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Epstein–Barr virus as a leading cause of multiple sclerosis: mechanisms and implications

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Abstract | Epidemiological studies have provided compelling evidence that multiple sclerosis (MS) is a rare complication of infection with the Epstein–Barr virus (EBV), a herpesvirus that infects more than 90% of the global population. This link was long suspected because the risk of MS increases markedly after infectious mononucleosis (symptomatic primary EBV infection) and with high titres of antibodies to specific EBV antigens. However, it was not until 2022 that a longitudinal study demonstrated that MS risk is minimal in individuals who are not infected with EBV and that it increases over 30-fold following EBV infection. Over the past few years, a number of studies have provided clues on underlying mechanisms, which might help us to develop more targeted treatments for MS. In this Review, we will discuss the evidence linking EBV to the development of MS and the mechanisms by which the virus is thought to cause the disease.

Furthermore, we will discuss the implications for the treatment and prevention of MS, including the use of antivirals and vaccines.

[H1] Introduction

Infections have long been suspected to have a role in multiple sclerosis (MS), a chronic inflammatory and neurodegenerative disease affecting young adults. In one of the earliest hypotheses proposed after MS was defined as a disease in 1868, Pierre Marie, one of Jean-Martin Charcot's former students, argued that the disease is caused by an infection and that it one day could be prevented by a suitable vaccine [1,2]. The hypothesis of an infective origin of MS is compelling, as it could explain several aspects of MS epidemiology, such as the geographical variation in incidence in different parts of the world [3], the change in risk that occurs with migration [4], and the sudden increase in incidence in some areas where MS incidence was low or where the disease had not previously been detected [5-7].

Over the past century, numerous viruses and other infectious agents have been implicated in MS, but the results from these early studies were inconclusive. However, in the 1980s, several studies found higher prevalence and titres of antibodies to the Epstein–Barr virus (EBV) in patients with MS compared with healthy controls [8-10]. Since then, strong and consistent evidence has emerged to indicate that EBV has an important role in the development of MS. Studies have linked both infectious mononucleosis (IM) [11] — a symptomatic primary EBV infection — and high titres of EBV-specific antibodies tto increased MS risk [12-15].This association is present even when the primary infection occurred decades before the first neurological symptoms [12,16]. The observation that almost all patients with MS have previously been infected with EBV adds further support

to the association between EBV and MS[17-19]. Most recently, in a cohort of individuals who were EBV-negative at baseline and were followed up longitudinally, the risk of MS increased 32-fold after EBV infection [20], providing compelling evidence of a causal link. In this Review, we provide a brief introduction to EBV and discuss evidence from epidemiological studies indicating that MS is a rare complication of EBV infection. Furthermore, we review the proposed underlying mechanisms of the EBV–MS association and discuss how the interplay between the virus and the immune system can contribute to the development of MS. Finally, we discuss the implications of these findings and how they could lead to the development of EBV-targeted MS treatments and preventative vaccines.

[H1] EBV infection

EBV is a double-stranded DNA virus belonging to the herpesvirus family and was the first virus shown to cause cancer in humans. This discovery was made in the 1960s when virus particles were found in cultured cells from rapidly growing tumours around the jawbones of children from specific regions of Africa[21,22]. Since then, EBV has been detected in all parts of the world, infecting more than 90% of the population, mostly during the first two decades of life [23]. The virus is transmitted primarily by saliva and infects B cells in the oral cavity, either directly or via EBV-infected oropharyngeal epithelial cells [24]. After infection, the virus establishes latency, wherein the expression of viral proteins is markedly lowered in comparison with active infection, thereby reducing recognition of infected B cells by cytotoxic T cells [24]. EBV can exhibit different types of latency; in the more restrictive patterns, only proteins essential for EBV are expressed, including

Epstein–Barr nuclear antigen 1 (EBNA1), which is needed for viral genome maintenance [25].

EBV persists in B cells for life, where it intermittently reactivates, enabling transmission of the virus to a new host. Primary infection during early childhood is usually asymptomatic, but up to 50% of individuals who are first infected during adolescence or adulthood develop IM [26]. As an oncogenic virus, EBV is known to cause several types of malignancies, including B-cell lymphomas (for example, Burkitt lymphoma and Hodgkin lymphoma), T cell and natural killer cell lymphoproliferative disorders, and epithelial malignancies (for example, nasopharyngeal carcinoma and gastric cancer). In addition, EBV has been linked to diseases that are thought to have an autoimmune component, such as systemic lupus erythematosus, Sjögren syndrome and MS [27,28].

[H1] Evidence of EBV involvement in MS

[H2] The link between IM and MS

EBV was first linked to MS more than 40 years ago when the disease was hypothesized to be a sequela of IM resulting from EBV infection during adolescence and early adulthood [29]. Since then, IM has consistently been associated with an elevated risk of MS. In a meta-analysis, results from both case–control and cohort studies showed that IM is associated with a twofold to threefold increased risk of MS [11]. The association seems to be specific for IM, as infection with other common viruses was not associated with MS risk [30]. Although one study reported that a higher number of hospital admissions related to bacterial infections during adolescence was associated with a higher MS risk, the association was modest, especially after individuals with a history of IM were excluded [31].

One hypothesis is that IM and MS are not causally related but could share the same aetiology. Factors associated with poor sanitary conditions, such as low socioeconomic status and crowded household conditions, are associated with an increased risk of primary EBV infection during childhood [32] and, therefore, a reduced risk of IM. The inverse relationship between infectious and immune disorders, including MS, has been linked to the hygiene hypothesis. This hypothesis proposes that high standards of sanitation during childhood affect the balance between immune cell populations and increase the risk of autoimmune diseases [33]. If IM and MS share causes (for example, good sanitary conditions), then the association between these conditions could reflect confounding bias rather than a causal effect of IM on MS risk. Paradoxically however, the results of a number of cross-sectional studies suggested that the risk of MS in EBV-seronegative individuals is extremely low [34].This finding does not support high levels of hygiene as the explanation for the link between IM and MS and is consistent with results from analyses comparing MS risk within families. IM was found to be a strong risk factor for MS in siblings who were discordant for IM [35]. The low MS risk in EBV-seronegative individuals and the increased MS risk following IM together supported the hypothesis that EBV infection causes MS. A rigorous and definitive demonstration that EBV-seronegative individuals had a low MS risk until after EBV infection was provided recently (Box 1).

[H2] Temporal association of EBV infection and MS

The temporal relationship between EBV and MS was addressed in a 2022 study of 35 individuals who were EBV-negative at baseline but later developed MS. Repeated serum samples to determine EBV status were taken over the course of the study[20]. All but one

of the participants became infected with EBV before developing the first neurological symptoms of MS. A subset of participants also underwent repeated measures of neurofilament light chain (NfL), a sensitive marker of neuroaxonal damage[36], serum levels of which can increase years before the first MS symptoms occur[37,38] (**Figure 1**). Results from these individuals showed that EBV infection also preceded elevations in this biomarker[20]. These results show that EBV infection precedes both the first neurological symptoms and early signs of neuroaxonal damage during the preclinical phase of MS. Therefore, EBV is unlikely to be a consequence of MS.

