# Epstein–Barr virus and multiple sclerosis

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Abstract | Epstein–Barr virus (EBV) is a ubiquitous human lymphotropic herpesvirus with a well-established causal role in several cancers. Recent studies have provided compelling epidemiological and mechanistic evidence for a causal role of EBV in multiple sclerosis (MS). MS is the most prevalent chronic inflammatory and neurodegenerative disease of the central nervous system and is thought to be triggered in genetically predisposed individuals by an infectious agent, with EBV as the lead candidate. How a ubiquitous virus that typically leads to benign latent infections can promote cancer and autoimmune disease in at-risk populations is not fully understood. Here we review the evidence that EBV is a causal agent for MS and how various risk factors may affect EBV infection and immune control. We focus on EBV contributing to MS through reprogramming of latently infected B lymphocytes and the chronic presentation of viral antigens as a potential source of autoreactivity through molecular mimicry. We consider how knowledge of EBV-associated cancers may be instructive for understanding the role of EBV in MS and discuss the potential for therapies that target EBV to treat MS.

Burkitt lymphoma
An aggressive form of non-Hodgkin lymphoma endemic to sub-Saharan Africa, where it is associated with Epstein–Barr virus infection.

Relapsing–remitting MS
(RRMS). A form of multiple sclerosis (MS) where disease exacerbations are interspersed with periods of disease inactivity.

Secondary progressive MS
(SPMS). A form of multiple sclerosis (MS) that follows relapsing–remitting MS where progressive disability accumulates with or without discernible relapse.

Primary progressive MS
(PPMS). A form of multiple sclerosis (MS) that lacks distinct periods of disease exacerbations.

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https://doi.org/10.1038/s41579-022-00770-5

Epstein–Barr virus (EBV) was the first human tumour virus identified, after its discovery in tumour cells of paediatric Burkitt lymphoma[1,2]. We now know that EBV is ubiquitous, establishing lifelong infection in more than 90% of adults worldwide[3,4]. Despite its typically subclinical persistence, EBV is consistently detected in numerous cancers, including nasopharyngeal carcinoma, subtypes of Hodgkin and non-Hodgkin lymphomas, a subtype of gastric carcinomas (EBV-associated gastric carcinoma), natural killer (NK)/T cell lymphomas and leiomyosarcomas. In addition, EBV has a profound effect on the immune system, and is the most common causal agent of infectious mononucleosis[5] as well as fatal lymphoproliferative disorders in various immunosuppressive conditions[6]. Increasingly, it is appreciated that EBV is also a major risk factor for several autoimmune disorders, notably multiple sclerosis (MS)[7,8].

MS is the most prevalent chronic inflammatory and neurodegenerative disease of the central nervous system (CNS). Approximately 2.8 million (35.9/100,000) people have MS worldwide[9]. MS incidence is also increasing in developing countries[9] and among children[10]. The neurological signs and symptoms of MS include impaired motor function; visual symptoms; fatigue; eye movement disorders; bladder symptoms; sensory symptoms; sexual dysfunction; ataxia; deafness; spasticity; dementia; and cognitive impairment[11]. The clinical progression of MS is variable and unpredictable, with three distinct clinical courses: relapsing–remitting MS (RRMS), (2) secondary progressive MS (SPMS) and (3) primary progressive MS (PPMS)[12,13]. In addition, clinically isolated syndrome often progresses to MS, especially when symptoms are accompanied by CNS lesions.

The aetiology of MS is complex and multifactorial, involving the interplay of known genetic susceptibility factors, predominantly in genes directing the immune system, and environmental factors, including infectious agents, lack of sun exposure and vitamin D, smoking and obesity[14]. Infectious agents were first suspected in the aetiology of MS soon after its classification as a discrete clinical entity in the late 1800s[15]. The heterogeneity and the evolution of the disease throughout a patient's lifetime and within the MS lesion itself have further obscured the identification of a single infectious agent as a consistent disease trigger. Nevertheless, epidemiological, serological and virological evidence has accumulated to support the role of EBV in the aetiology of MS, with recent large population-based studies demonstrating that EBV infection is likely a prerequisite for disease (reviewed in REFS.[7,16–18]; TABLE 1). In the most definitive epidemiology study on viruses and MS to date, more than ten million US army personnel were followed up over 20 years, and a 32-fold increased risk of MS diagnosis was shown in individuals who converted to EBV seropositivity compared with those who remained seronegative; this is the largest and most comprehensive study strongly suggesting that EBV infection is required for subsequent development of MS[18,19]. However, determining the precise mechanisms for EBV

Table 1 | **Selected studies providing evidence for a role of EBV in MS**

| Evidence | Result | Study | Refs. |
|---|---|---|---|
| Epidemiological | Low rates of MS in areas with more childhood infections | Review | 230 |
| | Increased risk of MS with a history of infectious mononucleosis | Review | 231 |
| | Increased risk of MS with EBV seroconversion | Human serum | 18 |
| | Decreased risk of MS in seronegative individuals | Human serum | 7,18 |
| Immunological | Increased levels of EBV-specific antibodies in MS | Review | 121,122 |
| | MS-risk alleles enriched for transcription control by EBNA2 | Computational GWAS | 176,178 |
| | Deficient cytotoxic T lymphocyte control of EBV in MS | MS $CD8^+$ T cells | 216 |
| | EBV-reactive OCBs | MS CSF | 85 |
| | Molecular mimicry between EBNA1 and CNS antigens | MS B cells | 17,116 |
| Virological | Increased shedding of EBV in saliva of paediatric patients with MS | Paediatric MS | 55 |
| | EBV BZLF1 in MS lesions | MS brain | 166 |
| | Prosurvival influence of EBV latency genes on memory B cells | In vitro | 232 |
| | EBV loads correlate with T-$bet^+CXCR3^+$ memory cells and IFNγ production | MS B cells | 171 |

CNS, central nervous system; CSF, cerebrospinal fluid; EBV, Epstein–Barr virus; GWAS, genome-wide association study; IFNγ, interferon-γ; MS, multiple sclerosis; OCBs, oligoclonal bands.

in the development of MS remains challenging because the virus is not always found in MS lesions. Related issues arise in the analysis of some EBV-associated cancers, in which EBV is present in only a subtype or subpopulation of cancer cells and oncogenesis depends on additional mutations or environmental cofactors[20]. Because most EBV infections do not cause disease, understanding the role of cofactors and aberrations in the normal infection process is key. One common cofactor in EBV disease is the disruption of normal immune control of EBV infection. Furthermore, as EBV infects and transforms B cells, we consider the intrinsic relationship of EBV with its host cells as a potential source of immune dysfunction.

## EBV biology and life cycle

EBV (human herpesvirus 4) is one of eight known human herpesviruses, with a large (173-kb) double-stranded DNA genome with approximately 100 protein-coding genes and numerous non-coding RNAs and microRNAs (miRNAs)[21,22]. Like all herpesviruses, EBV has both a productive (lytic) cycle and a non-productive (latent) phase. EBV establishes long-term latent infection of B lymphocytes and productive infection in the oral mucosal epithelium[23]. EBV DNA is packaged as a linear genome in the infectious viral particle, but persists in the nucleus of latently infected cells as a closed, circular chromatinized genome, referred to as an 'episome'. Although only two distinct EBV strains have been delineated, the impact of genetic variation on the pathobiology of EBV infection is poorly understood[22,24]. Specific strains of EBV may be associated with MS, but conclusive MS genotypes have not been identified[25–27].

EBV is typically acquired through oral secretory transmission before the age of 5–8 years in resource-poor regions, whereas in resource-rich environments, infection is frequently delayed until adolescence or young adulthood[5,28,29]. During primary infection, the virus enters squamous epithelial cells and replicates within them, subsequently crossing the mucosal epithelial cell barrier via transcytosis and infecting local infiltrating B lymphocytes of Waldeyer's tonsillar ring[30]. EBV infection of naive B lymphocytes initiates a developmental process and reprogramming similar to the germinal centre (GC) that results in long-lived memory B cells harbouring EBV episomes[23,31]. Lifelong persistence occurs through the establishment of latent reservoirs in these cells and periodic reactivation primarily in the oropharynx[32], but other sites of EBV persistence, such as the gut mucosa or meninges, have been reported but not extensively characterized[33,34].

