Epstein-Barr virus as a cause of multiple sclerosis: opportunities for prevention and therapy

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Multiple sclerosis is a chronic inflammatory disease of the CNS that results from the interplay between heritable and environmental factors. Mounting evidence from different fields of research supports the pivotal role of the Epstein-Barr virus (EBV) in the development of multiple sclerosis. However, translating this knowledge into clinically actionable information requires a better understanding of the mechanisms linking EBV to pathophysiology. Ongoing research is trying to clarify whether EBV causes neuroinflammation via autoimmunity or antiviral immunity, and if the interaction of EBV with genetic susceptibility to multiple sclerosis can explain why a ubiquitous virus promotes immune dysfunction in susceptible individuals. If EBV also has a role in driving disease activity, the characterisation of this role will help diagnosis, prognosis, and treatment in people with multiple sclerosis. Ongoing clinical trials targeting EBV and new anti-EBV vaccines provide hope for future treatments and preventive interventions.

Introduction

Multiple sclerosis is a chronic inflammatory and neurodegenerative disease of the CNS and is estimated to affect around 2·8 million people worldwide. The disease is commonly diagnosed between the ages of 20–40 years, and it is more prevalent in women than men. The past two decades have seen striking advances in the management of multiple sclerosis, including a better understanding of the involvement of the immune system in neuroinflammation and neurodegeneration, the identification of susceptibility genes, improved diagnostic and prognostic criteria based on MRI and new biomarkers, and the approval of highly effective therapies.[1]

A substantial body of evidence supports the multifactorial aetiology of multiple sclerosis. Besides genetic predisposition, several environmental risk factors acting mainly in early life, such as obesity in childhood or adolescence, infections, geographical latitude, and low vitamin D levels due to little sun exposure, have been associated with an increased risk of multiple sclerosis.[2] Viruses are the microbial agents that have received the greatest attention for triggering or exacerbating multiple sclerosis. Epstein-Barr virus (EBV) has emerged as the virus having the strongest, most consistent, and biologically plausible association with the disease.[3]

In this Review, we summarise the evidence from epidemiological studies that have led to pinpoint EBV as a necessary cause of multiple sclerosis, evaluate recent advances in the understanding of the mechanisms underlying the link between EBV and multiple sclerosis, and discuss how this progress could improve clinical management of the disease in the future and open new avenues for prevention and therapy.

EBV as a leading risk factor for multiple sclerosis

EBV, also known as human gammaherpesvirus 4, is recognised as one of the most successful viruses: it infects most people and persists throughout an individual's life. Although generally harmless, EBV is the causative agent of infectious mononucleosis and some lymphoid and epithelial cancers (panel 1). EBV has also been associated with autoimmune diseases.[3,4] The link with multiple sclerosis is mainly supported by longitudinal studies showing that EBV infection, history of infectious mononucleosis, and high titres of antibodies against EBV proteins—mainly EBV nuclear antigen 1 (EBNA-1) and the EBNA complex—increase the risk of developing multiple sclerosis, whereas the risk is negligible in EBV-seronegative individuals.[3,6–8] EBV infection and the rise in EBNA-1 IgG can precede the onset of multiple sclerosis by several years, suggesting a role for EBV in early disease pathogenesis.[3,6,7] Compared with 90–95% seropositivity in the general adult population, large cohorts of adults with multiple sclerosis have complete EBV seropositivity and higher concentrations of antibodies against EBV.[9,10] In paediatric multiple sclerosis, EBV seropositivity ranges from 85% to 99% in affected children, compared with 40% to 72% in age-matched control children.[11,12] Whether the variability of seropositivity findings in the paediatric population is due to differences in the sensitivity of different serological assays or to misdiagnosis is a moot point.

In 2022, a seroepidemiological study provided the most compelling evidence on EBV as an early and essential factor for the development of multiple sclerosis.[13] More than 10 million people in the US Army were followed up for more than 20 years to identify those diagnosed with multiple sclerosis and analyse longitudinally collected serum samples for the presence of anti-EBV antibodies. The individuals who became EBV seropositive had a 32-fold increased risk of developing multiple sclerosis, compared with those who remained seronegative. In people who developed multiple sclerosis, the study found that EBV infection preceded an increase in serum concentrations of neurofilament light chain, a biomarker of neurodegeneration (figure 1). A virome-wide serological screening found no association between multiple sclerosis and other viruses, indicating that people who develop disease do not have an altered susceptibility to viral infections in general.[13] Other risk factors could not have confounded the analysis since none has such a strong association with the disease.

Specifically, the strongest genetic risk factor for multiple sclerosis, the class II allele *HLA-DRB1*15:01*, increases the risk of developing multiple sclerosis by about three-fold in heterozygotes and by six-fold in homozygotes. Smoking habits, obesity in childhood or adolescence, and vitamin D insufficiency can double disease risk.[2,8]

Nonetheless, the low prevalence of multiple sclerosis (1–3 cases per 1000 people in North America and Europe) is difficult to reconcile with the ubiquity of EBV infection. As with other microbial agents, the biological effects of EBV might differ according to its genomic variability, which might be responsible for the different incidences of some EBV-associated diseases in different populations and geographical regions.[14] Multiple sclerosis might not be an exception; genetic variants of the EBV transcription factor EBNA-2, a master regulator of viral and cellular genes, have been associated with multiple sclerosis, but this finding requires confirmation in large cohorts from other geographical areas.[15]

EBV infection is necessary, but not sufficient to initiate multiple sclerosis

Gene–environment interactions could explain the discrepancy between the high prevalence of EBV infection worldwide and the relatively low prevalence of multiple sclerosis. Large-scale genome-wide association studies have identified more than 230 genetic variants that influence the risk of multiple sclerosis and implicate innate and adaptive immune cells in its pathogenesis.[16,17] This information can be used to identify disease-relevant environmental exposures (figure 1). Genomic and bioinformatic approaches suggest that, among the genes that predispose to multiple sclerosis, those interacting with EBV are significantly more represented than those interacting with other environmental risk factors.[18] A better knowledge of the interaction between EBV and disease-risk genes could then help to interpret the huge amount of data provided by genome-wide association studies, and to delineate gene modules associated with multiple sclerosis.[17,18] Their function could then be better understood thanks to the detailed knowledge about the capability of EBV for reprogramming the host B cell to support viral persistence during latent infection and the production of viral particles during lytic infection (figure 1). EBNA-2 is a major regulator of EBV gene expression and also regulates the concomitant expression of many host genes involved in B-cell growth and transformation during viral latency III. Numerous EBNA-2 binding sites are present throughout the human genome. Two studies that used a computational approach have demonstrated that EBNA-2 binding sites are present in a considerable proportion of multiple sclerosis risk loci, particularly in regulatory regions.[19,20] The overlap in multiple sclerosis risk loci between the binding sites of EBNA-2 and the vitamin D receptor suggests interactions between these risk factors in the transcriptional regulation of B cells.[19] Studies in EBV-infected B-cell lines indicate that multiple sclerosis risk alleles can alter EBNA-2 binding and EBV-induced gene expression, which might have consequences for B-cell growth and immune control of the virus.[21,22] Besides EBNA-2, other EBV proteins that function as regulators of B-cell transcriptional programmes (eg, EBNA-1) or as