Cytomegalovirus, another virus that causes IM, shares similarities with EBV, including the mode of transmission and associations with socioeconomic status [39,40]. For these reasons, cytomegalovirus was used as a negative control in one study to investigate the association between IM and MS risk [41]. Cytomegalovirus was not associated with an increased MS risk, which is consistent with previous observations[42]. Furthermore, a virome-wide screening revealed that only antibody responses to EBV antigens showed enrichment in individuals who later developed MS [20]. These observations do not support the hypothesis that the EBV–MS link is explained by an enhanced vulnerability to viral infections preceding the clinical onset of MS. In analyses comparing EBV infection over time in individuals with MS and age-matched and sex-matched controls, EBV was associated with a 32-fold increased risk of MS. The strength of the association suggests that the results cannot be explained by unmeasured confounders [43].

[H2] A rare complication of a ubiquitous virus

If MS is a complication of EBV infection, one might ask why MS is not more prevalent given that more than 90% of the population is infected with the virus. However, diseases developing as rare complications after viral infections seem to be the rule rather than the exception. For example, only a small subset of individuals infected with EBV will develop EBV-associated cancers, even though EBV is known to be a causal factor [28]. Similar phenomena have been reported for infections with other viruses, such as the link between polio and poliomyelitis [44] and between human papillomavirus and cervical cancer [45]. In these examples, the causal relationship between the viral infection and the disease has been established, but only a small proportion of those infected with the virus develop the disease. These considerations do not , however, preclude the existence of EBV variants that are more prone to cause MS [46].

[H2] EBV interaction with other MS risk factors

The consistent associations between MS risk and environmental factors such as vitamin D deficiency [47,48], smoking [49,50], and obesity during childhood[51] or adolescence [52] suggest that these factors might modulate the risk of MS after EBV infection (**Figure 1**). Additional arguments for a role for other risk factors have come from migration studies [53] and the dramatic changes in MS epidemiology over the past few decades. Examples include the disappearance of the latitude gradient within the USA [54,55] and the increasing incidence in African Americans[56] and women [57]. Higher age at EBV infection could have contributed to some of these changes but is unlikely to provide a full explanation.

Evidence exists for interactions between EBV and other risk factors for MS. Several studies have found interactions between EBNA1 antibody titres and *HLA*-

*DRB1*1501* [17], the strongest and most consistent genetic risk factor for MS [58]. In addition, EBNA2, an EBV viral protein involved in growth transformation of B cells [25], binds to genetic MS susceptibility sites more frequently than expected by chance [59,60]. Also, considerable overlap has been observed between EBNA2 and vitamin D receptor binding sites [59], which could provide clues to how vitamin D deficiency modulates the association between EBV and MS risk. Furthermore, genetic risk factors for MS may be involved in a dysregulated response to EBV infection [61]. Finally, most of the risk factors consistently associated with MS, including vitamin D deficiency, smoking and obesity, affect pathways involved in inflammation [62,63]. These factors could, therefore, modulate the immune response to the primary EBV infection or the lifelong chronic infection that follows via inflammatory mechanisms.

[H1] Mechanisms underlying EBV-related MS risk

[H2] Epidemiological clues

[H3] Elevated titres of antibodies to EBV antigens. Among apparently healthy individuals who are infected with EBV, the strongest marker of future MS risk is the titre of serum antibodies to EBV latent antigens. In a large prospective study, high levels of anti-EBNA complex antibodies measured by indirect immunofluorescence[64] predicted a 36-fold increased MS risk [14]. Anti-EBNA1 antibody levels also had a strong association with MS risk in the same population and other independent cohorts [12,15,65]. Furthermore, in a study including data on both IM and anti-EBNA1 antibody titres, both factors were independently associated with MS risk [66]. These observations are consistent with an altered immune response to EBV in patients with MS.

[H3] Lag time between primary EBV infection and MS onset. A lag time of several years between primary EBV infection and clinical MS onset has been reported,[20] This lag could correspond to the asymptomatic and prodromal phases of MS [67] (**Figure 1**), which are characterized by increased biomarker levels of neuroaxonal damage and non-specific symptoms years before the first neurological symptoms occur. Indeed, NfL levels might increase 10 years or more before the onset of clinically apparent MS [38]. Alternatively, the lag time could reflect an evolving host–virus dynamic. This evolving dynamic could include affinity maturation of memory B cells, clonal expansion, epitope spreading, gradual expansion of a poorly controlled EBV-infected B cell reservoir and/or exhaustion of cytotoxic T cells. Consistent with a genuine lag time (as opposed to MS prodrome) following EBV infection is the observation that MS risk remains elevated for decades following IM[16]. Furthermore, in people who develop MS, anti-EBNA antibody levels are reported to increase 15 to 20 years before the first symptoms [12]. Overall, these results indicate that the evolving interaction between the immune system and EBV contributes to MS risk. Alternatively, in analogy with EBV-associated neoplastic diseases, the lag time could hint at a role for genetic transformation in EBV-infected B cells.

[H3] Immunosuppression is not associated with MS risk.

Immunosuppression has been linked to an increased risk of EBV-associated lymphoproliferative diseases but not of MS. HIV infection causes progressive immune suppression and increases the risk of EBV-associated lymphomas, such as non-Hodgkin lymphoma [68], but is associated with a reduced risk of MS [69]. Although these observations

could be influenced by drugs used in antiretroviral therapy, which could have anti-EBV effects [70], they could also indicate that EBV increases the risk of lymphoproliferative diseases and MS through different underlying mechanisms. This supports a key role of the anti-EBV immune response in causing MS.

[H2] Immune imprint of altered primary EBV infection

[H3] Characteristics of EBV-specific immune control. Most EBV carriers will not develop EBV-related immune pathology or tumours, demonstrating the near-perfect control that the immune system has over the virus[71-73]. The rare occurrence of MS years after EBV infection could be a result of uncontrolled EBV infection. Understanding EBV-specific immune control could provide insights into the mechanisms that underlie the increased risk of MS due to EBV infection. Insights into this EBV-specific immune control have been gained from studies on peripheral blood and tonsillar mononuclear cells from EBV carriers who were healthy or had IM [71,74].

Primary immunodeficiencies that predispose individuals to EBV-associated pathologies have also provided clues to the underlying mechanisms of EBV-related MS risk. Genetic testing in these individuals has resulted in the identification of genes that are essential for asymptomatic virus-specific immune control [75-77]. Furthermore, studies in animal models, in particular, EBV-infected mice with reconstituted human immune system components and monkeys with EBV-related lymphocryptovirus infection, have uncovered protective functions for different lymphocyte populations and distinct surface receptors [78-80].