EBV can enter various cell types through different mechanisms. The viral proteins gp350/gp220 and gp42 are required for EBV entry into B lymphocytes. Engagement of gp350 with CD21 (also known as complement receptor type 2) is followed by endocytosis of the virus into a low-pH component, where fusion is facilitated by the virus's core fusion machinery (gB, gH and gL)[35]. gp42 subsequently binds to gH and interacts with HLA class II, which functions as a co-receptor[35]. Entry into epithelial cells occurs via a CD21-independent pathway. The EBV protein BMRF2 interacts with β1 integrin to trigger fusion and subsequent interactions between EBV gH/gL and αVβ6/8 integrins, thereby mediating endothelial cell fusion and entry[36,37]. More recently, ephrin receptor A2 (EphA2) was identified as an important entry factor for EBV in epithelial cells[38]. EphA2 genetic knockouts and inhibitors reduce infection of endothelial cells, and EphA2 interacts with gH/gL and gB[38]. In addition to B cells and epithelial cells, EBV can infect T cells, smooth muscle cells and NK cells, where the mechanism of entry is unclear[39]. EBV infection of T cells and NK cells is thought to be a rare event that can lead to the development of highly aggressive NK/T cell lymphomas and chronic active EBV infection[40]. EBV can also infect neuroblastoma cell lines and primary fetal astrocytes in vitro, although latent infection of neurons has not been unequivocally demonstrated in clinical specimens[41,42].

***EBV latent infection and B cell reprogramming.*** EBV infection efficiently reprogrammes naive B cells towards a developmental path recapitulating GC reaction, clonal expansion and differentiation towards a memory B cell phenotype[23,31]. These developmental stages correspond to different viral gene programmes termed 'latency types'. During the hyperproliferative phase, EBV adopts a type III latency in which most latency-associated genes (*EBNA1*, *EBNA2*, *EBNA3A*, *EBNA3B*, *EBNA3C*, *EBNA-LP*, *LMP1*, *LMP2* and multiple non-coding RNAs) are expressed[43]. Five latent genes (*EBNA1*, *EBNA2*, *EBNA3A*, *EBNA3C* and *LMP1*) are required for efficient B cell immortalization in vitro[44]. Different degrees

Clinically isolated syndrome
An initial episode of neurological symptoms associated with inflammation and demyelination with symptoms characteristic of multiple sclerosis that frequently, although not always, progresses to multiple sclerosis.

Waldeyer's tonsillar ring
A ring of lymphoid tissue surrounding the nasopharynx and oropharynx that includes the tonsils and adenoids.

Germinal centre
(GC). An area within lymph nodes and other secondary lymphoid organs, including the spleen, where T cell-dependent B cell activation, differentiation and proliferation occur. Germinal centres are concentrated areas of B cell somatic mutation and selection.

Chronic active EBV infection
A rare condition marked by poor control of Epstein–Barr virus (EBV) infection, resulting in high EBV plasma viral loads and systemic infiltration by EBV-positive B cells or EBV-positive T cells.

Oral hairy leukoplakia
A white lesion on the tongue with a 'hairy' appearance that is caused by Epstein–Barr virus lytic infection and that can occur in immunocompromised individuals, especially those with HIV/AIDS.

Infectious mononucleosis
A self-limiting disorder characterized by fever, extreme fatigue, sore throat and highly swollen lymph nodes; most frequently caused by immune response to primary Epstein–Barr virus infection, although milder forms are associated with cytomegalovirus infection.

of transcriptional silencing result in latency types II, I and 0, in which few or no viral genes are expressed. Importantly, all EBV-related cancers are associated with latent infection, and the different latency types correlate with different EBV-associated malignancies[45]. However, there can be considerable variation in viral gene expression among tumour cells and stages, including sporadic and abortive lytic reactivation. At present, it is unclear whether any specific latency type or lytic infection is associated with MS pathogenesis.

***Viral reactivation and lytic gene expression.*** EBV lytic cycle reactivation occurs in healthy individuals, and is required for transmission and potentially for replenishing the latent reservoir. However, aberrant lytic activity is associated with several diseases, including oral hairy leukoplakia[46] and chronic active EBV infection. EBV reactivation occurs through regulated stages with immediate-early genes controlling the expression of late genes and viral DNA replication, followed by virus assembly and egress[22]. Numerous cell signalling pathways can trigger the switch to lytic infection, depending on the host cell type. Many of these pathways are related to immune cell signalling, such as activation of B cell receptor (BCR) signalling with anti-immunoglobulin or activation of protein kinase C by phorbol esters[47,48]. In latently infected memory B cells, the switch requires two EBV-encoded transcription factors, BZLF1 (also known as ZTA, ZEBRA and Z) and BRLF1 (also known as RTA and R), which coordinately activate many of the EBV lytic genes[22]. Although EBV-related malignancies are associated with specific latency types, lytic gene expression has been shown in some tumour cells, and serology studies suggest that lytic antigen immunity precedes EBV-associated malignancies, particularly nasopharyngeal carcinoma, non-Hodgkin lymphoma and post-transplant lymphoproliferative disease[49,50]. In addition, highly sensitive genome-wide RNA sequencing methods have demonstrated expression of a subset of lytic genes in EBV-positive tumour cells[51], suggesting that aberrant lytic gene expression and abortive lytic replication may occur more frequently in EBV-associated cancers and autoimmune disorders[52]. Defects in the control of EBV lytic reactivation have been suggested for MS, but the findings remain controversial[53–55].

***Immune control of EBV.*** Immune responses to EBV infection differ widely and are influenced by genetics, the environment and age[56] (FIG. 1). While primary infection before the age of 5 years is often asymptomatic, primary infection in adolescence can result in infectious mononucleosis. During mononucleosis, $CD8^+$ T cells and NK cells rapidly expand in number. Most individuals maintain lifelong, effective immune control of the virus, where reactivation occasionally occurs but is quickly suppressed. This effective immune control is dominated by $CD8^+$ T cells that target latently infected cells and early lytic replication[57]. Various EBV

