efforts to generate EBV genome sequences for comparison are underway and have resulted in EBV genome sequences from NPC, GC, BL, Hodgkin lymphoma and NK/T-cell lymphoma, as well as from the blood or saliva of healthy individuals [38,50,74,81–86]. To date there are too few EBV genome sequences from tumours and healthy individuals from the same geographic location to make any strong conclusions on whether particular sequence changes are tumour-associated. However, there have been a number of interesting observations worthy of further investigation.

EBNA1 is a key latency protein expressed in all EBV cancers, with essential roles in replicating and maintaining latent EBV genomes, and additional roles in transcriptional activation and manipulation of cellular processes [27]. Therefore, it is interesting that several variants of this protein have been identified that are more common in NPC than in peripheral blood [87–89]. Several of these sequence changes occur in the DNA binding domain and are defined based on the amino acid encoded at position 487, with Val being the most common variant found in NPC. Several studies have examined the effect of these changes on EBNA1 properties. While there are reports of effects on DNA binding, transcriptional activation, episome maintenance and cell growth and survival, most reports indicate that DNA binding is not affected by these variations, and whether or not the other reported effects affect oncogenesis remains to be determined [90–95]. It also remains unclear as to whether these variants are truly cancer-associated or simply more common in NPC due to their higher prevalence in NPC-endemic regions. A study on EBV variants in GC and NPC attempted to address the effect of geographical variation by identifying variants that occurred frequently in both Asian and American Caucasian samples [50]. In this way, one nonconservative amino acid change was identified in EBNA (Thr85Ala) which was seen in 91% of NPC, 68% of GC but only 8% of non-carcinoma samples. This amino acid maps to sequences important for transcriptional activation, but the functional significance of this change remains to be determined.

Another EBV protein that is reported to vary considerably in sequence is LMP1. Variants with multiple amino acid changes in the N-terminal transmembrane domain of LMP1 have been found in NPC and GC isolates [50,96]. Their enrichment in these tumours relative to other EBV isolates suggests that these changes in LMP1 might promote oncogenesis, however this has not been conclusively determined. It was also found that the sequence of the BPLF1 deubiquitinase is commonly altered in two amino acids (Lys515Gly and Ser405GLy) in GC isolates [50]. Since this protein is expressed in GC and has multiple roles that could impact tumour growth (as discussed above), these sequence changes could potentially increase oncogenicity. In addition, a study of EBV variants in China identified two amino acid changes in the BALF2 ssDNA binding protein that increase the risk of NPC [86]. How these changes impact the DNA replication function or other roles of BALF2 remain to be determined.

Finally, variants with polymorphisms in noncoding RNA have also been reported to occur and are associated with EBV tumours. For example, a four base pair deletion downstream of EBER2, which would be expected to affect its secondary structure, was found to be more than twice as common in NPC samples from Hong Kong as compared to its prevalence in the general Hong Kong population [97]. Deletions in the BART miRNA cluster have also been found to be common in EBV isolates from NK/T-cell lymphoma and large B-cell lymphoma and were recently detected in Hodgkin's lymphoma [98,99]. This deletion promotes lytic infection and enhances B-cell tumourigenesis in humanized mice, so could be an important determinant in the induction of lymphoma [100]. Thus, there are multiple changes in EBV-associated isolates that could make these viruses more oncogenic.

#### 1.3.3. *Epstein-Barr virus and multiple sclerosis*

While EBV has long been recognized as a causative agent of cancer, its relationship to multiple sclerosis (MS) has been less clear. Multiple sclerosis (MS) is a chronic immune-mediated demyelinating disease of the central nervous system. Although its etiology is not well understood, strong evidence is accumulating that EBV plays an essential role in this disease. An initial connection between EBV and MS was made based on findings that MS patients are always EBV-positive, become EBV-positive prior to clinical onset of MS and typically have elevated levels of EBV antibodies (indicative of high viral load) that precede MS disease onset [101–103].

Memory B cells are the main sites of EBV persistence, and EBV infection results in their clonal expansion [104]. Therefore, it is intriguing that B cell depletion through monoclonal antibody treatment has proven to be an effective way to treat MS, with the loss of memory B cells thought to be of particular importance [103,105]. This raises the possibility that B-cell depleting therapies work due to reduction of the EBV-infected B cells. One hypothesis on how EBV might induce MS is that the EBV-positive memory B cells enter the CNS and activate $CD4^{+}$ T cells, which cause the damage seen in MS [106]. In keeping with this theory, EBV-positive B cells and plasma cells have been detected in the

brains of MS patients in some (but not all) studies, as have EBV-specific CD8 + T cells [107–109]. In addition, MS patients have increased spontaneous T cell proliferation, which is induced by memory B cells and results in higher levels of CD8 + T cells in the brain [110]. Interestingly, natalizumab, which is an effective treatment for MS, was shown to block the migration of $CD4^{+}$ T cells into the brain, further supporting this model of MS etiology [110].

In addition to the above circumstantial evidence, recent clinical studies strongly support a causative role of EBV in MS. Pender et al. [111] treated 10 MS patients with autologous EBV-specific T cells targeting the latency proteins EBNA1, LMP1 and LMP2A. Seven of the patients showed improvement in symptoms, with the biggest effects seen with the T cells with the strongest EBV reactivity. Since there were no serious adverse effects, this anti-EBV treatment is an exciting advance with a promising future as an MS therapeutic.

Like cancers associated with EBV, it is not yet understood why only a small fraction of people infected with EBV develop MS. While many factors may be at play, the question of whether particular EBV variants are more able to induce MS than others is an important one that is being investigated. Considerably more whole genome sequences of EBV MS isolates will be required before this possibility can be evaluated.

## 2. Summary

In summary, current studies are revealing a blurring of the lines between latent and lytic proteins and the types of infections to which they contribute. As discussed above, certain lytic proteins can be expressed in the context of latent infections, as seen in some cancers and in pre-latent infection of B lymphocytes. This raises the possibility that these lytic antigens might be useful therapeutic or vaccine targets for prevention of EBV-induced cancers. Whether or not any lytic proteins are expressed in other forms of latent infections merit further investigation. Conversely, the EBV latency proteins, EBNA1 and LMP1, are also expressed in the lytic cycle and have been reported to make important contributions to lytic infection [112,113]. Understanding how the expression of individual lytic genes is regulated in these latent infections and contribute to oncogenesis is an important area for future study.

In addition, characterization of specific EBV isolates has revealed that isolates can vary considerably in their infectious properties and hence in their oncogenic potential. Similarly, EBV sequencing efforts have identified a number of changes associated with cancer isolates, supporting the idea that EBV-associated diseases might be caused by specific EBV variants. Additional sequencing of EBV genomes and characterization of disease-associated variants will be required to determine the degree to which such changes contribute to EBV-induced cancers and MS.

## CRediT authorship contribution statement

**Lori Frappier:** wrote the entire review and made the figure. All funding was from CIHR to Lori Frappier.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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