Several surprising findings have emerged from studies of EBV-specific immune control. Immune control does not fail in individuals with gene mutations that affect the type I or type II interferon pathways or in people with combined variable immunodeficiency (CVID) who cannot produce antibodies [75,77,81]. Instead, cytotoxic lymphocytes that are found among natural killer cells, $\gamma\delta$ T cells, natural killer T cells and $CD4^+$ and $CD8^+$ T cells are essential for protective immune responses against EBV [82-85]. Populations of $CD8^+$ T cells that recognize lytic EBV antigens expand during IM [86], and T-cell lines, including those directed against the latent EBV antigens EBNA1, latent membrane protein (LMP) 1 and LMP2, have proven sufficient to control EBV-associated post-transplant lymphoproliferative disease [87]. Thus, cytotoxic lymphocytes are the cornerstone of EBV-specific immune control, and they maintain asymptomatic persistent infection with this virus for life after infection in the first two decades [75-77]. Diminished EBV-specific immune control by cytotoxic lymphocytes might lead to virus induced pathology that could lead to MS.

[H3] Molecular mimicry.

After primary infection, EBV-specific antibodies develop and can be used diagnostically to distinguish acute and persistent phases of EBV infection [88]. IgM antibodies to viral capsid antigens characterize the acute infection and then switch to viral capsid antigen-specific IgG responses. EBNA1-specific IgG responses, however, only emerge during convalescence from IM, sometimes up to 3 months after the initial [71].

Some studies have reported that antibodies to the EBV tegument protein BRRF2, the capsid antigen BFRF3, and EBNA1 exhibit cross-reactivity against CNS

autoantigens[89-92]. EBNA1-specific and BRRF2-specific antibodies are present in the cerebrospinal fluid (CSF) of a subset of patients with MS. These antibodies contribute to oligoclonal bands — bands of antibodies that are produced by clonal B-cell-derived plasma cells in the CNS and are characteristic of MS [89,93-97]. BRRF2-specific antibodies have been described to cross-react with mitochondrial proteins [92], whereas antibodies to EBNA1 have been found to cross-react with anoctamin 2, αB-crystallin, myelin basic protein (MBP), and glial cell adhesion molecule [89-91,98]. Primarily cross-reactivities have been detected for EBNA1-specific antibodies.

A region in the carboxyl half of EBNA1 (aa385–420) elicits elevated antibody levels in patients with MS and is most strongly associated with MS risk compared to antibody levels against other domains of EBNA1 [91]. These EBNA2 aa385-420 specific antibodies cross-react with αB-crystallin and glial cell adhesion molecule. Injection of a peptide from the aa395-420 region of EBNA1 into mice with experimental autoimmune encephalomyelitis (EAE) exacerbated the CNS autoimmunity[89].

The elevation of EBV-specific and autoantigen cross-reactive antibody levels in patients with MS, along with enrichment of these antibodies in oligoclonal bands, suggests that the antibodies are produced in the CNS (**Figure 2**). The antibody specificities might be primed during primary infection and then mature as a result of autoantigen recognition over several years. This maturation might enable CNS autoimmunity to be elicited without the need for high levels of EBV in the bloodstream. The antibody maturation might even be initially driven by EBV gene products; for example, both EBNA3C and LMP1 have been described to induce activation-induced cytidine deaminase (AID), which is required for somatic hypermutation of B-cell receptors

279 and antibodies [99,100]. Alternatively, autoreactive B cells could be rescued from apoptosis
280 during differentiation in the germinal centre reaction by EBV infection [101]. EBV infection
281 might then be lost from autoreactive cells, the persistence of which could be driven by
282 autoantigen recognition. In this way, both viral antigen stimulation and virus-dependent
283 AID induction could elicit autoreactive B-cell specificities that lead to CNS autoimmunity.
284 However, the pathogenic role of these antibodies remains unclear because successful B-
285 cell-depleting therapy for MS has no effect on antibody-producing plasma cells in the
286 short term. Furthermore, such treatment does not seem to diminish the oligoclonal bands
287 [102,103]. Alternatively to a direct pathogenic role, these antibodies could reflect poorly
288 controlled reservoirs of EBV infection and/or CNS tissue damage that stimulates their
289 production.

290 Uncontrolled initial EBV infection during IM and the associated lymphocytosis
291 might also prime cross-reactive $CD4^+$ T-cell populations. These cells might then drive
292 CNS autoimmunity, which could be independent of restimulation by persistent EBV
293 infection (**Figure 2**). The frequency of EBNA1-specific $CD4^+$ T cells was reported to be
294 consistently higher in patients with MS compared with healthy controls, even when viral
295 loads and $CD8^+$ T cells are possibly only altered during the first clinical manifestation of
296 the disease [104-108]. Some of these EBV-specific $CD4^+$ T cells have been shown to cross-
297 react with myelin autoantigens. This cross-reactivity has not been demonstrated for EBV-
298 specific $CD8^+$ T-cell clones, the T-cell receptor sequences of which were found to be
299 elevated in some studies of MS patients compared with healthy controls or individuals
300 with other inflammatory neurological diseases [109,110]. By contrast, other studies reported
301 that EBV-specific $CD8^+$ T-cell responses were unchanged or even diminished in patients

with MS [111-113]. EBNA1-specific CD4+ T-cell clones of patients with MS cross-reacted more frequently to a peptide mixture derived from the four myelin autoantigens — MBP, proteolipid protein, myelin oligodendrocyte glycoprotein, and 2',3'-cyclic nucleotide 3' phosphodiesterase — than to the diabetes mellitus autoantigen proinsulin that was used as a control [114]. In line with this observation, CD4+ T cells from EBV-infected humanized mice, which were selected for EBV-transformed B-cell recognition and recognize antigens presented on the MHC class II molecule HLA-DRB1*1501 that is the main genetic MS risk factor, cross-reacted with MBP peptides [115].

In addition to EBNA1, CD4+ T cells that recognize other EBV gene products have been found to cross-react with myelin autoantigens. For example, cells that recognize the tegument protein BPFL1 cross-react with RASGRP2 [116], and cells that recognize the viral DNA polymerase BALF5 cross-react with MBP [117,118]. The pathogenic relevance of these cross-reactivities, however, is difficult to demonstrate. Therefore, innovative experimental approaches are required, including new experimental animal models into which the respective human CD4+ T cell clones could be transferred to elicit CNS autoimmunity.