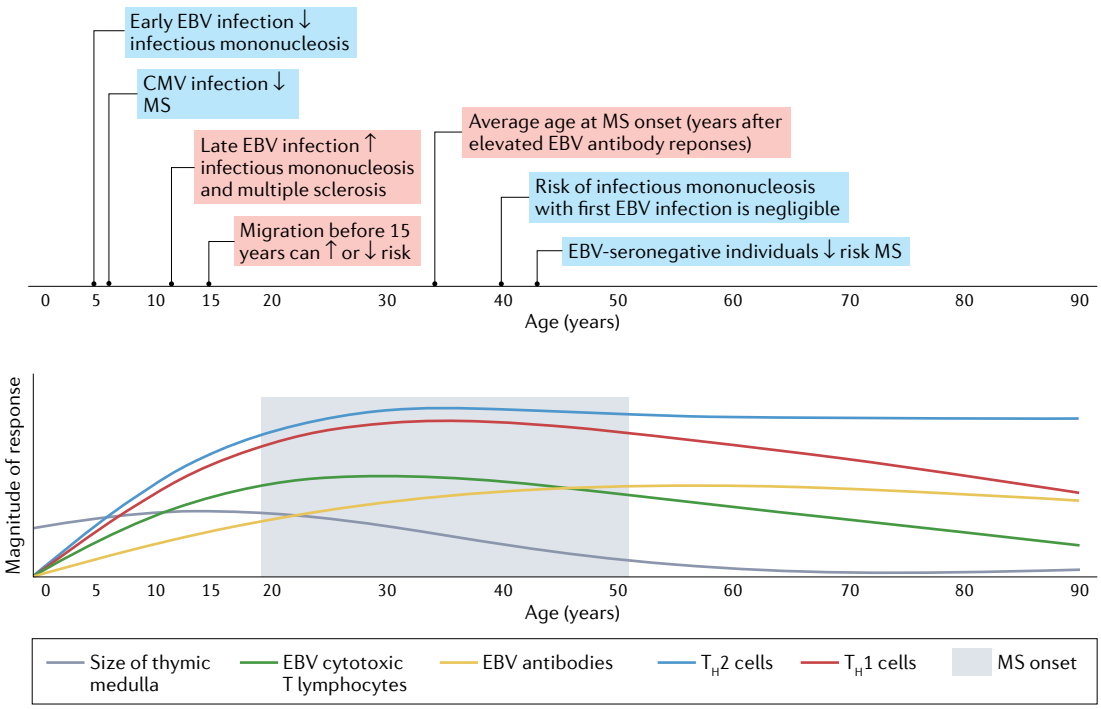



Fig. 1 | **The maturation of the immune system, EBV infection and the development of MS.** The consequences of Epstein–Barr virus (EBV) infection are influenced by the age and genetic background of an individual. The risk of both infectious mononucleosis and multiple sclerosis (MS) increases when primary EBV infection occurs after the age of 10 years, when thymic negative selection of autoreactive T cells slows and T helper 1 ($T_H1$) cell-mediated responses approach their peak. Most individuals receive a diagnosis of MS between the ages of 20 years and 50 years, years after EBV exposure. EBV infection increases the survival of memory B cells and causes lasting changes in the host cytokine response. There are many gaps in our understanding of how the maturation of the immune system triggers an evolving process of EBV-driven autoimmune reactivity leading to the development of MS. CMV, cytomegalovirus.

latency proteins, including EBNA2, EBNA3A, EBNA3B, EBNA3C and LMP2, are recognized by $CD8^+$ T cells through major histocompatibility complex (MHC) class I presentation[58]. By contrast, immune response to EBNA1 peptides is mediated predominantly by T helper 1 ($T_H1$)-polarized $CD4^+$ T cells[59,60]. In addition, NK cells play a supportive role in controlling primary and lytic infection, while NK T cells and γδ T cells restrict latency types I and II (REFS.[5,61–63]). These cytotoxic lymphocytes also successfully restrict EBV in preclinical models, indicating that the cytolytic arm of the immune system must be engaged for efficient control of EBV infection[45,64–66]. Study of primary genetic immunodeficiencies that are associated with an increased risk of EBV-associated disease has identified key immunoregulatory factors for controlling infection, such as the co-stimulatory proteins CD27, SLAM family members, magnesium transporter and the co-inhibitory CTLA-4 receptor[67,68].

***EBV deregulation of immune control.*** Despite a robust immune response to primary infection, EBV establishes a long-term latent infection in B lymphocytes, through a combination of viral reprogramming of B lymphocytes and disarming many innate and adaptive immune responses. EBV encodes numerous proteins that modulate the immune response. Some of these are expressed during the lytic or prelatent phase, while others are more consistently expressed during the latent infection. For example, EBNA1 can induce CXCL12 to recruit regulatory T cells[69] and suppress NK cell responses by downregulating NKG2D ligands[70]. EBNA2 transcriptionally activates numerous genes involved in immune regulation, such as those encoding tumour necrosis factor (TNF)[71], lymphotoxin-α[72], IL-18R[73] and PDL1 (REFS.[74,75]). EBNA2 also suppresses interferon responses[76] and HLA class II gene expression[77]. Virally encoded IL-10 (also known as BCRF1) suppresses pro-inflammatory cytokine secretion, such as secretion of IL-2 and interferon-γ (IFNγ), while viral BNLF2a inhibits the transporter associated with antigen processing (TAP)[78]. Multiple viral miRNAs target type I interferon pathways, such as IRF9, JAK1, JAK2 and RIG-I (REF.[79]). Functionally, EBV miRNAs suppress $CD8^+$ T cell response and are required for the establishment of latent infection in humanized mice[80]. Thus, EBV encodes numerous genes that deregulate innate and adaptive immunity, and it is not yet clear which, if any, of these pathways are most involved in the pathobiology of MS.

## Pathobiology of MS

The pathobiology of MS is notable for several immune abnormalities, which have been described extensively elsewhere. Briefly, oligoclonal bands in the cerebrospinal fluid (CSF) and elevated IgG concentrations in the CNS are hallmarks of MS, and can be used for diagnosis. Notably, oligoclonal bands are found in several neuroinflammatory disorders, and are typically directed against the pathogen implicated in the disease. By contrast, the oligoclonal bands in MS are reactive against multiple antigens, including viral antigens, bacterial antigens and self-antigens[81–86]. Several studies have provided evidence of EBV infection or elevated immune responses to EBV within the CNS, while others have not replicated these findings. The presence of EBV-reactive and human herpesvirus 6-reactive oligoclonal bands and antibody reactivity to EBNA1 and EBNA2 epitopes have been reported in MS CSF[85,87]. In addition, cytotoxic T lymphocytes (CTLs) reactive to EBV lytic proteins have been detected in the CSF of patients with MS[88]. The presence of serum antibodies to EBNA1 has been correlated with elevated intrathecal IgG levels in patients with early MS, suggesting a role for EBV at the onset of MS symptoms.

Cytokine production is highly perturbed in MS, with a characteristic upregulation of several pro-inflammatory cytokines, including IL-12, TNF, IFNγ, lymphotoxin-α and osteopontin[89]. Before disease relapse, IL-10 secretion is downregulated and both IL-10 and TGFβ levels increase with disease remission[89]. Inflammatory B cells secreting higher levels of IL-10 and GM-CSF have also been identified in peripheral blood from patients with MS[90–92]. The effects of immunomodulatory therapies in MS further underscore the role of the immune control. For example, IFNβ (type I interferon) treatment is therapeutic, while IFNγ (type II interferon) treatment exacerbates disease progression[93], and functional studies have confirmed that the IFNα/β pathway is downregulated in the peripheral blood mononuclear cells of untreated patients with MS[94]. More recently, the important role of B cells in MS pathogenesis was revealed by the success of B cell depletion therapy targeting B cells, including anti-CD20 (see later)[90].

Ultimately, the immune abnormalities in MS are associated with the development of focal demyelinating lesions (also known as plaques) in CNS white and grey matter and can be visualized by MRI. These lesions differ in size, distribution and cellular composition[95]. The neuropathological findings suggest that within the active lesion, inflammatory T cells, B cells, plasma cells, activated microglia and macrophages are associated with oligodendrocyte loss, demyelination and astrocyte activation as the lesion forms around veins and venules, expands into normal-appearing white matter and leads to the formation of gliotic scars[96–99]. Within the active lesion, macrophages contain both early and late myelin degradation products. Inflammation is greatest in active lesions, but is also observed in other stages of MS plaques. Interestingly, relatively little inflammation is observed in the initial stages of white matter lesions, leading to debate as to whether lesions are initiated by a neurodegenerative or an inflammatory process and raising the possibility that initial tissue injury is initiated by lymphocyte-derived soluble factors that induce damage directly or via activation of microglia[97]. Inactive MS lesions are hypocellular, with loss of oligodendrocytes and myelin, astrocytosis, fewer myelin degradation products within macrophages and loss of axonal density[96]. In addition to demyelination, axonal loss occurs in both white matter and grey matter and, over time, there is atrophy of the brain[100]. Remyelination may occur as new oligodendrocytes regenerate; the extent of remyelination depends on many factors, including the location in the brain. Circumstances that determine whether inflammation within a lesion resolves and remyelinates or if it 'smoulders' are incompletely understood.

