Epstein-Barr virus and multiple sclerosis: background virology

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Abstract

Epstein-Barr virus (EBV) is a ubiquitous human pathogen that has co-evolved with humans and shares considerable DNA homology, particularly with elements of our immune system. This has allowed it to be the ultimate stealth fighter, maintaining permanent latent infection within memory B cells. Active surveillance of cells with lytic infection is maintained by CD8+ cytotoxic T cells: T-cell immunosuppression can result in reactivation of EBV infection and proliferation of infected B cells. Conversely, latent infection of memory B cells is 'hidden' from CD8+ T cell control, maintaining a reservoir of potential infection. Here, in this online supplement, we provide a background to the nature of the virus, its interactions with the human immune system and diagnosis of EBV infection to support the published review of EBV and multiple sclerosis.

INTRODUCTION

This online supplement forms the background to the published review of the evidence linking Epstein Barr virus (EBV) infection with the risk of developing multiple sclerosis (MS). Here we briefly describe what is known about the virus itself and the course of EBV infection in humans, with particular focus on aspects of the virus that are pertinent to a possible role in MS and are relevant to researchers in this arena.

EPSTEIN-BARR VIRUS

EBV (also known as human herpes virus-4, HHV-4 or lymphocryptovirus) was first described in 1964, after isolation from continuous lymphoblastoid cell lines established from Burkitt's lymphoma biopsies.[1] It is a member of the Herpesviridae family, which includes three subfamilies: alphaherpesvirinae (herpes simplex virus (HSV) types 1 and 2, and varicella zoster virus), betaherpesvirinae (cytomegalovirus and human herpes virus types 6 and 7) and gammaherpesvirinae (EBV and Kaposi sarcoma-associated herpes virus or human herpes virus type 8).[2-3] Of the eight family members, human herpes virus 6 (HHV-6) is thought to have the genome closest to the progenitor herpesvirus[4] which is estimated to be at least 300 million years old.[5] Betaherpesviruses probably separated from this progenitor line first, followed by the gammaherpesviruses, with the alphaherpesviruses having the most recent origins. Genome analysis suggests that chickens may be the ancestral herpesvirus hosts but, of all the herpesviruses, EBV has the closest homology to human DNA:[4] the similarity between human and EBV genes suggests that DNA has been transferred between human hosts and the virus during

evolution. Up to one-third of the EBV genome may be derived from human host cells, including all of the latent genes.[4]

Structure (see Figure 1)

EBV consists of a protein core wrapped with double-stranded DNA and surrounded by a nucleocapsid, protein tegument and an outer glycoprotein envelope. The EBV genome is approximately 170kb in length[6] and encodes nearly 100 viral proteins,[5, 7] with importance in regulation of viral gene expression, viral DNA replication, formation of structural virion components and modulation of host immune responses.[7] Internal repeats (IR) divide the genome into four largely unique sequence domains (IR 1-4) - IR1, which lies between the unique short (U_S) and unique long (U_L) regions, and IR2, 3 and 4, which occur within the U_L region.[5] Within these sequence domains, over 70 different open reading frames have been identified, mapped and named according to their position on a *Bam*H1 restriction endonuclease genome map,[6] e.g. BHRF1 identifies the gene encoded by the *Bam*H1 H right reading frame. The number of IR and terminal repeated (TR) regions, located at the end of the genome, are stable for a given strain but vary greatly depending on the isolate. These repetitions account for the genome size variation for different EBV isolates.[5]

Genes & Proteins

EBV expresses different genes and proteins depending on the type of infection.[8] In healthy EBV 'carriers', there are more than 80 lytic and 8 latent EBV gene products,[9] including EBV nuclear antigens (EBNAs) EBNA-1 (encoded by the BKRF1 gene

segment); EBNA-2, -3A, -3B, -3C and Leader Protein (LP, also known as EBNA-5); viral nucleocapsid antigen (VCA); latent membrane proteins (LMPs 1, 2A and 2B);[10] and EBV-induced early antigens (EAs), including EA-restricted (EA-R, encoded by the BORF2 gene segment) and EA-diffuse (EA-D, encoded by the BMRF1 gene segment).[11] EA-D and EA-R differ by their staining patterns with different human sera: antibody to EA-D causes diffuse staining of the nucleus and cytoplasm, whereas EA-R staining is restricted to the cytoplasm.[12]