[H2] Uncontrolled EBV infection

[H3] EBV-infected B cells as potent antigen-presenting cells. Alongside the mechanisms discussed above, by which EBV infection, alters the immune system to cause CNS autoimmunity, EBV itself might also contribute to the promotion of MS pathogenesis. EBV exhibits a near-exclusive B-cell tropism, and during IM, a large pool of antigen-presenting B cells is generated through EBV-mediated activation. Some of these cells might persist over time and could continue to drive autoimmune or cross-

reactive T-cell responses (**Figure 2**). Antigen targeting to B cells, especially to EBV-transformed B cells, including B-cell targeting with EBV-derived virus-like particles was reported to efficiently stimulate CD4$^+$ T cells and support their expansion during primary immune responses [119-121]. Similarly, LMP1 transgenic mouse B cells can initiate cytotoxic CD4$^+$ T cell responses that are protective against tumour cell line implantation [122,123].

LMP1-induced upregulation of co-stimulatory molecules, such as CD70, OX40 ligand and 4-1BB ligand, contributes to the potent antigen-presenting function of B cells [123]. These LMP1 transgenic B cells are suggested to utilize endogenous antigens for presentation on MHC class II molecules to induce a CD4$^+$ T-cell response [122]. Along these lines EBNA2 has been described to transcriptionally activate gene alleles that are associated with increased MS risk and might enhance this CD4$^+$ T-cell stimulation [60,61,124]. The B-cell receptor (BCR) is the main entry point for antigens to be processed for presentation to CD4$^+$ T cells by MHC class II [125].Exogenous EBV antigens can be efficiently processed by EBV-infected B cells for MHC class II presentation to CD4$^+$ T cells [119]. Latent EBV infection promotes somatic hypermutation of BCRs, potentially generating myelin autoantigen-recognizing BCRs that take up CNS autoantigens, which could result in autoimmune CD4$^+$ T-cell stimulation. In particular, EBNA3C and LMP1 stimulate AID expression in infected B cells to drive somatic hypermutation of the host cell's BCR [99,100]. Latent EBV infection, and the LMP1 antigen in particular converts B cells into potent antigen-presenting cells and might induce mutation of some BCRs. This process could lead to autoantigen recognition by BCRs, resulting in efficient antigen uptake and MHC class II-restricted presentation to CNS autoantigen-specific CD4$^+$ T

cells. The precise roles of EBV and autoantigen-specific $CD4^+$ T cell responses in MS, however, remain to be defined.

[H3] Altered EBV-specific immune control in MS.

As we have already discussed, elevated EBV-specific antibody responses are common in patients with MS and are strongly associated with MS risk [12,13,66,126]. Indeed, these antibodies are emerging as a biomarker for MS. One study found that EBNA1-specific antibody titres at the time of the first clinical presentation of MS, correlated with CNS lesions and MS-associated disability as measures of disease progression[127], although another study produced conflicting results[128]. In other studies, MS disease activity, measured by brain lesions detected by MRI, correlated with EBNA1-specific antibody levels [129,130]. Furthermore, levels of these antibodies correlated with EBV viral loads in humanized mice [115]. The elevated EBNA1-specific antibody responses could reflect a poorly controlled reservoir of EBV-specific B cells in patients with MS (**Figure 2**).

Evidence has suggested an interaction between EBV and the main genetic risk factor for MS, the MHC class II molecule HLA-DRB1*1501. EBNA1-specific antibody levels were found to be higher in carriers of *HLA-DRB1*1501* than in *HLA-DRB1*1501*-negative individuals [131,132]. On an *HLA-DRB1*1501*-positive genetic background, a slight increase in EBNA1 antibodies significantly increased MS risk [131]. *HLA-DRB1*1501* and high EBNA1-specific antibody titres together augment the risk of MS more than 20-fold [91]. These findings suggest that in the context of *HLA-DRB1*1501*, even healthy individuals have elevated EBNA1-specific antibody responses that could indicate defective immune control of EBV. In EBV-infected humanized mice, *HLA-DRB1*1501*-

positive immune compartments were less able to control EBV viral load than were *HLA-DRB1*0401*-positive or *HLA-DRB1*1501*-negative immune compartments, resulting in elevated $CD8^+$ T cell expansion in the *HLA-DRB1*1501*-positive immune compartments [115]. Despite this evidence, however, the mechanism through which HLA-DRB1*1501 compromises EBV-specific immune control remains unclear. Further research is required to determine whether compromised EBV immune control leads to the establishment of a reservoir of EBV-infected B cells with potent antigen-presenting cell function and whether these cells can infiltrate anatomical locations that are isolated from the bloodstream, such as the CNS.

At distinct anatomical locations such as the brain, autoimmune T cells might be stimulated by B cells that are activated during acute or persistent EBV infection. Accordingly, meningeal B-cell follicles have been identified in patients with secondary progressive MS, suggesting an association with more severe disease manifestation [133,134]. These follicles might contain the oligoclonal B cell expansions and follicular helper T cells that have been identified by single-cell transcriptome analysis in the CSF of patients with MS[135,136]. Levels of C–X–C-chemokine ligand 13 (CXCL13), a chemokine that is involved in C–X–C chemokine receptor type 5 (CXCR5)-dependent migration of both B cells and follicular helper T cells, correlate with disease severity at the first clinical manifestation of MS [137,138]. In addition B cells are abundant in the CNS of patients with MS and might be equipped with superior brain homing capacity compared to other B cell subsets [139]. Levels of the CXCR3 ligand CXCL10 are also increased in the CSF of paediatric patients with MS at clinical disease onset [138]. CXCL10 production was found to be induced by EBNA3B in human lmyphoma cells [140]. The tertiary lymphoid structures,

resulting from this B and T cells homing to the CNS, might support EBV and/or autoantigen specific T-cell stimulation by B cells.

In addition to the CNS, intestinal secondary lymphoid tissues have been proposed to stimulate autoimmune T-cell responses. Autoimmune T-cell responses that are started in the intestine have been reported to elicit CNS damage in the EAE mouse model [141-144]. In these lymphoid tissues microbiota composition influences autoimmune T-cell and B-cell stimulation, possibly through cross-reactivity among bacteria, EBV and autoantigens [116]. Thus, B cells activated during EBV infection, either by EBV gene products or in response to viral antigens, might stimulate autoimmune T cells. This stimulation could occur either directly in the CNS or in the extensive network of intestinal secondary lymphoid organs.