Box 1 | **Critical questions and knowledge gaps**

- How do developmental changes in the human immune system impact the long-term control of Epstein–Barr virus (EBV) with respect to T cell responsiveness and latent B cell reservoir? And how does this inform our understanding of the timing of EBV infection and its subsequent lifetime latency and immune control?
- What, if any, are the pathogenic roles of EBV in the central nervous system (CNS)? Do CNS-infiltrating immune cells harbour EBV or EBV-reactive immune cells, especially in multiple sclerosis (MS)? What are the specific dynamics of EBV infection in the CNS? Which cells are involved, and does this differ in patients with MS compared with healthy controls?
- How does EBV reprogramming of B cells contribute to MS risk? Does EBV alter B cell antigen presentation and T cell miscommunication to drive autoimmunity? Does EBV rescue autoreactive B cells?
- How do MS-risk alleles compound the effects of EBV latent infection in B cells? Is enhanced EBNA2 binding at MS-risk alleles sufficient to drive B cell autoimmunity?
- How do EBV infection and the HLA-DR15 allele compound the risk of MS? Is there an altered presentation of EBV antigens or EBV-induced factors in this HLA haplotype?
- Which EBV factors are most consistently associated with MS pathogenesis, and can this inform more selective drug design and immunotherapies?
- How do effective MS therapies (for example, CD20 depletion, cladribine and CD52 depletion) affect EBV-positive cells, EBV infection cycle, the frequency of EBV-positive cells and EBV loads? Does deficient cytotoxic T lymphocyte control in MS lead to EBV reactivation and increased EBV antibody responses and CNS inflammation?

Immune cell composition within the lesion differs with respect to the type of MS and the stage of the lesion. T cell and B cell infiltration is greatest in active lesions in patients with RRMS. $CD8^+$ T cells consistently outnumber $CD4^+$ T cells in all sites of the MS lesion except for the perivascular and meningeal cuffs, where $CD4^+$ T cells, $CD20^+$ B cells and plasma cells predominate[98,99], suggesting that $CD8^+$ T cells are more important effectors in the immunopathogenesis of MS than previously appreciated. Notably, fewer brain lesions, fewer inflammatory cells and more spinal cord lesions are found in PPMS than in RRMS.

## Immunogenetics of MS

The genetic contribution to MS susceptibility is complex and is extensively reviewed elsewhere[101,102]. The strongest genetic risk factor for MS is a specific haplotype of the highly polymorphic MHC[103]. Specifically, an increased risk of MS exists in individuals with the MHC class II alleles HLA-DR2 and HLA-DQw1 (REF.[104]), with the primary risk allele being HLA-DRB1*1501. Genome-wide studies have identified more than 200 MS-associated loci across the human genome, and approximately 30 are associated with the MHC locus[105–109]. Most of these loci have well-ascribed functions in the immune system, while some are associated with myelin structure or mitochondrial function[110–113]. Importantly, these studies also reveal shared genetic risk factors with other autoimmune conditions.

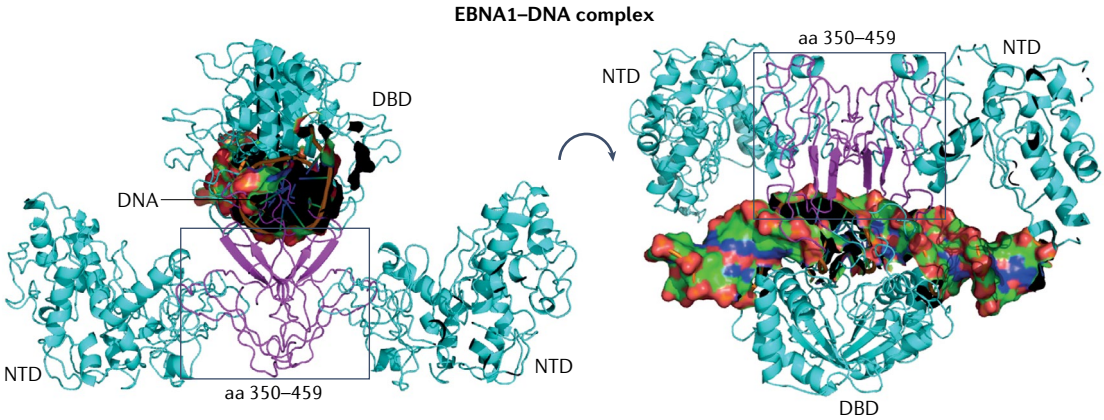
## How does EBV increase the risk of MS?

MS has a complex aetiology, with multiple causative factors that can be further defined as either drivers or triggers[114]. EBV is a trigger (that is, it must be acquired before the onset of disease); however, its role as putative 'driver' of disease progression is poorly defined. Ongoing clinical studies using antivirals, vaccines and cell-based approaches targeting EBV in patients with MS (discussed later) are likely to elucidate the role of EBV as a driver of disease activity. The risk of MS increases approximately 32-fold with EBV infection, and more with symptomatic to severe infectious mononucleosis and HLA-DR2b (HLA-DRB1*1501b and HLA-DRA1*0101a)[18]. How these genetic and environmental factors compound risk in MS is not fully understood, and there remain many plausible mechanisms. Determining which of these are the most frequent drivers and how best to therapeutically intervene remain challenges. In this section, we discuss the evidence for EBV as a trigger and/or a driver in MS pathogenesis, and highlight critical questions that may elucidate the role of EBV as a trigger and potential driver of MS (BOX 1).

***Molecular mimicry and mistaken self.*** Latent and persistent infection is a chronic source of viral antigenic stimulation. Several EBV antigens are the target of cross-reactive autoantibodies found in MS. This cross-reactivity between self-antigens and EBV antigens involves both cellular and humoral immune responses. Early studies found that patient-derived T cells autoreactive to myelin basic protein (MBP) were also cross-reactive to a wide range of viral peptides, including peptides from EBV[115]. Subsequent studies identified MBP-reactive T cells in patients with MS that cross-react with EBNA1 (REF.[116]). Similar cross-reactivities with EBNA1 were found for T cells autoreactive to anoctamin 2 (REF.[117]), α-crystallin B chain (CRYAB)[88,118] and most recently glial cell adhesion molecule[17]. Mimicry has been reported for the lytic proteins BHRF1 and BPLF1 (REF.[119]). Peptides from these viral lytic proteins were found bound to the HLA-DR15 haplotype and were cross-reactive with the self-protein RASGRP2 as a target autoantigen, which is expressed in the brain and B cells and is targeted by brain-homing, autoreactive $CD4^+$ T cells[119].

Autoreactive antibodies in MS also cross-react with viral proteins, especially EBNA1 (REF.[120]). Higher levels of antibodies to EBNA1 are typically observed in both serum and CSF of patients with MS[121,122]. Elevated titres of antibodies to EBNA1 were found to have a genetic component beyond just HLA type[123], and high titres of antibodies to EBNA1 are associated with an increased risk of MS[124]. Many of these EBNA1-specific antibodies are polyreactive, and it is not clear which antigen initiates the immunogenicity. In addition to viral mimicry, virus infection in peripheral tissue induces cellular stress proteins, such as CRYAB, that can mimic CNS tissues and elicit an autoimmune reaction to proteins such as myelin[125]. Interestingly, CRYAB-specific antibodies from patients with MS cross-react with EBNA1 (REF.[126]). Despite these correlations, the pathogenic role of autoreactive and EBV-cross-reactive antibodies in MS is not well established.