Panel 1: The Epstein-Barr virus (EBV)

Epidemiology and serology

Infection with EBV is almost ubiquitous and generally occurs during childhood, when it is usually asymptomatic. If infection occurs in adolescence or later, it often causes infectious mononucleosis—a self-limiting lymphoproliferative disease— that is more common in countries with high socioeconomic status and better hygiene conditions. EBV serology is used to define the stage of infection: viral capsid antigen (VCA) IgM is produced during acute infection, while VCA IgG and EBNA antibodies persist for life and indicate previous infection.

Lifecycle of EBV

EBV is transmitted through saliva, lytically infects oropharyngeal epithelial cells, and then establishes a lifelong latent infection in human B cells.[4] In lymphoid tissue, EBV infection of resting B cells induces B-cell proliferation and transformation due to the expression of the complete set of viral latent transcripts, which includes EBV nuclear antigens (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, and EBNA-LP), latent membrane proteins (LMP-1 and LMP-2), and several non-coding RNAs (latency III or growth programme).

Then, EBV enters a restricted form of latency, in which only EBNA-1, LMP-1, LMP-2A, and LMP-2B are expressed (latency II). LMP-1 mimics CD40 and LMP-2A mimics B-cell receptor signalling. LMP-mediated survival of B cells promotes entry into the mature memory pool. EBNA-1 is essential for replication of the episomal EBV genome and is the only viral protein expressed in memory B cells during latency I. All EBV gene products are downregulated in circulating, resting memory B cells (latency 0), allowing the virus to elude immune surveillance. EBV can reactivate periodically in the tonsils, resulting in new infection of B cells and viral shedding into saliva. EBV reactivation is associated with expression of more than 80 lytic genes that encode proteins implicated in production of viral particles, immune evasion, and inhibition of apoptosis.[4]

Immune control of EBV

Efficient immune surveillance is essential to maintain EBV–host homoeostasis and avoid the development of EBV-associated cancers.[5] Early control of EBV infection involves the expansion of natural killer cells, cytotoxic CD8 T cells and, to a lesser extent, CD4 T cells. During persistent infection, memory CD4 T cells are present at low frequency and recognise mainly EBV latent proteins, whereas EBV-specific CD8 T cells are more frequent and expand vigorously in response to lytic proteins.[5]

Pathogenicity

EBV is causally linked to about 1·5% of human cancers, particularly Hodgkin lymphoma, Burkitt lymphoma, diffuse large B-cell lymphoma, primary CNS lymphoma, natural killer or T-cell lymphoma, and nasopharyngeal and gastric carcinomas.[4] EBV is the causative agent of infectious mononucleosis and chronic active EBV infection, a serious condition with persistence of infectious mononucleosis-like symptoms. Several rare primary immunodeficiencies compromising natural killer and cytotoxic T-cell function result in failure to control EBV infection and predispose to B-cell lymphomas, fulminant infectious mononucleosis, and haemophagocytic lymphohistiocytosis.[4,5] EBV-associated lymphoproliferative disease can develop in individuals receiving aggressive immunosuppressive therapies, like transplant recipients.