Lytic proteins, including VCA and EAs, are categorised into 'immediate-early', 'early' and 'late', and are expressed in the various replicative stages of infection. In contrast, latent genes are expressed in the growth phases of infection and include LP, EBNAs and LMPs, as well as non-coding RNAs (Epstein Barr encoded RNAs, EBERs).[6]

Two distinct EBV types (1 & 2, or A & B) have been described,[5] based on differences in their geographical distribution and *in vitro* biological properties.[13] Type 1 (A) is ubiquitous in Western countries and in Asia,[14-19] infects oropharyngeal epithelial cells and B cells, and efficiently immortalizes B cells *in vitro*. Type 2 (B) is more common in equatorial Africa and New Guinea,[15-18] and is less efficient at *in vitro* immortalization, compared to Type 1.[18, 20] Specific viral types have not been associated with particular disease entities.

As noted, there has been close contact between humans and EBV, with exchange of DNA resulting in considerable homology that may be important for infection and subsequent disease (summarized in Table 1).

Homology between EBV and other herpesviruses varies. The 85-kDa protein of EA-R shares extensive homology with the ribonucleotide reductase of HSV, and there is also limited homology between HHV-6 and EBV DNA.[11, 21] The relevance of these homologies for human disease is unclear.

Human Infection with Epstein Barr virus

EBV infection is limited to man and non-human primates and has a narrow cell tropism, infecting only B cells and epithelial cells.[22] Infection of B cells leads to two possible outcomes, one culminating in latently infected memory cells and the other in lytic viral replication with the production of free virions.[23] Transmission occurs almost exclusively via saliva,[6, 11] but vertical, sexual and blood transmission have also been described.[24] EBV infection of the tonsillar epithelial cells has not been demonstrated in primary infection[6] and it is possible that the virus bypasses these cells to directly access local B cells in tonsillar lymphoid tissue.[6]

During primary infection, EBV enters the oropharynx and binds to B cells via the major envelope glycoprotein gp350/gp220 and the CR2 complement receptor[25] (also known as CD21), which is expressed by mature B cells. This binding triggers endocytosis of infected virions.[26] A second envelope glycoprotein, gp42, which binds to the β_1 domain

of the HLA class II protein HLA-DR[26] to act as a co-receptor, is also important in this process.[7, 22, 25] Within infected B cells, the EBV genome transforms from a linear structure to a circular episome (see Figure 1). This form is maintained by the EBNA-1 protein through sequence-specific binding to short sequences at the origin of latent replication, OriP.[25, 27]

Infected naïve B cells are driven out of the resting state to become activated B blasts[28] that enter the germinal centre (GC). Here they use the normal processes involved in B cell activation to proliferate and differentiate into latently infected memory B cells[28-29] that persist within the circulation.[9, 25]

In vivo, EBV-infected B cells show five possible patterns of EBV gene expression (and hence proteins produced), that depend on the location and differentiation state of the infected B cell, and allow the establishment of different types of infection within the human host:[6, 9]

1. A lytic program that produces infectious virus;[6]
2. Four latency programs that do not produce infectious virus:
    a. The growth program (Latency III) involves the full spectrum of latent viral gene expression including EBNAs (1-6), LMPs (1, 2A, 2B) BART and EBERs (1 and 2);[30]
    b. The default program (Latency II) in which only 4 genes are expressed – EBNA-1, LMP-1, LMP-2A and LMP-2B; [10, 31]
    c. The latency program (Latency I), in which only EBNA-1 is expressed;[10]

d. Latency 0 where no EBV genes are expressed within a subset of memory B cells in healthy virus carriers.[10]

The growth program (Latency III) stimulates rapid proliferation of the infected B cell.[30] However, recent work suggests that it is the Latency II program (particularly expression of EBNA-2 and LMP-1)[6] that is expressed within the GC and allows infected B cells to survive and differentiate into memory B cells, by mimicking the required B cell receptor signaling and T cell help.[29, 31] EBV-infected memory B cells then persist within the circulation, and EBV transmission to daughter B cells is ensured through EBNA-1-induced linking of the viral episome to the cellular chromosome. The virus is thus replicated in dividing cells as though it were part of the cellular genome.[31] In normal adults, the virus remains in its latent form within the memory B cells and the number of infected cells (~ 1-50 infected B cells/million in the circulation) remains relatively stable over many years.[7]

Latently infected memory B cells returning to the tonsil can terminally differentiate into plasma cells, initiating the lytic (replicative) transcription program with the production of infectious virus.[32] The resulting free virions infect tonsil epithelial cells, where the virus replicates at a high rate. Shedding of virus into saliva facilitates transmission to new hosts,[33] and repeated EBV infection of the tonsil may cause tonsillar hypertrophy and recurrent tonsillitis.[34-35] Newly formed virus can also infect additional naïve B cells in the same host.