Why then do so many self-reactive immune responses in MS cross-react with EBV peptides and EBNA1 in particular? Peptide library analyses have identified several domains of EBNA1 that are recognized by autoreactive immune responses (FIG. 2). EBNA1 amino acids 391–410 peptide mimics CRYAB amino acids 1–15




| EBNA1 (aa) | Sequence | Mimic | Immune response |
|---|---|---|---|
| 386–405 | SQSSSSGSPPRRPPPGRRPF | GlialCAM | IgG |
| 411–426 | EADYFEYHQEGGPDGE | MBP | IgG |
| 504–518 | VAGGRVYGGSKTSLY | β-SYN | HLA-DR2b |
| 431–440 | PGAIEQGPAD | ANO2 | IgG |
| 473–593 | | | HLA-DRB1*15 |
| 385–420 | PPPGRRPFFHPVGEA | CRYAB | IgG + HLA-DRB*1501 |
| 407–418 | HPVGEADYFEY | | CD8 + HLA-B35 |
| 90–330(GA)*n* | GAGGGAGAGG | | IgG |

Fig. 2 | **EBNA1 sequences and their potential role in molecular mimicry and autoreactivity.** Computational model of EBNA1 full-length protein[233] indicating the most frequent peptide epitopes associated with multiple sclerosis autoimmunity. DNA is shown as solid, protein as ribbon. Epitopes are highlighted in magenta and boxed. A partial list of EBNA1 peptides and their autoimmune properties, including immune response type and cellular protein mimic. aa, amino acids; ANO2, anoctamin 2; β-SYN, β-synuclein; CRYAB, α-crystallin B chain; DBD, DNA-binding domain; (GA)*n*, glycine-alanine repeats; glialCAM, glial cell adhesion molecule; MBP, myelin basic protein; NTD, amino-terminal domain.

with an overlapping sequence of RRPFF[126]. A similar domain of EBNA1 (amino acids 386–405) mimics glial cell adhesion molecule[17]. In the case of glial cell adhesion molecule, post-translational modification of the host protein increased cross-reactivity, providing a mechanism for epitope evolution and spreading in response to environmental signals. Some reactivity to EBNA1 was associated with germ line, unmutated BCR, suggesting that early antibodies have innate affinity for a region of EBNA1 (REF.[17]). Other studies have pointed to the glycine-rich regions of EBNA1, which generate repetitive, low-complexity peptides[127]. Autoreactive antibodies also react with peptides derived from the exposed surface of the EBNA1 DNA-binding domain, but not the DNA-binding interface itself, suggesting that the intact EBNA1–DNA complex is an important antigenic stimulus[128]. Paradoxically, EBNA1 also has immune-evasive properties. The internal Gly-Ala repeats (amino acids 90–303) suppress HLA presentation through multiple mechanisms, including inhibition of peptide processing[129–131], suppression of autophagy[132] and translational suppression[133,134] owing to the mRNA structure[134–136]. How these activities are related to the high exposure of EBNA1 in autoimmune disease and what aspects of EBNA1 peptide presentation differ in patients with MS are unclear.

***Rescue of autoreactive and inflammatory B cells.*** EBV is highly efficient at immortalizing naive and resting B cells (FIG. 3). However, it is not fully established which B cell subtypes may or may not be susceptible to EBV. EBV immortalization of a 'forbidden' autoreactive B cell clone has been proposed as a potential mechanism triggering MS[137]. EBV transformation could bypass the normal process of elimination of autoreactive B cells, although most of this selection occurs in the bone marrow at early stages of B cell development[138]. Similar mechanisms of immune evasion are proposed for EBV-associated cancers[139] (BOX 2). EBV immortalization bypasses many of the requirements for T cell help through the virally encoded CD40-like receptor LMP1 and BCR-like receptor LMP2 (REFS.[23,31]). Their combined expression is sufficient to drive lymphomagenesis in transgenic mice[140], and it is likely that these ligand-independent receptors provide signals that can rescue autoreactive B cells. EBV-infected B cells also express mature BCR and IgG without necessarily passing through GC selection, further enabling the survival of B cell clones reactive to self[141]. EBV-infected B cells alter T cell interactions mediated by CD70–CD27 and OX40L that disable T cell control and enable B cell lymphomagenesis[142,143]. Whether these forbidden B cells are antigen-presenting cells or antibody-producing cells is not yet known. However, recent B cell depletion studies suggest that B cell subtypes, and not plasma cell numbers or overall circulating antibody levels, best correlate with CNS pathogenesis in patients with MS[90].

***EBV infiltrating the CNS.*** EBV-infected B cells migrate to the CNS, where they may have altered immune reactivities and are associated with EBV-associated diseases, including primary CNS lymphoma (FIG. 4). EBV-positive B cells and plasma cells have been identified after death

in MS lesions in the CNS of patients with MS, but not in controls[144–146]. EBV gene expression was a variable mixture of both latent transcripts (EBV-encoded small RNAs (EBERs), EBNA3A, LMP2A and LMP2B) and lytic transcripts (BZLF1 and gp350) in these brain lesions[33,144,146–148]. In situ hybridization experiments revealed a significant number of EBER$^+$ B cells and a small number of BZLF1$^+$ cells, although some EBV-positive B cells were also found in the brains of controls[148]. EBV-infected plasma cells in the CNS have been found synapsed with cytotoxic CD8$^+$ T cells, suggesting a local inflammatory interaction initiated by EBV-positive B cells in the CNS[149]. There is evidence that EBV establishes an extralymphatic viral sanctuary in the CNS[150], especially in vulnerable individuals during infectious mononucleosis, in which approximately 50% of memory B cells can be EBV positive[151]. However, several studies failed to find evidence of EBV-positive B cells in the CSF of patients with MS or MS lesions in the CNS[152–157]. Some of these conflicting findings may be due to technical challenges of detecting transient EBV gene expression in migratory B cells in the CNS of patients with MS and post-mortem samples[158].

***Deficient CTL control of EBV infection.*** T cell control of EBV infection is required for homeostatic viral persistence, and immune dysregulation is observed in all EBV-associated disease. In healthy carriers of latent EBV infection (more than 90% of the adult population), nearly 1% of all T cells are reactive to EBV latent or lytic antigens[159,160]. Immune response to EBV is frequently skewed in patients with MS. Higher titres of EBNA1-reactive IgG are found several years before the onset of MS symptoms and correlate with MS risk[8,161,162]. EBNA1-specific T cell frequencies increase and specificities broaden in MS. CD4$^+$ T cells show $T_H1$ polarization and CD8$^+$ T cell responses correlate with disease activity[163–166]. MS progression correlates with a decreased functionality of EBV-specific CD4$^+$ T cells and CD8$^+$ T cells, as measured by IFNγ production and cytotoxic activity[167–169]. T cell exhaustion may partly account for the failure to control chronic EBV infection. Developmental changes in the immune system are also critical for control of EBV infection. Childhood experience (time of exposure to EBV and geographical risk) indicate that immune system maturation, exposure and education are important components of MS