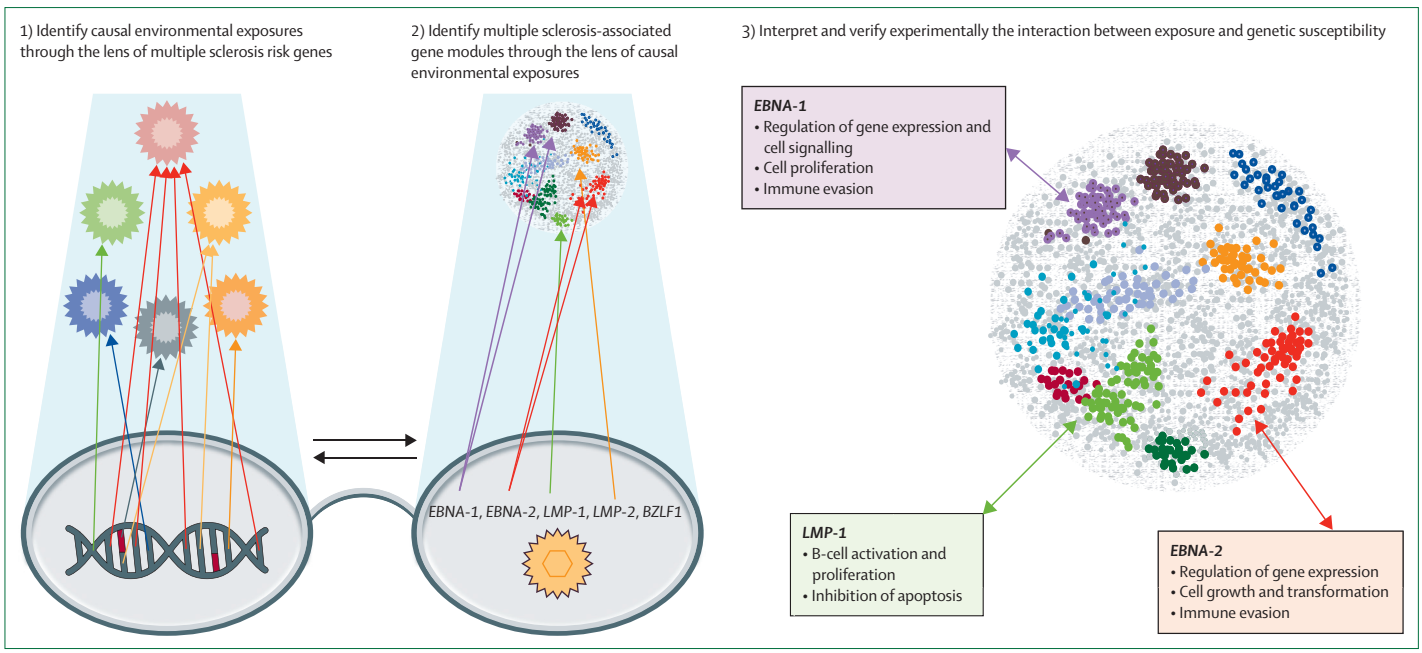

Figure 1: **A three-step approach to reveal the role of risk–environment interactions in pathogenesis**
(1) Disease-associated genetic variants identified in genome-wide association studies might be found to preferentially interact with one environmental factor. (2) The lead factor (ie, EBV) could then be used to identify gene modules that are relevant for pathophsiology. (3) Eventually, the interaction between multiple sclerosis genes and EBV can be verified experimentally. The three EBV latent genes shown in this part of the figure (*EBNA-1*, *EBNA-2*, and *LMP-1*) epitomise how knowledge on the ability of the virus to influence B cell biology could help with interpretation of the functional relevance of multiple sclerosis-associated gene modules. The transcription factor EBNA-1 is expressed in all stages of EBV latent infection (latency 0, I, II, and III) and regulates genes involved in purine metabolism that are essential for B-cell proliferation. The transcription factor EBNA-2 is expressed only in the initial phase of EBV infection (latency III) and promotes key metabolic pathways that support proliferation of newly infected B cells and regulates many cellular genes, including genes associated with multiple sclerosis and other autoimmune diseases. *LMP-1*, expressed in latency II and III, is a homologue of the multiple sclerosis gene *CD40* that is implicated in T cell-mediated B-cell activation. LMP-1 contained in small vescicles (exosomes) and released from EBV-infected B cells can influence the function of neighbouring and distant cells.[4]

constitutive, B-cell activating receptors (eg, LMP-1) need to be explored to fully understand how EBV exploits genetic susceptibility to orient B-cell function towards a multiple sclerosis phenotype.

Besides the effect of genetic variants on EBV reprogramming of B cells, increasing evidence suggests that genetic susceptibility can contribute to the development of multiple sclerosis by influencing the function of subsets of immune cells that control EBV infection. An interaction between HLA alleles and EBV infection is supported by studies showing that the risk of developing multiple sclerosis conferred by the class 2 allele *HLA-DRB1*15:01* is strikingly increased in the presence of high concentrations of EBNA-1 IgG and a history of infectious mononucleosis, and in the absence of the class 1 allele *HLA-A*02*, which confers protection from multiple sclerosis.[23] Experimental data in humanised mice reconstituted with immune system cells from *HLA-DRB1*15* (*HLA DR15*) or *HLA-DR4* donors suggest that *HLA-DRB1*15* might be less effective in presenting EBV antigens to CD4 T cells, resulting in reduced control of EBV infection.[24] In addition to their role in antigen presentation, class 2 HLA molecules, including *HLA-DRB1*15:01*, function as co-receptors for EBV entry into B cells.[25] *HLA-A*02* is one of the most frequent MHC class 1 alleles and is implicated in antigen presentation to cytotoxic CD8 T cells, which is required for the elimination of virus-infected cells. A variant of the perforin gene that reduces the amount of perforin, a pore-forming molecule essential for the elimination of virus-infected cells by cytotoxic T cells and natural killer cells, is associated with multiple sclerosis.[26] This variant is also implicated in late-onset familial type 2 haemophagocytic lymphohistiocytosis, a genetic disease characterised by uncontrolled immune activation driven by infections which, in many cases, are EBV infections.

Evidence is accumulating in support of the synergistic action of some modifiable risk factors and *HLA-DRB1*1501* or aspects of EBV infection (ie, high EBNA-1 IgG titres or previous infectious mononucleosis).[8,27] In genetically susceptible individuals, obesity and cigarette smoking might promote chronic, low-grade inflammatory states and modulate the anti-EBV immune response, conferring an excess risk for multiple sclerosis. Other factors influencing the immune system, such as early-life immune modulating exposures, including the gut microbiota or transient immunosuppression due to environmental stressful events or co-infections, might modify multiple sclerosis risk by affecting the immune control of EBV or directly inducing EBV reactivation.

Human herpesvirus-6 can transactivate EBV and human endogenous retroviruses, whereas an interaction

between a multiple sclerosis-associated human endogenous retrovirus and EBV has been implicated in the production of neurotoxic substances and induction of EBV replication.[28] The lower seroprevalence of cytomegalovirus in adult patients with multiple sclerosis (compared with age-matched healthy people), and in EBV-seropositive children who will later develop multiple sclerosis (compared with children with monophasic acquired demyelinating syndromes) points to a protective effect of cytomegalovirus infection, but its effect on the immune response to EBV remains to be investigated.[2,13,29]

Mechanisms linking EBV and multiple sclerosis pathology

Despite EBV infection being necessary for the initiaton of multiple sclerosis, the identification of the mechanisms linking the virus to CNS inflammation and neurodegeneration remains challenging. The most relevant connection between EBV and multiple sclerosis pathology involves the intertwining of the virus with B cells (panel 2). On the one hand, EBV exploits the B-cell machinery to propagate and establish a lifelong latent infection within its human host. On the other hand, persistent B-cell activation in the CNS is a diagnostic hallmark of multiple sclerosis, but its pathological significance, including its link to EBV infection, remains to be clarified. Despite knowledge gaps, the efficacy of B-cell-depleting anti-CD20 antibodies has unravelled a key role of B cells in pathogenesis, stimulating research on B-cell functions (eg, antigen presentation, T-cell activation, and cytokine production) as potential mechanisms driving disease activity.[34] Two main hypotheses are being investigated (figure 2): (1) that EBV acts as trigger of CNS-directed autoimmune responses; and (2) that EBV is the antigenic driver of multiple sclerosis through the activation of a detrimental antiviral immune response.