Persistent stimulation of T cells by EBV could lead to inflammation in the CNS. EBV-infected B cells preferentially home to the submucosal secondary lymphoid organs, such as the tonsils in Waldeyer's ring, and to the CNS [145-148]. This preferential homing could drive EBV-specific T cell stimulation and inflammation at these sites (**Figure 2**). Indeed, EBV-infected B cells were found in meningeal tertiary lymphoid structures and brain lesions in patients with MS in some studies, [149-151] although not in others [152,153]. B cells might restimulate EBV-specific $CD8^+$ T cells at these sites and thereby contribute to inflammation in the CNS of the patients[154]. Numbers of EBV specific $CD8^+$ T cells were found to be increased in the CSF of patients with MS, but possibly not more than in other inflammatory neurological diseases [110,112]. In patients with MS, upregulation of TBX21, CXCR3 and CXCL10 expression through EBV infection of B cells [155,156], as well as proliferation of $CXCR3^+$ memory B cells following

EBV reactivation [157], might allow these memory B cells to gain access to meningeal tertiary lymphoid follicles and the brain parenchyma [139,158]. Blocking the anti-viral cytokine IFNγ compromised the antigen presenting capacity of this subset of B cells [139]. Therefore, $CXCR3^+$ EBV-infected memory B cells might restimulate viral antigen-specific and autoimmune T-cell responses in intestinal secondary and CNS tertiary lymphoid tissues during MS.

[H1] EBV-targeted therapeutic approaches for MS

[H2] EBV vaccines

[H3] Prophylactic vaccines. Vaccines that prevent EBV infection or IM might reduce the incidence of MS. Prophylactic vaccines were first shown to be effective in animals by Anthony Epstein, who used purified EBV membrane antigens from virus-infected cells to vaccinate cottontop tamarins. When the animals were challenged with EBV, the vaccine was able to protect them from EBV lymphomas[159]. Most studies of EBV vaccines have focused on EBV glycoprotein 350 (gp350), the main viral glycoprotein on the virion and on infected cells. This glycoprotein is an important target for antibodies that neutralize EBV infection of B cells in human plasma. In the largest clinical trial of EBV vaccines to date, EBV-seronegative students received either a soluble gp350 vaccine in an alum–monophosphoryl lipid A adjuvant or a placebo, in a randomized format. Although the vaccine reduced the incidence of EBV IM by 78% in an intention-to-treat analysis, it did not reduce EBV infection [26] and was not developed further.

Ferritin nanoparticles displaying gp350 have also been used for EBV vaccination. The nanoparticles feature a high-density array of gp350 , which enhances the antibody

response compared with soluble gp350 [160]. Vaccination of mice and non-human primates with gp350–ferritin nanoparticles induced higher titres of B-cell neutralizing antibody than did soluble gp350.

Vaccines containing other EBV glycoproteins, including the EBV gH–gL–gp42 glycoprotein complex, have also been investigated. gH–gL is the principal target of antibodies that neutralize EBV infection of epithelial cells in human plasma, and these antibodies also contribute to neutralization of the virus infection in B cells [161]. Vaccination of mice and non-human primates with gH–gL–gp42–ferritin nanoparticles induced high titres of antibodies, which neutralized EBV infection and blocked glycoprotein-mediated fusion in both B cells and epithelial cells. One study investigated a combined gp350–ferritin and gH–gL–gp42–ferritin nanoparticle vaccine. Transfer of immunoglobulin from vaccinated mice into naive humanized mice protected the mice from viraemia and EBV lymphomas following challenge with EBV[162].

Other EBV vaccines in preclinical studies include trimeric constructs of glycoprotein B and recombinant gH–gL[163], Newcastle disease virus-like particles expressing gp350, gH–gL–gp42, glycoprotein B [164], and EBV virus-like particles deleted for EBV latency and lytic genes [165]. In 2022, clinical trials of two EBV vaccines— an NIH trial of gp350–ferritin nanoparticle vaccine in Matrix-M adjuvant (NCT04645147) and a Moderna trial of an mRNA vaccine expressing gp350, gH–gL and gp42 (NCT05164094) — began enrolling healthy participants.

The ultimate goal of an EBV vaccine would be to block infection and thereby prevent IM and other EBV-associated diseases, including MS. However, a vaccine that enables the immune system to control the virus, without completely preventing infection

might also reduce the incidence of many EBV-related diseases (**Figure 3**). The symptoms of IM are caused by an exuberant immune response to the virus, including release of numerous cytokines and a large T and natural killer cell response. The soluble EBV gp350 vaccine did not prevent infection with the virus; however, it did reduce the incidence of IM [26]. Thus, if MS is attributable to an aberrant immune response to EBV, a vaccine that better controls the immune response to the virus and/or prevents IM, even without fully preventing infection, might reduce the incidence of MS.

[H3] Therapeutic vaccines. An effective therapeutic vaccine for EBV, that decreases the number and proliferation of virus-infected cells, might reduce the frequency of MS recurrence (**Figure 3**). Therapeutic EBV vaccines that were designed to treat patients with EBV lymphomas or nasopharyngeal carcinoma use viral latency proteins, which are expressed in EBV-associated cancers to induce T-cell response. These vaccines include replication-defective vectors, such as modified vaccinia Ankara which express portions of EBNA1 and LMP2 [166]; dendritic cells from patients infected with recombinant adenovirus that express LMP2; [167] and dendritic cells from patients infected with adenovirus that express LMP2 and a portion of LMP1 [168]. Although such vaccines have been reported to expose virus-specific $CD4^+$ and $CD8^+$ T cells to the latency proteins and some responses have been observed, no controlled trials have been conducted. Future trials could combine therapeutic vaccines with checkpoint inhibitors that block PD1 or PDL1 to enhance the immune response. However, increases in reported relapses in patients with MS receiving checkpoint inhibitors[169] indicate that patients treated with these drugs will need to be closely monitored for disease enhancement.

[H3] Potential risks of EBV vaccines. EBV vaccines could potentially induce an 'unnatural' or 'excessive' immune response to the virus, which might trigger or exacerbate autoimmune diseases such as MS. Alternatively, an EBV vaccine that induces only transient sterilizing immunity could, in theory, delay the onset of primary infection and result in more severe IM or MS at an older age.

As noted above, molecular mimicry in which antibodies or T cells to EBV proteins cross-react with CNS proteins has been proposed as a possible mechanism for the association of EBV with MS. If this hypothesis is correct, immunizing with certain portions of EBV proteins that cross-react with CNS proteins, such as EBNA1, might induce an immune response that increases the risk of MS. Therefore, careful consideration should be given to which viral antigens should be included in EBV vaccines.