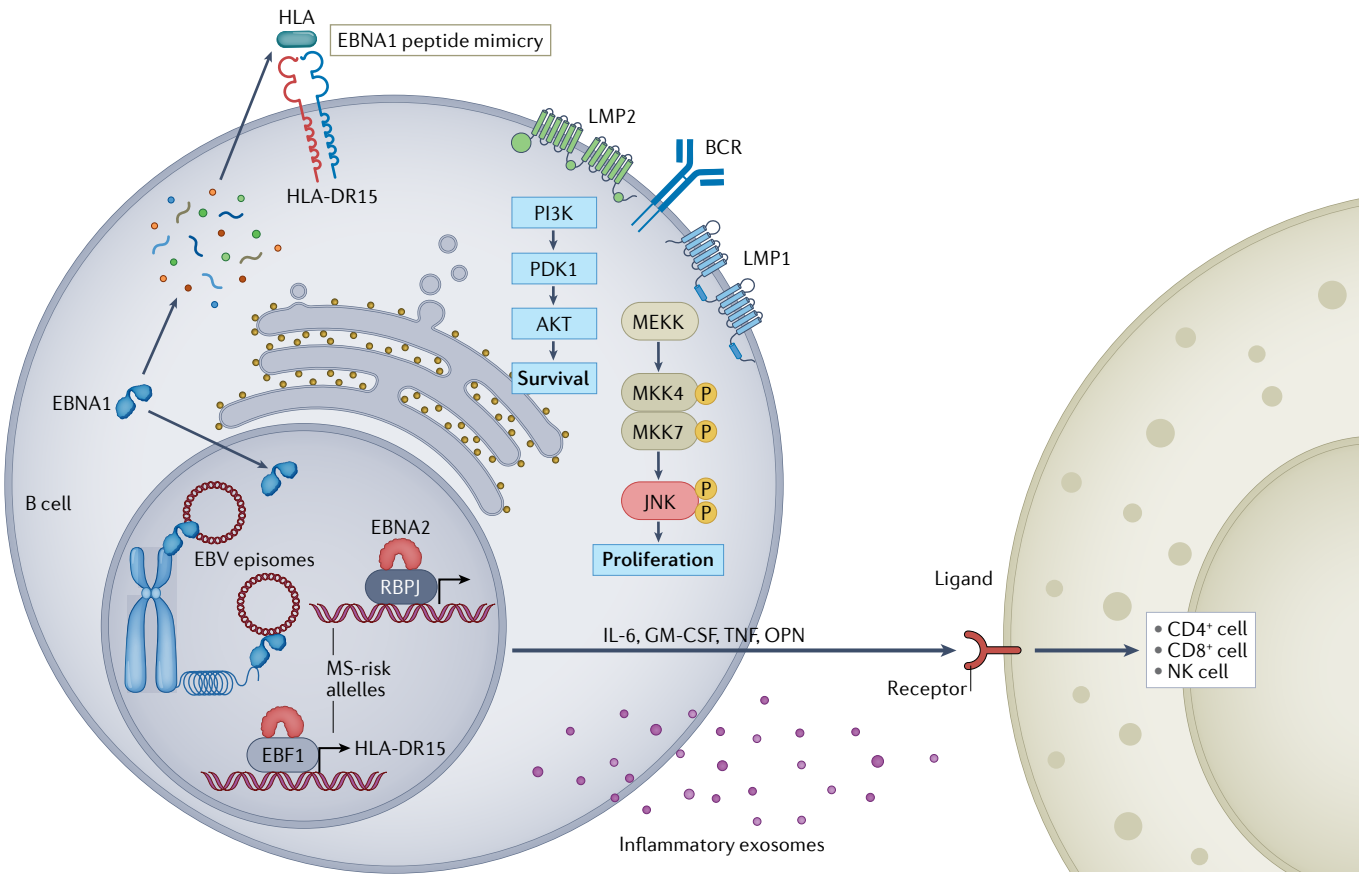



Fig. 3 | **EBV latency drives B cell survival of inflammatory B cells.** Epstein–Barr virus (EBV) infection promotes the proliferation and survival of memory B cells that may alter T cell control of EBV infection and autoimmune reactive B cells and T cells. EBV LMP1 and LMP2 function as CD40-like and B cell receptor (BCR)-like mimics to bypass T cell-dependent germinal centre reactions. EBNA1 and EBNA2 drive gene regulatory changes that may affect preferentially multiple sclerosis (MS)-risk alleles. EBV-induced viral and cellular factors may promote inflammation, driving autoreactivity through direct interaction with T cells or natural killer (NK) cells, as well as through soluble factors, including exosomes. EBNA1 is frequently processed as an antigenic epitope that can stimulate autoreactive B cell and T cell development. Although EBV-positive cells are shown as antigen-presenting cells, it is not known whether they actually present EBNA1 peptides or whether these are presented by uninfected antigen-presenting cells, including uninfected dendritic cells and macrophages that captured infected cell debris. OPN, osteopontin; TNF, tumour necrosis factor.

aetiology[8]. Poorly defined, idiosyncratic CTL deficiencies may also enable EBV-positive B cells to proliferate, migrate to the CNS and express inflammatory viral and host factors[169].

***EBV-associated inflammation.*** Both B cells and T cells from patients with MS have atypical inflammatory features. Patients with MS with high EBV loads have T-bet$^+$CXCR3$^+$ memory B cells induced by IFNγ and TLR9 signals and EBV-reactive CTLs autoreactive to neuronal tissue[170]. EBV load correlated with the early emergence of CXCR3$^+$ class-switched memory B cells, GC-like B cell development and trafficking of these cells to the CNS in mice[170]. These CXCR3$^+$ B cells had enhanced ability to secrete anti-EBNA1 IgG[171]. It is important to note that EBV-negative B cells from patients with MS also have inflammatory features, and memory B cell subsets, in particular, were found to secrete higher levels of GM-CSF in patients with MS relative to healthy controls[92]. EBV-infected B cells produce high levels of inflammatory cytokines and exosomes that contain inflammatory components, including small viral nucleic acids, such as EBERs and miRNAs[172]. Exosomes containing EBERs with 5′-triphosphate pathogen-associated molecular patterns stimulated dendritic cell antiviral inflammatory activity, similar to systemic lupus erythematosus[172]. EBV miRNAs, which can be transported in exosomes, can target MS risk-associated genes, such as *ZC3HAV1* regulating interferon response[173]. Exosomes may cross the blood–brain barrier, and are endocytosed by brain microvascular endothelial cells[174]. Therefore, it is possible that EBV-positive B cells in the periphery produce exosomes that cross into the CNS and/or that EBV-positive B cells in the CNS are a source of these inflammatory exosomes (FIG. 4). EBV-positive B cells may also induce autoreactive T cells through modification of their antigen presentation[8,90].

***Deregulation of B cell gene expression and autoimmune control.*** EBV encodes several transcriptional regulators and signalling molecules that reprogramme B cell gene networks implicated in cancer and autoimmunity. The latency nuclear regulatory factor EBNA2 is essential for EBV immortalization and drives B cell proliferation[175]. EBNA2 interacts with several host transcription factors, and studies involving chromatin immunoprecipitation followed by sequencing revealed that EBNA2 binds to almost half of the risk alleles for seven autoimmune disorders[176]. Genome-wide chromatin accessibility (assay for transposase-accessible chromatin using sequencing) and DNA looping (HiC) further demonstrated the role of EBNA2 in altering chromatin structure at many autoimmune genetic risk alleles[177]. Risk alleles were enriched for EBNA2 binding relative to non-risk alleles, as demonstrated for a few specific examples, such as *ZMIZ1* (REF.[177]).

Genome-wide linkage studies have further implicated EBV as a regulator of MS-risk alleles[178]. Expression quantitative trait locus analysis found that genes located near MS-risk SNPs were linked with EBV type III latency. These genes include *BATF*, *IRF5*, *IRF7* and STAT genes. In a related study, EBNA2 bound preferentially to five of six MS-risk alleles, relative to non-risk alleles, and a peptide inhibitor that disrupts EBNA2 interaction with the cellular transcription factor RBPJ altered high-risk allele expression[71]. Thus, MS-risk alleles could increase the efficiency of EBNA2 to promote B cell survival and immortalization[71]. EBNA2 targets also overlap with those of vitamin D receptor, which is another risk factor for MS[179]. Furthermore, polymorphisms in *EBNA2* correlate with MS risk, suggesting that the virus strain may also be a risk factor[180]. The precise mechanism of gene deregulation in MS may be further nuanced and influenced by epigenetic control. DNA methylation and genomic imprinting of alleles associated with MS have been implicated in MS[181,182]. For example, HLA-DRB*1501 is hypomethylated and expressed at high levels in antigen-presenting cells in patients with MS[183,184]. Alternative splicing has been seen in MS B cells, and may be related to EBV transcriptional reprogramming[185].

EBV encodes several other transcription regulatory factors that can influence B cell biology. The EBV lytic activator BZLF1 is a potent transcriptional regulator of numerous viral and cellular genes. BZLF1 expression has been identified in plasma B cells in post-mortem brain samples from patients with MS and has been associated with reactive cytotoxic CD8$^+$ T cell infiltration[166]. EBV-induced G protein-coupled receptor 2 (EBI2; also known as GPR183) is a G protein receptor for dihydroxycholesterol, which is overexpressed in MS lesions and involved in migration of CD4$^+$ T cells[186].