Molecular mimicry is a mechanism whereby infectious agents can induce autoimmunity. Sequence similarities between exogenous and self-antigens can induce autoreactive T-cell or B-cell responses in susceptible individuals. Multiple sclerosis is considered to be an autoimmune disease caused by loss of immune tolerance toward myelin proteins, but the list of autoantigens has grown to include proteins whose expression is not confined to the CNS. In support of EBV-mediated molecular mimicry, several studies have identified antibodies cross-recognising peptides from EBNA-1 and myelin basic protein or other proteins expressed in the CNS.[30,35,36] Intrathecally produced anti-EBV antibodies are part of the intrathecal immune response against various neurotropic viruses, and oligoclonal CSF IgG from patients with multiple sclerosis bind EBV proteins, including EBNA-1.[30] A recent study showed that a monoclonal antibody generated by a B-cell clone from the CSF of a patient with multiple sclerosis recognised EBNA-1 and GlialCAM, a protein expressed in glial cells.[36] Antibodies cross-recognising EBNA-1 and GliaCAM were also found in sera of some patients.[36] CD4 T cells cross-recognising peptides from EBNA-1 and myelin proteins were identified in patients with multiple sclerosis more frequently than in people from the healthy control group.[37] By use of an unbiased antigen-discovery approach

Panel 2: B-cell involvement in multiple sclerosis pathogenesis

CNS

Persistent intrathecal B-cell activation and immunoglobulin synthesis are typical pathological features of multiple sclerosis. More than 90% of patients with multiple sclerosis have a characteristic oligoclonal pattern of IgG in their CSF. Oligoclonal immunoglobulins are produced by a few B-cell clones that expand in the CNS and CSF. The specificity of intrathecally produced oligoclonal IgG is largely unknown, but some studies have reported recognition of EBV proteins by intrathecal oligoclonal bands.[30] Polyspecific immunoglobulins recognising common viruses (eg, rubella, measles, varicella zoster, or, less frequently, EBV) are also synthesised in the CNS of most patients, probably resulting from non-specific bystander B-cell activation.[30]

Mature B cells—mainly memory B cells and plasmablasts—are present in the CSF and CNS of patients with multiple sclerosis, but not in healthy individuals. B cells can organise into rudimental B-cell follicle-like structures in the subarachnoid space between the pial and arachnoid membranes. Intrameningeal lymphoid-like structures have been best characterised in post-mortem brain and spinal cord from patients with secondary progressive multiple sclerosis and are associated with cortical damage and more severe disease course.[31]

CNS-draining lymph nodes

Little information is available on B-cell abnormalities in deep cervical lymph nodes of patients with multiple sclerosis. These lymph nodes are probably the site where CNS-derived antigens activate pathogenic T-cell responses. Analysis of the B-cell receptor repertoire in CNS tissue, cervical lymph nodes, and peripheral blood of patients with multiple sclerosis has unravelled bidirectional trafficking of activated B cells between these compartments and preferential B-cell clonal expansion in deep cervical lymph nodes.[32,33]

Peripheral blood

No major differences in peripheral B-cell subsets, proportion, or phenotype have been reported between untreated patients with multiple sclerosis and healthy individuals. Ex vivo studies suggest that B cells from people with untreated multiple sclerosis display a more pro-inflammatory phenotype than B cells from healthy individuals when exposed to molecules mimicking infectious or inflammatory stimuli.[34] Most disease-modifying therapies for multiple sclerosis have marked effects on circulating B cells.

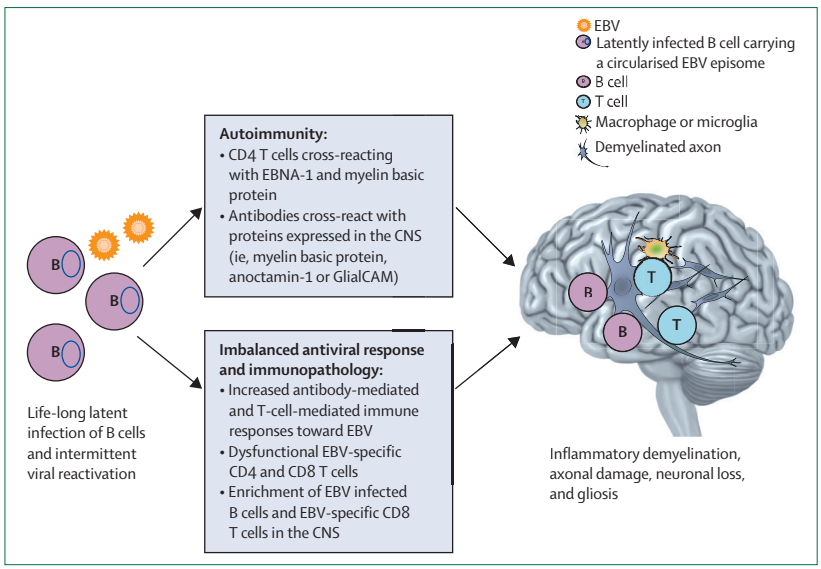

Figure 2: **Mechanisms linking EBV infection to multiple sclerosis**
Following infection of a susceptible individual who will later develop multiple sclerosis, EBV establishes a lifelong latent infection that could cause CNS pathology through two mechanisms, for which varying evidence exists. Due to structural similarities between EBV and some host proteins (molecular mimicry), EBV could break immunological tolerance and trigger self-perpetuating autoimmunity. This scenario is supported by studies showing that antibodies and CD4 T cells cross-recognising the EBV latent protein EBNA-1 and proteins expressed in the CNS are more frequently found in people with multiple sclerosis than in healthy people. In another scenario, altered control of EBV might favour the establishment of a dysregulated viral infection, possibly in the CNS and deep cervical lymph nodes, that stimulates an excessive antiviral immune response and immunopathology in the target organ. This scenario is supported by studies showing changes in anti-EBV immune reactivity in individuals with multiple sclerosis compared with healthy people. Dysregulated EBV infection in the CNS remains controversial. Further studies are needed to identify the antigenic drivers of the CNS-targeted immune response in multiple sclerosis. EBV=Epstein-Barr virus.

in *HLA-DR15*-positive individuals, a study identified autoreactive CD4 T cells that were activated by B cells and cross-reacted with the RAS guanyl releasing protein 2, an autoantigen expressed in B cells and neurons, and EBV and *Akkermansia*, a commensal gut bacterium associated with multiple sclerosis.[38] This activation of CD4 T cells might explain the link between genetic predisposition, B-cell antigen presentation, and EBV-induced molecular mimicry in people with multiple sclerosis. Other potential mechanisms involve induction of a putative autoantigen (alpha B crystallin) in EBV-infected B cells and stressed oligodendrocytes, and survival of autoantibody-producing EBV-infected B cells that migrate in the CNS, ultimately leading to stimulation of CNS-targeting autoreactive T cells.[30] Regardless of a viral trigger, it remains to be demonstrated whether autoimmunity to CNS antigens causes—or is the consequence of—tissue damage in people with multiple sclerosis. Whether B cells contribute to pathogenesis as sources of autoantibodies is also uncertain, since anti-CD20 therapies markedly reduce disease activity without affecting intrathecal and peripheral immunoglobulin titres.[34]