[H2] EBV antiviral therapies

[H3] Inhibitors of lytic replication. Another therapeutic approach for MS might be to inhibit EBV replication and thereby reduce viral antigen exposure to the immune system (**Figure 3**). Antiviral drugs might reduce MS relapses if these relapses are driven by active virus replication associated with virus reactivation; however no definitive evidence for such a mechanism is available at present. Several antivirals that are licensed for treatment of other viral infections, such as aciclovir, ganciclovir and tenofovir, inhibit EBV replication [70]. However, these drugs have no effect on latently infected cells, which is the predominant mode of EBV infection. Nonetheless, by blocking virus replication, one might limit the production of new virions, thereby reducing infection of additional cells. Antiviral

509 therapy would need to be tested in controlled clinical trials, both for efficacy and safety of
510 long-term therapy.
511
512 ***[H3] Inhibitors of latent viral replication.*** Certain EBV latency proteins are essential for
513 the virus to maintain persistent infection in cells. EBNA1 is expressed in all EBV
514 malignancies and is required for the virus to be passed to daughter cells when latently
515 infected cells divide. VK2019 is an inhibitor of EBNA1 activity [170] and is currently being
516 tested in a clinical trial in patients with nasopharyngeal carcinoma (NCT04925544). Other
517 inhibitors of EBV latency proteins are in development. By reducing the number of latently
518 infected cells, fewer EBV antigens and EBV-infected B cells should be present to drive
519 an aberrant immune response to autoantigens and certain viral proteins, such as EBNA1.
520
521 ***[H3] Treatments to destroy EBV-infected cells.*** Destruction of virus-infected cells might
522 reduce targets or stimulators of aberrant immune responses to latent EBV proteins and
523 autoantigens, thereby reducing the severity or recurrence of MS. Three approaches have
524 been used in humans to destroy EBV latently infected cells. In the first approach,
525 monoclonal antibodies (mAbs) to CD20 are used to kill B cells that harbour latent EBV
526 infection. Such mAbs have been approved to treat patients with MS. Targeting of mAbs
527 to $CXCR3^+$ EBV-infected memory B cells and/or to CD20 expressed on B cells in the CNS
528 might improve their specificity and reduce adverse effects.
529 The second approach to destroying latently infected B cells involves treatment with
530 histone deacetylase inhibitors such as arginine butyrate [171], romidepsin [172] or bortezomib
531 [173]. These treatments induce reactivation of the cells and expression of viral lytic proteins,

including the viral protein kinase. When lytic induction therapy is used in combination with ganciclovir, the viral protein kinase phosphorylates the drug. Phosphorylated ganciclovir is toxic and kills the virus-infected cells. Treatment of patients with EBV malignancies with a combination of arginine butyrate and ganciclovir resulted in partial remissions in some patients [171].

In a third approach, autologous virus-specific T cells are used to recognize and destroy cells that express EBV latency proteins, such as EBNA1 or LMP2. This approach is widely used to treat EBV-related lymphoproliferative disease in individuals who have received a haematopoietic stem cell or organ transplant and exhibit rapidly rising EBV levels in the blood [174]. EBV-specific T-cell therapy is also under development for treating nasopharyngeal carcinoma [175]. A small unblinded clinical trial of autologous virus-specific T cells directed to EBV latency proteins was conducted in patients with MS. Following treatment, improvements in MRI findings and symptoms — particularly fatigue — were reported in some patients [176], and sustained improvements were noted for up to 3 years after treatment in some participants[177].

An additional method to target EBV-infected cells would be a therapeutic agent that could reduce trafficking of the infected B cells to the brain. An EBV-positive lymphoma cell line selected for neuroinvasiveness showed reduced trafficking to the CNS in mice when the animals were treated with an antibody to a protein that was specifically upregulated in cells with the more neuroinvasive phenotype [158].

[H3] Targeting of cross-reactive antibodies or T cells. As mentioned above in the section on molecular mimicry, T cells or antibodies that cross-react with myelin or glial

proteins might become primed during asymptomatic EBV infection or IM. This priming could result in an autoimmune attack on the CNS. Such cross-reactive B cells might be targeted by antigen–toxin conjugates. Alternatively, cross-reactive T cells could be treated with peptide epitopes displaying tolerogenic antigen formulations, such as peptide-coated apoptotic cells or erythrocytes [178-180]. This approach is currently limited, however, as the autoantigen targets have not yet been well-defined.

Conclusions

The evidence from epidemiological studies indicate that MS is a rare complication of infection with EBV. Considerable efforts have been made to better understand the mechanisms that underlie the association; however, these mechanisms are not yet fully understood. Nevertheless, the epidemiological evidence suggests that the risk of MS could be markedly reduced by targeting EBV, for example with a vaccine. Furthermore, if persistent lifelong EBV infection also contributes to MS disease activity, EBV could be a target for novel MS treatments.

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This small, uncontrolled study found that some patients with MS who received EBV-specific T cells, particularly those who showed strong reactivity, had reduced fatigue and other neurological symptoms

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Competing interests:

J.C. is named as an inventor on patent applications on Epstein–Barr virus vaccines, which have been filed by the NIH. A.A. has received an honorarium as a speaker from Moderna. K.B. and C.M. declare no competing interests.

Key Points:

- In a longitudinal study following Epstein–Barr virus (EBV)-seronegative individuals over time, multiple sclerosis (MS) risk increased more than 30-fold after EBV infection. The results are unlikely to be explained by reverse causation or confounding factors.

- Among individuals who are EBV-positive, those with a history of infectious mononucleosis or high antibody titres against EBV nuclear antigens have an increased risk of developing MS.

- Several mechanisms have been proposed to explain the link between EBV and MS, including molecular mimicry and an altered immune response to poorly controlled EBV infection.

- Vaccines that may prevent EBV infection are currently being developed. If effective, these vaccines would most likely prevent the large majority of MS cases.

- Targeting EBV with therapeutic vaccines or antivirals may represent novel treatment strategies for MS.

Figure legends

Figure 1. Multiple sclerosis risk factors and disease course. Multiple sclerosis (MS) is caused by an interplay between environmental and genetic factors. Before Epstein–Barr virus (EBV) infection, the risk of MS is negligible. Infection with EBV increases the risk more than 30-fold, indicating that EBV is a leading cause of MS. Infectious mononucleosis and high levels of antibodies to EBV latent antigens remain associated with an increased MS risk decades after the primary infection. Therefore, pre-existing preclinical or prodromal MS (a phase characterized by increased biomarker levels of neuroaxonal damage and vague/non-specific symptoms years before the first neurological symptoms occur) at the time of EBV infection is unlikely to be a confounding factor. Other risk factors, including low vitamin D levels, smoking, obesity during childhood and adolescence, and genetic factors have consistently been associated with MS risk and are likely to modulate the risk of MS after EBV infection.

Figure 2. Mechanistic links between EBV infection and multiple sclerosis. The figure shows putative mechanisms through which Epstein–Barr virus (EBV) infection might contribute to the development of multiple sclerosis. **A:** During primary EBV infection, especially infectious mononucleosis, autoimmune T and B cell responses could be primed (1). The population of primed cells could then expand as a result cross-recognition of myelin and other autoantigens (2). The T-cell priming might be performed directly by EBV-infected B cells (3) or through presentation of released viral antigens by dendritic cells (4). Migration of these autoimmune B and T cell populations to the CNS allows oligodendrocyte destruction, thereby compromising neuronal function (5). **B:** In individuals predisposed to MS by their genetic background, primary EBV infection could

also establish a reservoir of EBV-infected B cells that are not efficiently immune controlled (6) . These potent antigen-presenting cells could stimulate autoreactive T cells for myelin recognition or virus-specific T cells that then promote inflammation in the CNS (7). EBNA1, Epstein–Barr nuclear antigen 1; GlialCAM, glial cell adhesion molecules; LCL, lymphoblastoid cell line; MBP; myelin basic protein.