EBV genomes are also regulated by epigenetic modification, especially DNA methylation, which can impact viral gene expression and latency type[187,188]. Epigenetic control of EBV is an important component of EBV cancer aetiology, but its role in autoimmune disease is

Box 2 | **Common themes of EBV-associated cancers and MS**

There are several common features of Epstein–Barr virus (EBV) infection as an aetiological agent in both cancer and multiple sclerosis (MS). Most EBV-associated cancers result from EBV prolonging the survival of a cell that acquires additional oncogenic mutations or epigenetic changes that drive cancer cell evolution. Cancer may also arise from EBV entering a cell with precancerous mutations that may enable EBV to establish an oncogenic infection, such as a type II latency in an epithelial cell. It is also possible that EBV acquires mutations and induces epigenetic changes in the host cell that drive oncogenesis. These rare events amount to a significant incidence of cancer cases owing to the high prevalence and persistence of EBV. Similar types of aberrations may need to be considered for MS. Does EBV infect a rare 'forbidden' B cell? If so, what are the B cells that are infected in patients with MS, and how may these differ from non-pathogenic EBV-positive B cells that do not drive MS? Could EBV have acquired rare mutations or polymorphisms that drive MS? Because EBV is so ubiquitous and because it is usually acquired early in life, the question of how the virus may be tolerated as 'self' versus chronically rejected as 'non-self' may depend on the age at primary infection. Antigens acquired before a certain stage of immune development and presented in the appropriate HLA context may be considered self-antigens and acquire tolerance. Similarly, foreign antigens that mimic self-antigens may escape immune recognition by posing as self or exhausting T cells. EBV modulation of many B cell immunoregulatory genes is also likely to contribute to pathogenesis in both cancer and MS. Indeed, similarly to MS, infectious mononucleosis in adolescence increases the risk of developing Hodgkin lymphoma (an approximately fourfold increase). In Hodgkin lymphoma, EBV rescues defective germinal centre B cells from apoptosis and initiates early events in lymphomagenesis by altering normal B cell gene expression programmes[139]. Therefore, it is possible that an analogous EBV-mediated rescue of autoreactive B cells or other B cell subsets may set the stage for the development of MS.

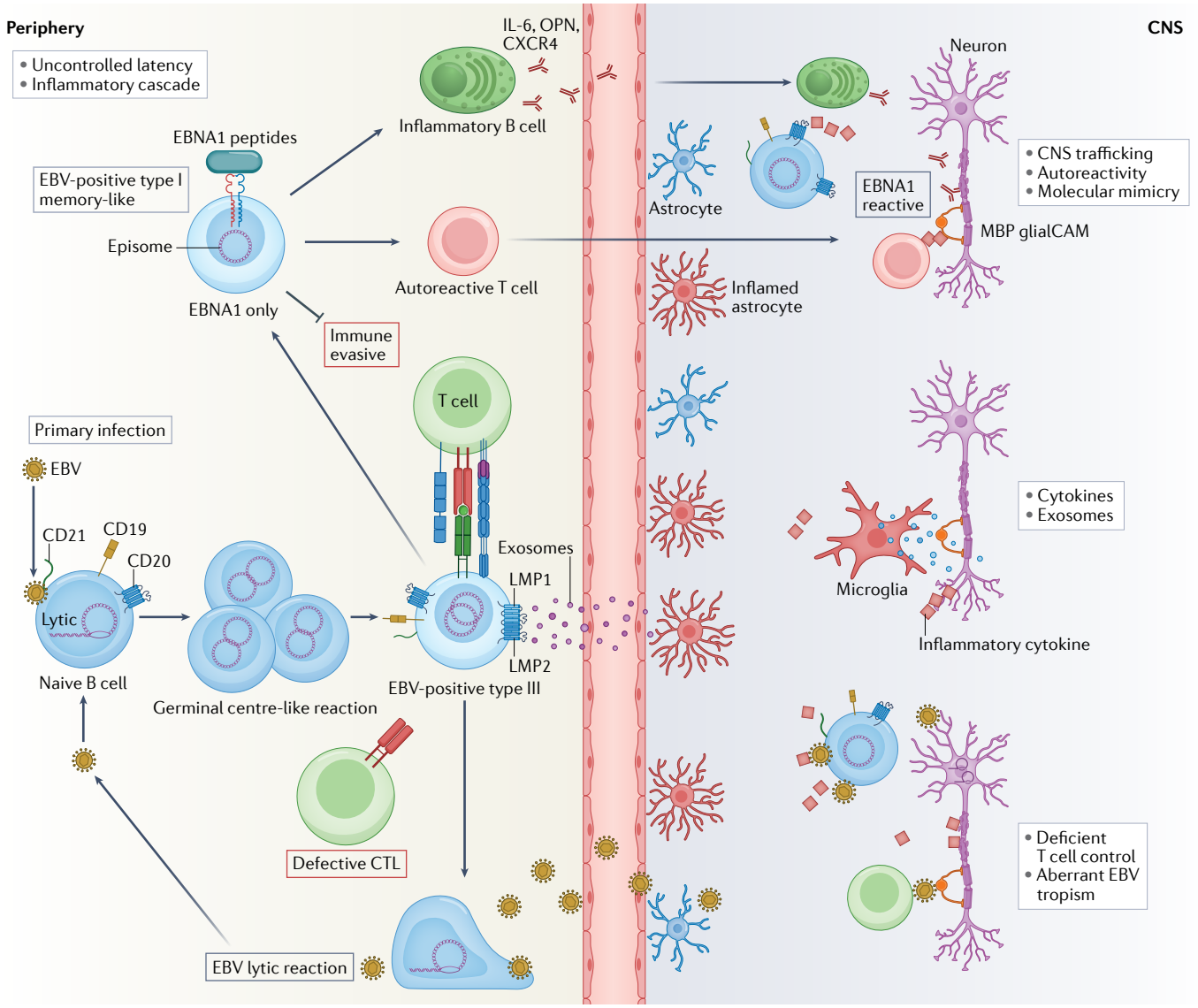



Fig. 4 | **Mechanisms of EBV-mediated inflammatory cascades in periphery and CNS.** Epstein–Barr virus (EBV) may drive inflammatory events in both the periphery and the central nervous system (CNS), leading to the development of the multiple sclerosis lesion in the CNS. EBV immune-evasive features and risk-associated immune deficiencies promote EBV inflammatory cascades in the periphery. CNS pathogenesis may be initiated through multiple mechanisms, including natural and EBV-driven CNS trafficking of autoreactive B cells and T cells, molecular mimicry driven by chronic EBV infection, EBV-driven inflammatory cytokines and exosomes, and aberrant EBV lytic infection and tropisms owing to deficient immune control. CTL, cytotoxic T lymphocyte; glialCAM, glial cell adhesion molecule; MBP, myelin basic protein; OPN, osteopontin.

not well described. Studies in MS patients and animal models have identified gene variants, miRNAs and viral co-factors that exert epigenetic control to increase inflammation, immune cell differentiation and myelin breakdown[189]. Epigenetic modification of genes that promote neuroinvasion of EBV-positive B cells, including the genes encoding osteopontin and CXCR4, has been described in some experimental models, suggesting that EBV may affect epigenetic mechanisms driving MS[190].

***EBV interactions with HLA.*** HLA alleles have different binding affinities and specificities for antigenic peptides that impact T cell immunogenicity and functionality[191]. Antigenic peptides derived from MBP have been identified from B cells from patients with MS and correlate with higher levels of MBP-specific T cells in patients with MS than in controls[81]. Related studies implicate variant peptide binding of the high-risk HLA-DRB1*15 allele in the presentation of various autoreactive peptides. Some of these peptides may be derived from EBV proteins, providing a potential mechanism to explain the combined risk of EBV infection and HLA-DRB1*15. For example, humanized mice reconstituted with HLA-DR15 had elevated $CD8^+$ T cell responses and $CD4^+$ T cells cross-reacting with MBP[192]. In addition, some studies have found that HLA-DR15 and HLA-DRB*07 patients with MS have higher EBV viral loads, whereas HLA-A*02 individuals have lower viral loads, suggesting that class I and class II MHC molecules modulate EBV latency control[193,194]. However, other studies did not

find increased EBV viral loads in MS or changes that immediately precede or coincide with relapses[195–197]. Nevertheless, HLA-A*02 correlates with a decreased risk of MS (reviewed in REF.[198]). Alternatively, but perhaps related, MS-associated risk alleles, including HLA-DRB5, are also correlated with differentially regulated gene expression[199,200]. Higher expression levels of HLA alleles may also affect peptide selection and presentation that contribute to peptide mimicry and autoreactivity. Another intriguing finding is that the HLA-DR15 allele can serve as a co-receptor for EBV entry into B cells, raising the possibility that viral entry pathways may also contribute to MS risk[201].