Alternatively, EBV could act as the antigenic driver of a cytotoxic T-cell response sustaining CNS inflammation.[39] EBV DNA load in CSF, peripheral blood, and saliva does not differ significantly between patients and controls, suggesting that EBV dysregulation might occur in other body compartments, probably CNS tissue and CNS-draining lymph nodes.[40,41] Migration of EBV-infected B cells into the CNS occurs in several clinical conditions, such as EBV encephalitis and primary CNS lymphoma, and experimental work suggests that EBV-induced epigenetic changes increase the propensity of infected B cells to migrate into the CNS.[42] Due to inadequate immune control in susceptible individuals, EBV might establish a persistent infection in the CNS and stimulate a chronic immunopathological response, causing collateral demyelination and neurodegeneration. This hypothesis remains controversial due to the discrepant results obtained in different studies. Although several research groups have reported absence or paucity of EBV-infected B-lineage cells in post-mortem brain samples from patients with multiple sclerosis,[39,43] other studies showed accumulation of EBV latently and lytically infected B cells,[44–46] particularly within meningeal B-cell follicles,[44] and CD8 T cells recognising EBV peptides or EBV-positive B-cell lines.[47,48] The presence of EBV lytic markers in brain samples from patients with more severe disease suggests a link between viral reactivation and immune-mediated CNS inflammation.[39,44,49] The compatibility of this hypothesis with typical pathological features, including persistent intrathecal B-cell activation, lesion reactivation, and predominance of cytotoxic CD8 T cells in brain lesions, should stimulate research efforts aimed at its refutation or confirmation.[50]

EBV involvement in multiple sclerosis onset and progression

The clarification of whether EBV's role in multiple sclerosis is limited to early disease or extends to ongoing disease is important to support the rationale for therapies that directly target EBV. There is no evidence to suggest that EBV involvement in multiple sclerosis subsides after disease onset. Furthermore, it is plausible that the events triggering disease onset might be the same as those sustaining disease chronicity. However, the evidence is still insufficient to link EBV status or anti-EBV immunity to disease course.

Most studies have reported none or weak associations between EBV DNA load in peripheral blood cells, plasma, or saliva and relapse, disability, progression in disability, and lesion number or volume.[30,41] EBV serology is the best available biomarker of viral dysregulation in multiple sclerosis. Compared with healthy people, people with multiple sclerosis or clinically isolated syndrome (a condition suggestive of multiple sclerosis) have increased EBNA-1 IgG titres before disease onset; high concentrations, with no remarkable changes, have been also reported during the course of the disease. In the largest 2–5-year follow-up studies, no associations were found between EBNA-1 and VCA IgG antibody titres and conversion from clinically isolated syndrome to definite multiple sclerosis, disease progression, or clinical and

radiological activity.[10,41,51,52] In smaller follow-up studies, a correlation was found between anti-EBV antibody titres and cortical atrophy and lesion load, and disease activity on brain MRI.[30,53] These findings require confirmation in independent larger studies.

Regarding the cellular immune response against EBV, EBV-specific CD4 and CD8 T cells have been shown to be more numerous and recognise a wider array of antigenic epitopes from latent EBNA-1 and primarily EBV lytic proteins, respectively, in the peripheral blood of patients with clinically isolated syndrome or relapsing-remitting multiple sclerosis than in control-group participants.[39,54–56] In some studies, expansion of EBV-specific T cells has been associated with clinical and radiological disease activity.[57–59] However, there is also evidence that EBV-specific T cells display reduced cytotoxicity, proliferation, and cytokine release in patients with multiple sclerosis and a decrease in number with disease duration, which could be explained in part by immune exhaustion induced by a persistent viral infection.[55,59,60] Some studies have provided evidence for enrichment of CD8 T cells recognising EBV, mainly the viral lytic proteins, but not other common viruses or putative CNS autoantigens, and for the presence of activated EBV-specific CD8 T cells in the CSF of patients with multiple sclerosis, compared with control-group participants.[56,61–63] These findings suggest that migration of EBV-specific CD8 T cells into the CNS might be stimulated by an ineradicable intracerebral EBV infection, but better characterisation of the antigenic specificity and functional phenotype of reactivated T cells in the CNS is needed. Unbiased techniques, such as single-cell transcriptomics and T-cell receptor sequencing coupled with high dimensional flow cytometry, will be instrumental in sketching the landscape of lymphocyte populations in the CNS, CSF, and peripheral blood.[64]

Effects of pharmacological therapies on EBV

Understanding whether disease-modifying therapies interfere with EBV infection or the anti-EBV immune response could help verify whether EBV is needed after disease onset and, hence, could advance therapeutics, even in the absence of specific anti-EBV treatments. EBV persists in the host through virus-driven expansion of latently infected B cells. Remarkably, many disease-modifying therapies target the EBV reservoir or other aspects of the EBV lifecycle (table 1).

Anti-CD20 monoclonal antibodies deplete all CD20-positive B-cell subsets, implying that the B-lymphocyte population harbours key effectors in multiple sclerosis pathogenesis.[34,71] Memory B cells, the main reservoir for EBV, get depleted along with other CD20-positive B-cell subsets, reinforcing the idea that lowering EBV load could have an effect on pathophysiology. A consequence of reducing EBV load in patients receiving treatment with ocrelizumab is a decrease in serum anti-EBV antibodies, and possibly in anti-EBV T-cell response.[73,74] The poor penetration of anti-CD20 antibodies in the CNS indicates that these drugs mainly eliminate a reservoir of pathogenic peripheral B cells that are prone to migrate to the CNS or exert a pro-inflammatory function in lymphoid tissues (probably CNS-draining lymph nodes).[32,33,39] In line with this effect on peripheral B cells, the results of exploratory trials assessing the clinical and immunological effects of intrathecally administered rituximab, another anti-CD20 monoclonal antibody, have not been encouraging.[78,79] Regarding trafficking of EBV-infected B cells and EBV-specific T cells to the CNS, massive EBV reactivation and cytotoxic activity were observed in post-mortem brain samples from two fulminant cases of multiple sclerosis after withdrawal of natalizumab, a drug that blocks entry of circulating lymphocytes into the CNS.[49,80]