The immune control of EBV infection

The lytic, growth and default programs are immunogenic as EBV protein is expressed. In contrast, the Latency I and Latency 0 programs, in which few or no genes are expressed, allow the virus to evade immune detection within resting memory B cells.[31] In addition to the formation of memory B cells, short-lived, locally differentiated, plasma cells produce an initial antibody response, which is maintained in the long-term by long-lived plasma cells, probably resident within the bone marrow, that can produce antibody without further antigenic stimulation.[36] CD4+ T cells also respond to multiple latent cycle epitopes, particularly within EBNA-1.[37]

Both $CD8^+$ and $CD4^+$ T cells are thought to have cytotoxic activity against infected B cells to control EBV infection and reactivation.[38] Cytotoxic $CD8^+$ T cells recognize short peptides that are derived from the intracellular breakdown of viral proteins and presented at the surface of the infected cell by MHC class 1 molecules,[39] to eliminate proliferating and lytically infected B cells.[37] They may also destroy B cells in the GC (during the default program) and this may explain the apparently contradictory findings that EBV-infected memory B cells appear to undergo multiple rounds of proliferation within the GC, but the usual massive clonal expansion is not apparent.[29] The cytotoxic role $CD4^+$ T cells is less well-understood. They may have particular relevance in relation to the EBNA-1 antigen that is characterized by a sequence of glycine-alanine repeats. This impairs presentation of EBNA-1 by MHC class 1 molecules (to $CD8^+$ T cells), but processing and presentation by MHC class II molecules (to $CD4^+$ T cells) is preserved. [37, 38]

Primary infection with EBV causes a strong cellular immune response (resulting in lymphocytosis in peripheral blood) with hyperexpansion of the cytotoxic $CD8^+$ T cell pool. T cell receptors (TCRs) are markedly oligoclonal, containing EBV-epitope-specific reactivities, of which those to the immediate early and early antigens (lytic program) are usually dominant.[25, 37] *In vitro* studies suggest that these T cells with TCRs reactive to lytic program antigens require ongoing antigenic stimulation to avoid apoptosis.[37] As antigen becomes limited (after the immediate early stages of infection), both absolute numbers and the relative proportion of T cells bearing these TCRs falls and a greater proportion of T cells bear TCRs to latent program antigens.[37] $CD8^+$ T cells maintain surveillance during chronic viral infection, with TCRs specific for latent antigens, particularly EBNA-3A, -3B and -3C (although other latent antigens may be important in the context of different HLA class I alleles). EBV latent within memory B cells avoids detection by cytotoxic T cells by switching off all antigen expression.[40]

Primary infection is invariably accompanied by permanent seroconversion.[22] In children under 10 years of age, primary EBV infection is generally unrecognized and may be asymptomatic.[22] In later childhood and adulthood, 30-40% of primary infections[22] result in the clinical syndrome of infectious mononucleosis (IM), characterized by malaise, anorexia and chills, pharyngitis, fever and generalized lymphadenopathy. However, this constellation of symptoms can also be caused by infections with other pathogens, such as cytomegalovirus, toxoplasma, HIV or HHV-6.[6]

Diagnosis of EBV infection

EBV infection is diagnosed serologically, with patterns of antibodies against specific EBV antigens defining particular types of infection, e.g. latent vs. active, recent vs. distant. Up to 12 commonly occurring serological patterns involving anti-VCA IgG and IgM, anti-EBNA-1 IgG and heterophile IgM have been described, allowing for the categorization of EBV infection into naïve, primary acute infection, past infection and recovery/reactivation.[41] In general, acute EBV infection in immunocompetent individuals is based on the detection of anti-VCA IgM and IgG and anti-EA-D IgG.[42] Antibodies to EBNA (primarily EBNA-1) and EA-R are detected 4-8 weeks after infection.[43] Following primary infection, anti-VCA, anti-EA and anti-EBNA complex IgG antibodies persist[22] at readily detectable and nearly constant levels.[44] Host reactivity to EBNA-1 and EBNA-2 occurs separately[42] and, although anti-EBNA-1 antibodies persist, anti-EBNA-2 antibodies appear in the acute phase of infection and decline with convalescence.[42] Reactivation of EBV infection is supported by elevated antibodies to EA-R, which are found at low levels in 10-20% of healthy carriers, but are more commonly found (>50%) in immunosuppressed patients.[45] In these patients, anti-EBNA-2 titres increase, whilst anti-EBNA-1 titres decrease, resulting in an inversion of the anti-EBNA-1/anti-EBNA-2 ratio (which is greater than 1 in healthy individuals).[42]