## Opportunities for therapeutic intervention

***Existing immunomodulatory therapies and their potential effect on EBV.*** The effectiveness of immunosuppressive and anti-inflammatory therapies in MS supports the autoimmune component in disease pathogenesis. Corticosteroids effectively treat MS flares[202], but are too immunosuppressive for long-term use. Several immunosuppressive and chemotherapeutic drugs that dramatically decrease the levels of circulating immune cells, including cyclophosphamide, cladribine, mitoxantrone, methotrexate and teriflunomide, have been used with variable success[203,204]. It is now appreciated that B cells play an essential role in MS pathogenesis, on the basis of the success of CD20-specific depletion. Monoclonal antibodies to the B cell antigen CD20 (ocrelizumab and ofatumumab) reduce MS relapse and lesion formation, while a monoclonal antibody (anti-IL-12 p40 and anti-IL-23 p40, ustekinumab) that targets both $T_H1$ cells and $T_H17$ cells did not show similar efficacy[205–207]. Importantly, additional therapeutics that broadly target B cells, including anti-CD52 monoclonal antibody and cladribine act as B cell-depleting drugs and are of therapeutic use in MS. By contrast, treatments that target naive and plasma B cells (for example, atacicept) or boost memory B cells (for example, infliximab) further aggravate MS via TNF blockade[208]. The effects of these treatments on EBV load is not yet known. Interestingly, teriflunomide has been shown to reduce both EBV-induced lymphoproliferation and lytic viral replication[209].

***EBV-specific CTL therapy.*** Cell-based immunotherapies, including EBV-specific CTL lines, have proven successful in the treatment of post-transplantation lymphoproliferative disorder and EBV-associated lymphomas and nasopharyngeal carcinoma, with low rates of graft-versus-host disease[210–212]. Therefore, the use of autologous T cell therapy has been expanded to clinical trials in MS[213–216]. These therapies attempt to compensate for deficient CTL control of EBV-infected B cells. Phase I trials using ATA188, an allogenic T cell therapy using T cells from healthy donors, have been initiated to evaluate allogenic EBV CTL therapy in PPMS and SPMS (NCT03283826), and the first clinical episode highly suggestive of MS (NCT02912897). Initial reports have demonstrated increased circulation of LMP-reactive and lymphoblastoid cell line (LCL)-reactive effector $CD8^+$ memory cell populations. Notably, patients with PPMS have reported clinical improvement after autologous EBV-specific T cell therapy targeting EBNA1, LMP1 and LMP2A[214]. Early results suggest that ATA188 is safe and well tolerated, with a decrease in Expanded Disability Status Scale (EDSS) score[217].

***Antivirals, vaccines and their potential to target EBV in MS pathogenesis.*** Specific antivirals for treating EBV infection have not, to date, been approved for treatment of MS. Moreover, several clinical trials testing the efficacy of antivirals, specifically those with broad antiherpesvirus activity, including acyclovir and valacyclovir, did not demonstrate a clear benefit in MS[218–220]. IFNβ, a cytokine with broad antiviral, antiproliferative and anti-inflammatory effects, was the first immunomodulatory therapy to successfully modify the disease course of MS, and is still one of the most frequently used therapeutic options for MS; it is considered a first-line therapy with modest efficacy in controlling ongoing disease[221]. The exact mode of action of IFNβ in MS is only partly understood. IFNβ has potent antiviral activity and is known to counteract many immunomodulatory actions of EBV[222,223]. Antiviral nucleoside analogues may also be effective for treating EBV infection in MS. Recent studies have shown that the non-cyclic nucleoside analogue tenofovir alafenamide (TAF), which was developed as a specific inhibitor for the HIV and hepatitis B virus reverse transcriptases and is frequently used in HIV pre-exposure prophylaxis, also inhibits the EBV DNA polymerase[224]. Notably, TAF was twice as potent as ganciclovir in direct inhibition of EBV replication and DNA polymerase activity[225]. In addition, anecdotal reports and case studies have suggested that there may be a clinical benefit and decreased relapses in patients with RRMS receiving TAF regimens[226]. A clinical trial (NCT04880577) has been initiated to test the ability of TAF as an add-on therapy to ocrelizumab to reduce symptoms and promote neuroprotection in RRMS.

There are distinct challenges for EBV vaccine development. Sterilizing immunity to EBV may not be possible given the efficiency of EBV transmission and persistence, and merely delaying the time of infection is undesirable, because it increases the risk of mononucleosis and MS. Furthermore, identification of the most appropriate viral antigens is complex for both prevention of infection and treatment of existing disease. Vaccine approaches to block early events in EBV primary infection would require neutralizing antibodies that target components of viral entry proteins (including gp350, gp42, gH, gL and gB). Therapeutic vaccines for various EBV-associated cancers or autoimmune disease may need to target multiple viral proteins, as both latent and lytic viral genes have been implicated in disease pathogenesis. In addition to careful consideration of the viral antigens included in the vaccine, a successful vaccine strategy for EBV must stimulate both the humoral arm and the cell-mediated arm of the adaptive immune system and induce production of effector and long-lived memory cells. The development of a vaccine targeted at preventing the development of mononucleosis in EBV-seronegative children could potentially reduce the likelihood that these individuals will later develop MS. A small trial examining the efficacy

Graft-versus-host disease
A condition in which the donor's immune system (the graft) rejects the recipient (the host) as non-self.

of vaccination with the HLA-B*0801-restricted CD8$^+$ T cell epitope FLRGRAYGL demonstrated a reduced likelihood of developing mononucleosis in those children who seroconverted[227]. Similarly, vaccination of EBV-seronegative young adults with a recombinant gp350 subunit vaccine prevented the development of mononucleosis, although it did not decrease rates of asymptomatic EBV infection[228]. Phase I/II trials demonstrated that this gp350 subunit vaccine was well tolerated and immunogenic, inducing robust gp350 antibody responses as well as EBV-neutralizing antibody responses[229]. Following the success of its severe acute respiratory syndrome coronavirus 2 vaccine, Moderna launched a vaccine trial (NCT05164094) using mRNA encoding EBV gp350, gB, gH/gL and gp42 in seronegative 18–30-year-old adults. Further studies are required to determine whether this approach or other vaccine approaches could ultimately decrease the likelihood of developing mononucleosis and, subsequently, MS.

## Conclusions

Despite years of controversy, the role of EBV infection and seropositivity as essential co-factors for most forms of MS may now be settled. As the severity of EBV primary infection strongly correlates with the development of MS many years later, it is likely that MS depends on the initial immune response to EBV infection. Failure to control this primary infection may lead to colonization of resident memory B cell and T cell follicles in CNS accessible sites, such as tertiary lymphoid structures, that are uniquely prone to inducing immune pathology in the CNS. The time of infection likely contributes to immune system elimination of viral, autoreactive T cells and antibodies that target CNS components. These events must be further exacerbated by numerous genetic risk alleles, especially HLA-DRB1*1501, that may compound the effects of EBV infection through aberrant presentation of autoreactive peptides. Other alleles can cooperate with EBV transcription regulatory factors, such as EBNA2, through altered binding specificity and gene programmes promoting inflammatory B cell proliferation. Whether there are any special features of EBV antigens, such as EBNA1, that induce high rates of polyreactivity and self-mimicry needs to be further investigated. Among the most pressing questions is whether EBV-infected cells or viral products act within the CNS or indirectly through inflammatory events in the periphery. Ultimately, how autoreactive immune cells and antibodies form and accumulate in the CNS remain high-priority questions. Knowing that EBV is a likely driver of inflammatory autoimmune disease provides a target for future therapies.

Published online: 05 August 2022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### Author contributions
The authors contributed equally to all aspects of the article.

### Competing interests
P.M.L. founded and is an adviser to Vironika LLC. P.M.L. is named on a patent for inhibitors of EBNA1. S.S.S. declares no competing interests.

### Peer review information
*Nature Reviews Microbiology* thanks Christian Münz and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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