Other disease-modifying therapies, such as cladribine and alemtuzumab, deplete B cells in addition to other lymphocyte populations, with long-lasting reduction of memory B cells, and possibly EBV.[34,71] Among disease-modifying therapies that can directly interfere with EBV biology, type 1 interferons (beta interferons, widely used as first-line treatment) are antiviral cytokines that inhibit viral replication and orchestrate the antiviral immune response. Notably, a defective type 1 interferon response has been documented in people with multiple sclerosis.[81] Cladribine interferes with DNA synthesis and cell proliferation through mechanisms that involve resistance to degradation by adenosine deaminase, a detoxifying enzyme that prevents apoptosis of proliferating cells and is induced by EBNA-1 in EBV-transformed B cells.[72] Molecular docking studies have shown that teriflunomide and the S1P agonists ozanimod and siponimod bind with high affinity to EBNA-1, an EBV-encoded DNA-binding protein that maintains the EBV episomal genome and is crucial for EBV replication.[68] Proliferation of EBV-transformed B cells and EBV reactivation are inhibited by teriflunomide.[69] New drugs that could target B cells and EBV infection within the CNS are Bruton's tyrosine kinase (BTK) inhibitors, small molecules that cross the blood-brain barrier and specifically target B cells and myeloid cells.[82] BTK inhibitors do not deplete cells, but interfere with BTK, a key enzyme involved in signalling from the B-cell receptor and Fc receptors in B cells and myeloid cells resulting in their activation.[82] Several BTK inhibitors are being tested in late-stage clinical trials (NCT04742400, NCT03889639, NCT02975349, NCT04410991, NCT04410978, NCT04458051, NCT04411641).[75] BTK inhibitors block activation of the B-cell receptor pathway, which induces EBV lytic gene expression in EBV-transformed B cells, and could therefore interfere with LMP-2A, an EBV mimic of the B-cell receptor.[77]

New EBV-targeting treatments in multiple sclerosis

Trials that specifically target EBV should clarify whether lowering viral load reduces antigenic stimuli driving

	Effect on memory B cells	Effect on EBV life cycle	Effect on anti-EBV immunity
Interferon beta	Induces apoptosis in memory B cells, reduces B-cell count, and decreases EBV gene expression in peripheral blood[65]	Binds to the interferon receptor and activates a signal cascade that results in the transcriptional induction of hundreds of antiviral genes, inhibiting viral replication and stimulating antiviral innate and adaptive immune responses; inhibits EBV entry and proliferation of EBV-transformed B cells[66]	No effect on serum levels of EBNA1 IgG;[67] reduces or has no effect on EBV-specific CD8 T-cell response[56,57]
Glatiramer acetate	Reduces circulating memory B cell count[34,60]	Unknown	Increases the number of circulating EBV-specific CD8 T cells[60]
Teriflunomide	Mild lymphopenia with modest reduction of circulating B cells[34]	Binds to EBNA-1 with high affinity;[68] inhibits EBV lytic infection and viral DNA replication[69]	Reduces serum EBNA-1 and VCA IgG levels[70]
Dimethyl fumarate	Variable degree of lymphopenia, with marked reduction of circulating memory B cells[34]	Binds to EBNA-1 with low affinity[68]	Not reported
Sphingosine 1-phosphate receptor modulators*	Reduces lymphocyte counts, with marked reduction of circulating B cells, mainly memory B cells[34]	Stable, high-affinity binding of siponimod and ozanimod to EBNA-1, which could make EBNA-1 unavailable for DNA interaction[68]	Not reported
Cladribine	Reduces lymphocyte counts with marked and persistent reduction of circulating B cells, mainly in the memory subset[34,71]	Cladribine is a purine analogue that is resistant to degradation by adenosine deaminase, which is a direct target of the EBV-directed immortalisation process[72]	Not reported
Anti-CD20 monoclonal antibodies†	Reduces B cells in CSF and causes marked, long-term depletion of peripheral B cells[34,71]	Decline in EBV DNA load in peripheral blood during ocrelizumab treatment[73]	Reduces serum EBNA-1 IgG levels;[74] reduces or has no effect on cytotoxic T-cell response toward EBV in the peripheral blood[56,73]
Alemtuzumab	Marked decrease of all lymphocyte populations, with marked long-term depletion of circulating memory B cells[34]	Not reported	Not reported
Natalizumab	Reduces leukocyte count and numbers of T cells, B cells, and plasma cells in CSF; increases lymphocyte count in peripheral blood, particularly for B cells and natural killer cells, and pro-inflammatory B cells[34]	Not reported	Increases EBV-specific CD8 T-cell response in peripheral blood[56,57]
Bruton's tyrosine kinase inhibitors[75]	Not yet tested in patients; a study in healthy volunteers shows a reduction in B-cell metabolism and expression of co-stimulatory molecules, with a shift to an anti-inflammatory B-cell phenotype[76]	Blockade of B-cell receptor-mediated activation of the EBV lytic cycle[77]	Not reported

EBV=Epstein-Barr virus. EBNA-1=Epstein-Barr virus nuclear antigen 1. VCA=Viral caspid antigen. *Fingolimod, siponimod, or ozanimod. †Rituximab, ocrelizumab, or ofatumumab.

Table 1: **Effects of disease-modifying therapies for multiple sclerosis on B cells, EBV, and anti-EBV immunity**

pathogenic immunity and disease activity. Some therapies used for viral infections or EBV-associated cancers are now being tested in exploratory clinical trials in patients with multiple sclerosis (table 2).

All licensed antivirals act by inhibiting viral replication. Antiviral drugs used to treat herpes viruses and other viral infections, such as aciclovir, valaciclovir, and ganciclovir, also inhibit EBV replication; however, their clinical efficacy in acute and chronic EBV-associated diseases is unclear.[83] In phase 2 trials, neither aciclovir nor valaciclovir significantly affected disease activity or disability progression in patients with relapsing-remitting multiple sclerosis, although a reduction in number of clinical relapses and new lesions was observed in patients with high relapse rates.[84] A clinical trial of famciclovir, an antiviral used to treat several herpesvirus infections, has recently started.

Molecules that target EBV latent infection, such as EBNA-1 inhibitors, have been developed to treat EBV-associated cancers.[85] EBNA-1 is essential for viral DNA replication during latency and inhibition of its binding to cellular DNA can lead to tumour cell death. A small molecule EBNA-1 inhibitor, VK-2019, is being tested in patients with EBV-associated cancers. Ongoing research efforts entail different approaches to interfere with EBV latency or reactivation,[86,87] and progress in this field could also benefit multiple sclerosis therapy. However, as for other viral infections (eg, hepatitis C virus), the path leading to safe and effective therapy for EBV-associated diseases, including multiple sclerosis, could take years, until directly acting antivirals are developed.