EBV DNA load in whole blood, serum, plasma or memory B cells can be measured quantitatively by real-time PCR techniques.[46, 47] EBV DNA can be detected soon after primary EBV infection, with levels spiking at about 2 weeks and then falling to low or

undetectable levels over the following several weeks to months.[46] Cell-free body fluids (serum, plasma) contain negligible amounts of EBV DNA except during reactivation or EBV-related disease. In contrast, the cellular compartment accumulates latent viral load.

Interaction with other viruses

There is growing evidence that EBV interacts with other viruses, either activating, or being activated by, concurrent infection. For example, EBV transactivates a human endogenous retrovirus (HERV-K18, located on chromosome 1q21.2-q22) to encode a superantigen that stimulates a large fraction of primary T cells.[48] Alternatively, infection with HHV-6 (possibly restricted to the HHV-6A variant[49]) can upregulate (up to 10-fold) the expression of an immediate early antigen of EA-R and EA-D, and possibly VCA, suggesting a direct effect on the EBV replicative cycle.[11] HHV-6 can infect many cell types but, at least *in vitro*, human B cells are particularly susceptible if they have been previously infected with EBV.[11] Cytomegalovirus infection can cause an antibody profile suggestive of reactivation of both EBV and HHV-6.[50]

Other factors affecting EBV infection/reactivation

Factors that induce suppression of cell-mediated immune function, such as elevated stress hormones,[51] post-transplantation immunosuppression,[52] and acquired immunodeficiency, e.g. due to HIV infection,[53] may allow opportunistic reactivation of latent EBV infection. This can be manifested by increased production of anti-EA and anti-VCA IgG[51] or by the development of EBV-infected B cell proliferation and tumour development.[52]

CONCLUSION

EBV infects most humans by the time they reach adulthood. Co-evolution with humans and exchange of DNA between humans and the virus, has resulted in homologies with several elements of the human immune system. These assist the virus in infecting human B cells and surviving in a latent form, with minimal antigen production, that allows EBV to evade immune destruction. In healthy individuals memory T cells provide active surveillance against lytic antigens that protects against the reactivation of EBV infection. These characteristics provide a background whereby EBV infection can cause human disease, including cross-reactivities with human tissue proteins and/or other viruses, impairment of immune surveillance and recurrent reactivation, or direct infection of specific body compartments with limited immune surveillance.

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Table 1. Homology between EBV and human proteins, likely arising from exchange of DNA during evolution

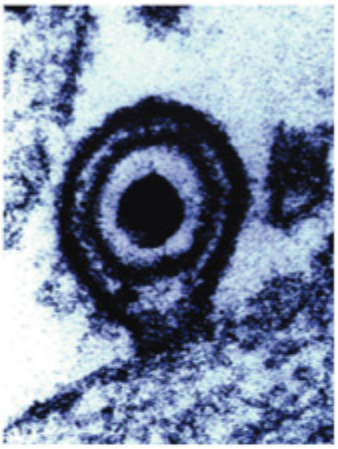
EBV protein	**Human protein**	**Comments**
viral IL-10 (vIL-10) (BCRF1)	IL-10 (hIL-10)	• 70% homology in amino acid sequence[4, 54-55] • Mimics hIL-10 to inhibit interferon-γ synthesis by peripheral blood mononuclear cells *in vitro*[7] • Lacks mast-cell stimulatory activity of hIL-10[55] • vIL-10 is detected within hours of EBV infection and may be required for B cell transformation and escape of infected B cells from T-cell surveillance[27]
EBV DNA polymerase (BALF5) epitopes	Myelin basic protein (MBP)	• Cross-reactivity between EBNA-1 -specific T cells and MBP antigens has been demonstrated, particularly among people with multiple sclerosis[56] • A T cell receptor recognises MBP amino acids 85-89 (the immunodominant peptide for human MBP-specific T cells, particularly in HLA-DRB1*1501 positive individuals[57]) and an epitope of BALF5[58-59] • Similar conformation with respect to binding to HLA class II molecules
RRPFF pentapeptide of EBNA-1	αB-crystallin (CRYAB)	• CRYAB is a heat shock protein but is normally absent in human lymphoid tissue[60]