Figure 3. EBV-targeted strategies to prevent or ameliorate multiple sclerosis. **a** | Natural infection with Epstein–Barr virus (EBV) results in a large percentage of B cells being infected with the virus, which establishes a latent infection with episomal viral DNA (green rings). In a host predisposed to multiple sclerosis (MS), this latent infection could result in an increased risk of generating EBV-specific antibodies or T cells that cross-react with nervous system proteins to increase the risk of MS. **b** | Vaccination against EBV might prevent infection or reduce the number of cells latently infected with EBV after infection, resulting in a reduced risk of cross-reacting EBV antibodies or T cells and reducing the risk of MS. **c** | Treatment of patients with MS with therapeutic agents to kill EBV-infected cells results in fewer latently infected cells after infection. This approach could diminish the antigenic stimulus for virus-specific antibodies and/or the production of T cells that cross-react with nervous system proteins, thereby reducing disease activity. EBNA1, Epstein–Barr nuclear antigen 1; HDAC, histone deacetylase.

Box 1: Epidemiological evidence supporting that EBV causes MS

Direct evidence

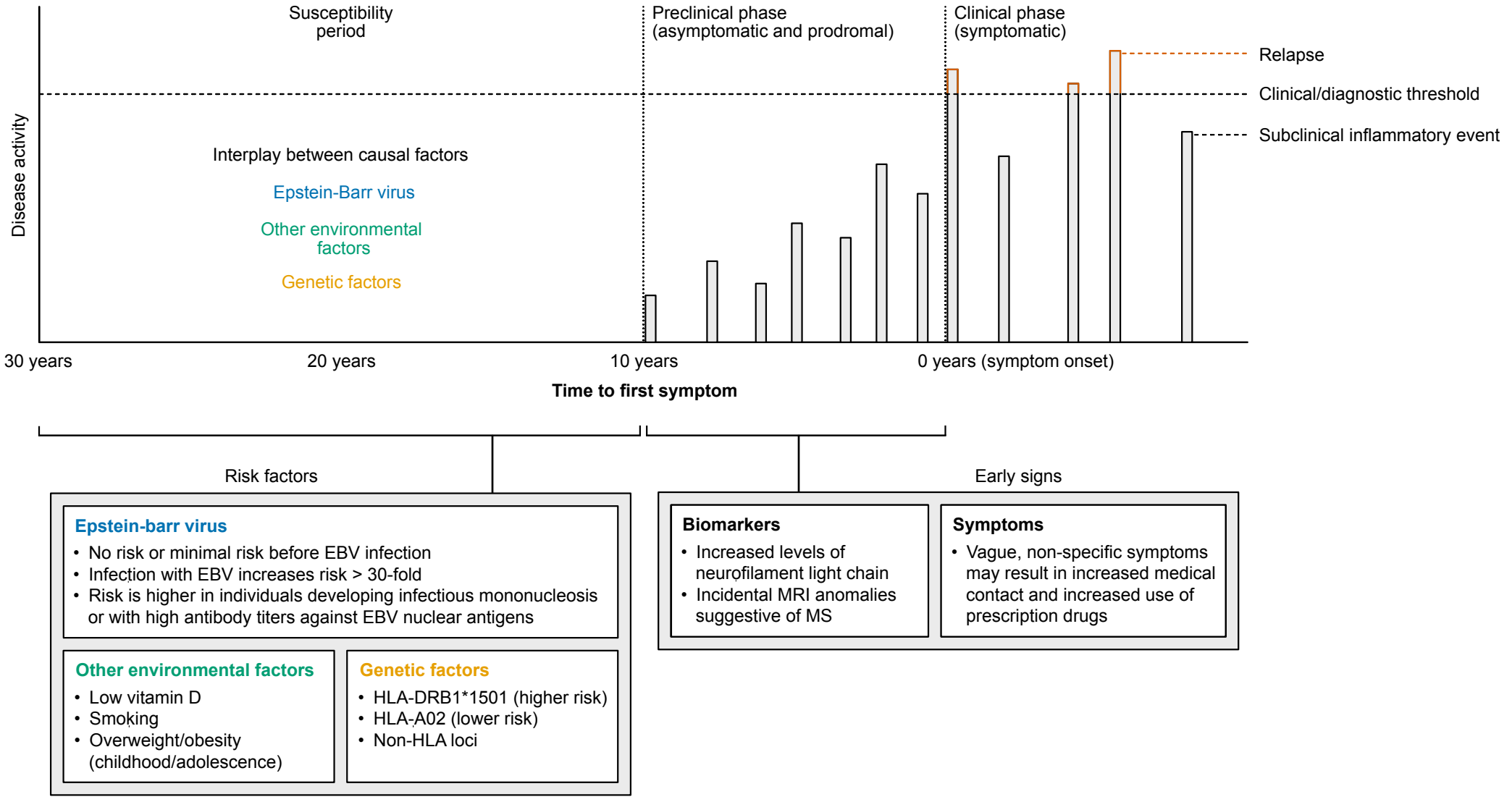
This evidence is based on the extended longitudinal study of individuals who are Epstein–Barr virus (EBV)-negative.