Adoptive T-cell immunotherapy (infusion of in vitro generated EBV-specific cytotoxic T lymphocytes) has been developed for the treatment of EBV-associated

	ClinicalTrials.gov identifier, status	Phase	Condition	Primary outcome
Antiviral drug				
Famciclovir	NCT05283551, recruiting	2	Definite multiple sclerosis	Quantification of EBV shedding in saliva; quantification of serum levels of anti-EBV antibodies and EBV replication in peripheral blood
Adoptive cell therapy using allogenic EBV-specific cytotoxic T lymphocytes	NCT03283826, active, not recruiting	1/2	Primary and secondary progressive multiple sclerosis	Safety and clinical (expanded disability status scale) improvement
Adoptive cell therapy using autologous EBV-specific cytotoxic T lymphocytes	NCT02912897, recruiting	1	Clinically isolated syndrome	Evaluation of treatment-related adverse events as assessed by CTCAE
EBNA-1 inhibitor VK-2019	NCT04925544, recruiting	2	Patients with EBV positive nasopharyngeal cancer or other EBV-associated cancers	Response rate using RECIST
Prophylactic EBV vaccine				
EBV multimeric RNA vaccine	NCT05164094, recruiting	1	Healthy adults aged 18–30 years with or without previous EBV infection	Evaluation of immunogenicity, adverse events, and laboratory abnormalities
Adjuvanted EBV gp350-ferritin vaccine	NCT04645147, recruiting	1	Healthy adults aged 18–29 years with or without previous EBV infection	Evaluation of immunogenicity, adverse events, and antibody response to EBV
Therapeutic vaccine				
Bacillus Calmette-Guérin vaccine	NCT03888924, recruiting	2	Radiologically isolated syndrome	MRI evaluation of the cumulative number of new gadolinium T1-weighted lesions and non-enhancing new and newly enlarging T2-weighted lesions

CTCAE=Common Terminology Criteria for Adverse Events. RECIST=Response Evaluation Criteria in Solid Tumors. EBNA-1=Epstein-Barr virus nuclear antigen 1.

Table 2: **Ongoing clinical trials of EBV-targeting drugs and vaccines**

cancers, aiming to boost anti-EBV immunity and eliminate EBV-positive tumour cells. The effects of adoptively transferred autologous EBV-specific cytotoxic T cells targeting EBV latent proteins have been tested in small numbers of patients with progressive multiple sclerosis.[88] The results of these early studies suggest mild reduction of some symptoms, which needs to be confirmed in larger studies. Two early phase trials of adoptive immunotherapy using autologous or allogenic EBV-specific T cells are ongoing in patients with clinically isolated syndrome or progressive multiple sclerosis, respectively. Choice of target antigens, survival, and trafficking of the transferred T cells into CNS compartments could be limiting factors dampening therapeutic efficacy.

Anti-EBV immunity could be boosted with vaccines, aiming to promote virus-specific CD4 and CD8 cytotoxic responses with anti-tumour activity.[89] Immune checkpoint inhibitors are licensed for cancer therapy and have been proposed for the treatment of virus-associated cancers, including EBV-positive primary CNS lymphomas.[90] However, evidence is mounting that these drugs can induce or aggravate multiple sclerosis in some individuals, suggesting that the mechanisms through which they promote protective anti-tumour immunity might amplify immune dysfunction in multiple sclerosis.[91]

Multiple sclerosis prevention

As for other diseases with a viral cause, the most promising intervention for multiple sclerosis is primary prevention through vaccination (figure 3). The two potential outcomes of a prophylactic EBV vaccine are induction of sterilising immunity that prevents infection or non-sterilising immunity that does not prevent infection, but reduces the risk of developing the disease. Even in the absence of sterilising immunity, an effective vaccine could limit EBV dissemination, prevent excessive immune system activation, and reduce viral load during primary infection, which are all factors potentially contributing to the development of EBV-associated diseases.[92] A prophylactic EBV vaccine should therefore be effective in reducing the incidence of multiple sclerosis.[93]

EBV vaccines are being tested in clinical trials. These vaccines have been designed to target the viral glycoproteins that mediate EBV entry into B cells and epithelial cells.[94] Early EBV vaccines targeted gp350, the most abundant glycoprotein expressed on the viral envelope and on infected B cells, and the major target of antibodies blocking EBV infection of B cells. In an EBV vaccine trial, EBV-seronegative students received soluble EBV gp350 or placebo; the anti-gp350 EBV vaccine did not reduce EBV infection, but reduced the incidence of infectious mononucleosis by about 80%.[95] Since 2019, prophylactic vaccines against EBV have been effective in inducing