(amino acids 402-406)		
BHRF-1	Bcl-2 (cellular)	• Bcl-2 inhibits host cell apoptosis[7, 61-62]
gp350	C3dg complement fragment	• gp350 is the major envelope glycoprotein of EBV and critical to B-cell infection[26]
LMP-1	CD40	• CD40 is a key receptor on germinal centre B cells • Engagement by CD154 on T-helper cells delivers a survival signal preventing apoptosis and driving proliferation, allowing the B cell to enter the memory pool[31]
LMP-2A	B cell antigen receptor (BCR)	• LMP-2A mimics the survival signal usually provided by the BCR, allowing B cells to survive the germinal centre reaction without encountering their specific antigens[31]

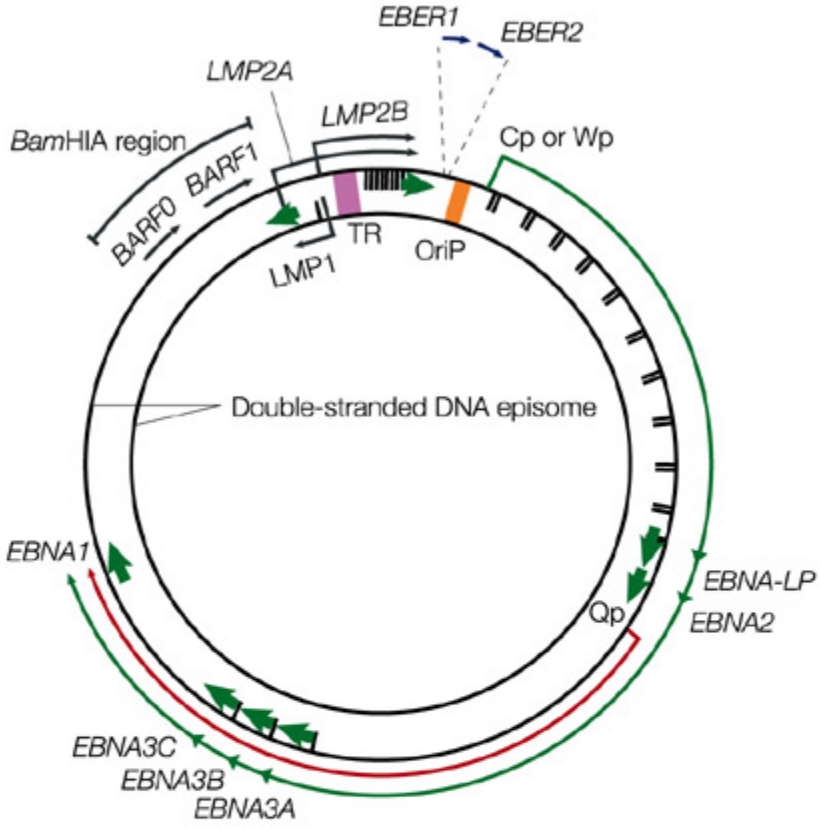
Figure legends

Figure 1.**a**. Electron micrograph of the Epstein-Barr virion. **b.** Diagram showing the location and transcription of the EBV latent genes on the double-stranded viral DNA episome. OriP is the origin of plasmid replication. The large green solid arrows represent exons encoding each of the latent proteins, and the arrows indicate the direction in which the genes encoding these proteins are transcribed. The long outer green arrow represents EBV transcription during latency III. c. Location of open reading frames for the EBV latent proteins on the *Bam*H1 restriction-endonuclease map of the prototype B95-8 genome. The BamH1 fragments are named according to size, with A being the largest. Lowercase letters indicate the smallest fragments. Adapted by permission from Macmillan Publishers Ltd: Nature Reviews, Figure 1 in[25], copyright 2004.

a EBV electron micrograph



b EBV genome: latent genes



c Open reading frames for the EBV latent proteins