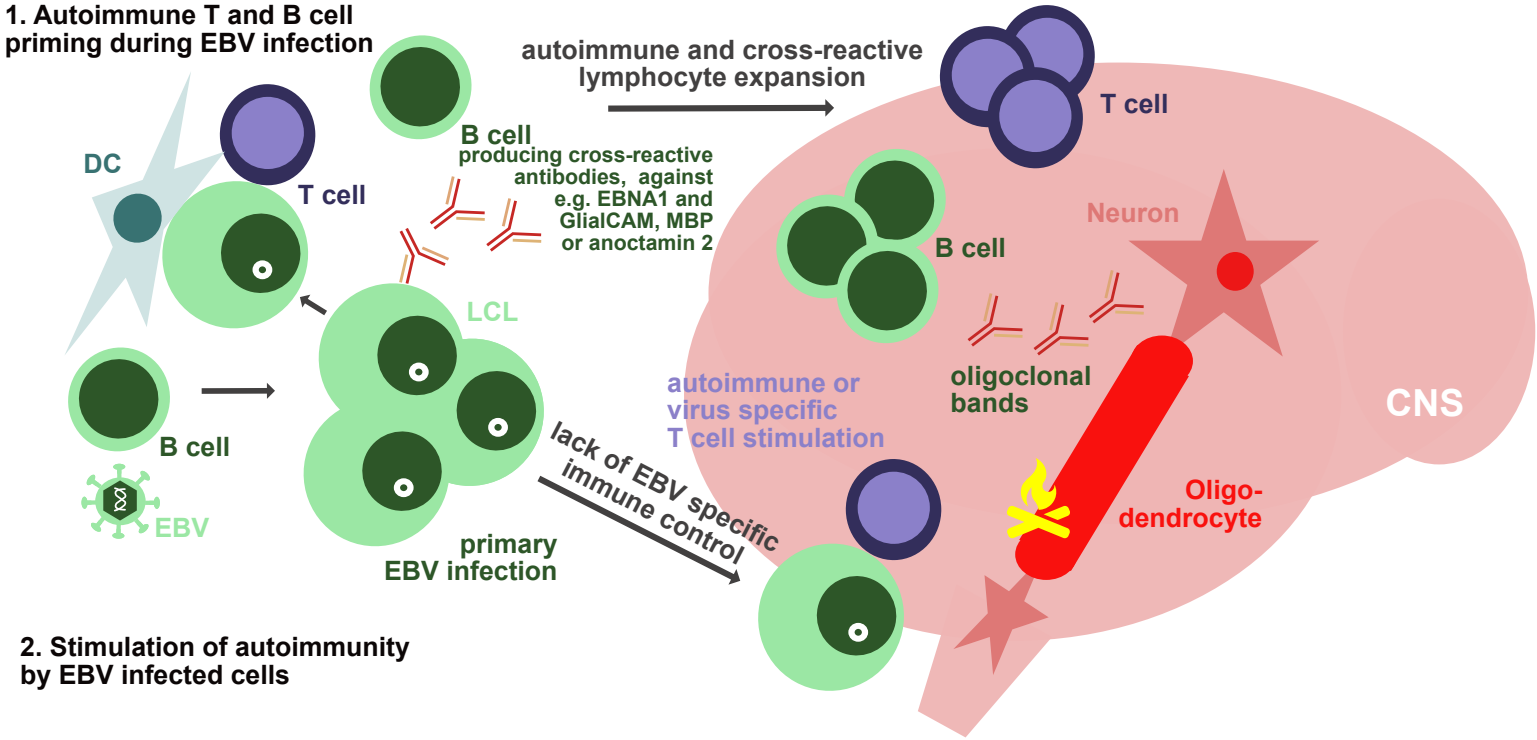
- The risk of multiple sclerosis (MS) before EBV infection is negligible.
- Infection with EBV increases MS risk more than 30-fold.
- EBV infection precedes elevations of neurofilament light chain, a marker of neuroaxonal damage.
- Infection with cytomegalovirus, which is transmitted similarly to EBV, is not followed by an increase in MS risk
- In a virome-wide analysis, only antibodies to EBV peptides showed enrichment in patients with MS compared with matched controls.

Collectively, the above results rule out confounding and reverse causation, thus providing compelling evidence of causality

Corroborating evidence

- Infectious mononucleosis, a symptomatic EBV infection, has consistently been associated with a twofold to threefold increased MS risk, which persists for decades.
- In individuals infected with EBV, titres of antibodies to EBV are the strongest markers of future MS risk.

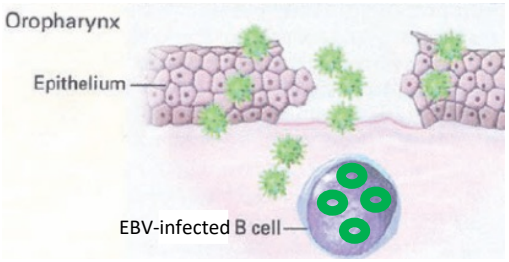

Susceptibility period
Preclinical phase (asymptomatic and prodromal)
Clinical phase (symptomatic)
Relapse
Clinical/diagnostic threshold
Subclinical inflammatory event
Disease activity
Interplay between causal factors
Epstein-Barr virus
Other environmental factors
Genetic factors
30 years
20 years
10 years
0 years (symptom onset)
Time to first symptom
Risk factors
Early signs
Epstein-barr virus
• No risk or minimal risk before EBV infection
• Infection with EBV increases risk > 30-fold
• Risk is higher in individuals developing infectious mononucleosis or with high antibody titers against EBV nuclear antigens
Other environmental factors
• Low vitamin D
• Smoking
• Overweight/obesity (childhood/adolescence)
Genetic factors
• HLA-DRB1*1501 (higher risk)
• HLA-A02 (lower risk)
• Non-HLA loci
Biomarkers
• Increased levels of neurofilament light chain
• Incidental MRI anomalies suggestive of MS
Symptoms
• Vague, non-specific symptoms may result in increased medical contact and increased use of prescription drugs


1. Autoimmune T and B cell
priming during EBV infection
DC
T cell
B cell
producing cross-reactive
antibodies, against
e.g. EBNA1 and
GlialCAM, MBP
or anoctamin 2
LCL
B cell
EBV
primary
EBV infection
autoimmune and cross-reactive
lymphocyte expansion
lack of EBV specific
immune control
T cell
B cell
Neuron
autoimmune or
virus specific
T cell stimulation
oligoclonal
bands
CNS
Oligo-
dendrocyte
2. Stimulation of autoimmunity
by EBV infected cells

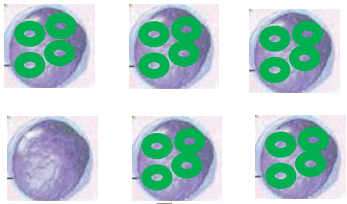
Figure

A

EBV infection



Numerous EBV-infected B cells



Antibodies or T cells recognizing some EBV proteins may cross-react with nervous system proteins

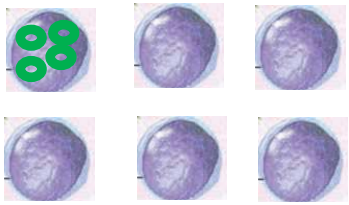
Risk of MS

B

EBV vaccination

Followed by exposure to EBV

Few or no EBV-infected B cells



Less likelihood of antibodies or T cells cross-reacting with nervous system proteins

Reduced risk of MS

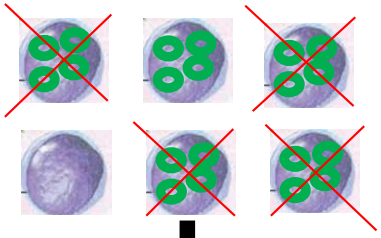
C

Killing EBV-infected cells in patients MS

Methods:

Infusion of EBV-specific T cells
Therapeutic EBV vaccine
HDAC inhibitor + ganciclovir
EBNA1 inhibitor

Few EBV-infected B cells



Reduced antigenic stimulus for EBV-specific antibodies or T cells to recognize nervous system proteins

Reduced MS disease