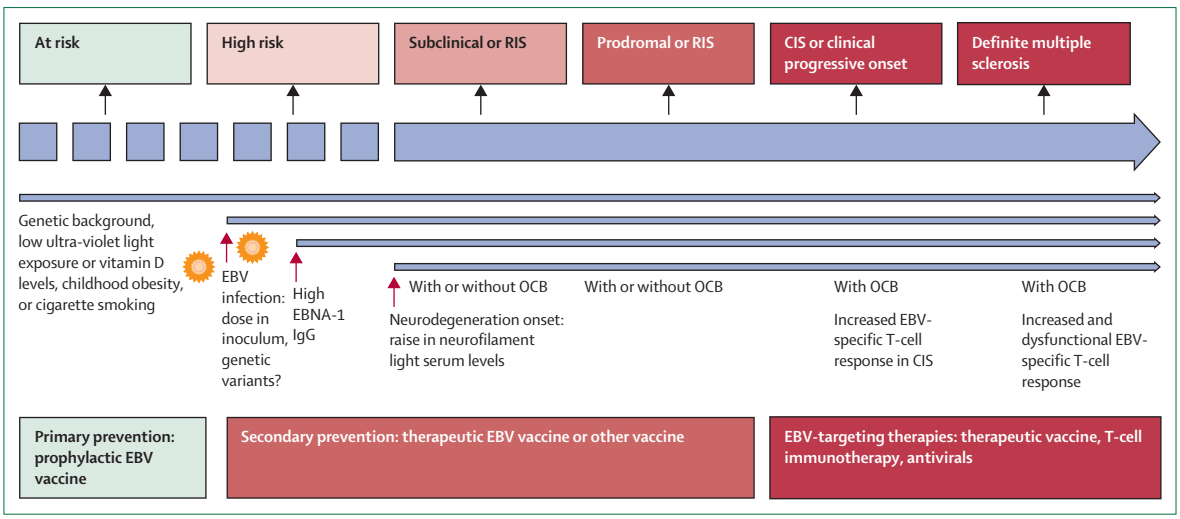

Figure 3: **Multiple sclerosis stages and EBV infection: therapeutic opportunities**
EBV infection greatly increases the risk of multiple sclerosis. However, dose of virus in the initial inoculum and viral genetic variants might modify the effect of EBV infection on the immune system. Genetic susceptibility, age-related and sex-related factors, environmental exposures, and lifestyle habits with a probable major effect in adolescence (eg, low ultra-violet light exposure or vitamin D levels, or childhood or adolescent obesity), or later in life (eg, cigarette smoking) can influence the functionality of the immune system and the immune response to EBV.[2] EBV infection (left red arrow) is followed by the appearance of serum EBNA-1 IgG (middle red arrow), which strongly correlates with high risk of disease. Subsequent increase in serum concentrations of neurofilament light chain (right red arrow) marks the onset of neurodegeneration in the CNS.[13] These biological changes can occur during the subclinical stage of multiple sclerosis and persist during the following stages that lead to overt disease. OCB in the CSF are a sign of intrathecal B-cell activation. OCB might appear during the subclinical and prodromal stages of multiple sclerosis, are present in about 70% of patients with CIS and 90% of patients with multiple sclerosis, and persist throughout the disease course. So far, the EBV-specific T-cell response has been investigated only in patients with CIS or definite multiple sclerosis. The bottom portion of the figure shows EBV-targeted interventions whose efficacy could be investigated before EBV infection and in the different multiple sclerosis stages. RIS=radiologically isolated syndrome. OCB=oligoclonal immunoglobulin bands. CIS=clinically isolated syndrome.

high titres of neutralising antibodies to multiple EBV glycoproteins (bivalent EBV gp350-targeting nanoparticle vaccine with gH/gL or gH/gL/gp42 complex) that block EBV infection of both B cells and epithelial cells.[96] Trials of the multivalent mRNA vaccine, including gp350/gH/gL/gp42, and of the EBV gp350 nanoparticle vaccine started in 2022, the primary outcome being the evaluation of safety and reactogenicity (table 2).

The risk of infectious mononucleosis, and possibly also of multiple sclerosis, is increasing in high-income countries, probably due to older age at infection and changes in lifestyle, hygiene, and social conditions.[92] The efficacy of a vaccine that prevents infectious mononucleosis in EBV-seronegative adolescents and young adults can be assessed in a relatively short time; however, its efficacy for preventing diseases with clinical onset years or even decades after EBV infection, such as multiple sclerosis or EBV-associated cancers, is challenging and requires very long studies. Other concerns relate to failure to induce lifelong immunity, the potential need for booster vaccines, and the postponement of primary infection at an older age, with the risk of developing infectious mononucleosis and subsequent multiple sclerosis.[93]

As for other neurological diseases, emerging evidence indicates that a prodromal phase exists in multiple sclerosis, raising the possibility of secondary intervention to prevent or delay progression. Criteria to reliably identify individuals with prodromal multiple sclerosis are not available, but recent evidence from epidemiological, immunological, and MRI studies disclose the possibility of detecting markers of CNS damage and immune dysfunction at this early phase.[97] These biomarkers might include serum neurofilament light chain, EBV serology, and radiological and CSF abnormalities.[13,97]

Concerning secondary prevention, it has been proposed that the Bacillus Calmette-Guérin (BCG) vaccine might provide protection against autoimmune diseases, such as multiple sclerosis and type 1 diabetes, due to its immune modulating activity.[98] BCG is a potent stimulator of trained innate immunity, a recently discovered form of innate immune memory, which confers protection toward subsequent infections.[99] A BCG vaccine trial in patients with radiologically isolated syndrome, a condition in which white matter lesions are detected on MRI scans in individuals without a history of a clinical demyelinating attack or alternative cause, is ongoing (table 2).

Another strategy to intercept subclinical and prodromal multiple sclerosis is by monitoring cohorts of children and adolescents with infectious mononucleosis in search of serological and radiological abnormalities suggestive of multiple sclerosis.[100] Follow-up of paediatric populations at increased risk of multiple sclerosis should increase the likelihood of identifying children at early disease stage in whom secondary prevention can stop or delay disease development.

Search strategy and selection criteria

We identified references by searching PubMed and from relevant articles. We used the search terms "multiple sclerosis" or "clinically isolated syndrome", and "herpesvirus", "Epstein-Barr virus", "therapy", or "vaccine", without language restrictions. The final reference list includes articles selected on the basis of relevance to this Review and published between Jan 1, 2016, and Oct 31, 2022. We included references published before 2016 if deemed relevant to the scope of our Review.

Conclusions and future directions

The establishment of EBV as a cause of multiple sclerosis has spurred interest in the mechanistic connections between viral exposure and disease. Within this framework, technological advances in the fields of viral and human molecular genetics, genomics, and immunology are expected to provide a deeper understanding of how EBV initiates disease. Large-scale genomic sequencing of EBV to search for pathogenic EBV variants associated with multiple sclerosis, and the study of the interactions between EBV and human genetics, will lead to a better understanding of EBV-induced disease mechanisms. Pinpointing changes in EBV load and anti-EBV immunity during treatment with different disease-modifying therapies will be important for the identification of infection-related disease biomarkers with predictive value.

Clarifying how EBV causes multiple sclerosis—either by autoimmunity (via molecular mimicry) or defective control of EBV infection (due to host factors or viral genetic variants)—is essential for identifying new therapeutic approaches. Although prophylactic EBV vaccines are a great hope for preventing multiple sclerosis, more immediate progress in preventing or delaying disease could be driven by improved criteria for prodromal multiple sclerosis and evidence from trials with antiviral drugs.

Contributors

All authors contributed to the writing of the original draft and the editing of the final manuscript. FA and MS created the figures and tables.

Declaration of interests

GG declares consulting or speaker fees from AbbVie, Aslan, Atara Bio, Biogen, Bristol Myers Squibb–Celgene, GlaxoSmithKline, GW Pharma, Janssen–Actelion, Japanese Tobacco, Jazz Pharmaceuticals, LifNano, Merck & Co, Merck KGaA–EMD Serono, Moderna, Novartis, Sanofi Genzyme, Roche-Genentech, and Teva Pharmaceuticals. MS declares speaking honoraria, research support from, and participation on an advisory board for Biogen, Bristol Myers Squibb, Merck, Novartis, Sanofi, Hoffmann-La Roche, and Viatris, and a patent: Epstein-Barr virus genotypic variants and uses thereof as risk predictors, biomarkers and theraputic targets of multiple sclerosis. FA declares participation on an advisory board for Hoffmann-La Roche.

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