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SPECIAL FEATURE REVIEW

The interaction between Epstein–Barr virus and multiple sclerosis genetic risk loci: insights into disease pathogenesis and therapeutic opportunities

Ali Afrasiabi[1,2], Chantelle Ahlenstiel[3,4], Sanjay Swaminathan[1,5] & Grant P Parnell[1,6]

[1]EBV Molecular Lab, Centre for Immunology and Allergy Research, Westmead Institute for Medical Research, University of Sydney, Sydney, NSW, Australia
[2]The Graduate School of Biomedical Engineering, University of New South Wales, Sydney, NSW, Australia
[3]Kirby Institute, University of New South Wales, Sydney, NSW, Australia
[4]RNA Institute, University of New South Wales, Sydney, NSW, Australia
[5]Department of Medicine, Western Sydney University, Sydney, NSW, Australia
[6]Biomedical Informatics and Digital Health, School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia

Correspondence
GP Parnell, EBV Molecular Lab, Centre for Immunology and Allergy Research, Westmead Institute for Medical Research, University of Sydney, Sydney, NSW, Australia.
E-mail: grant.parnell@sydney.edu.au

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Abstract

Multiple sclerosis (MS) is a chronic neurodegenerative autoimmune disease, characterised by the demyelination of neurons in the central nervous system. Whilst it is unclear what precisely leads to MS, it is believed that genetic predisposition combined with environmental factors plays a pivotal role. It is estimated that close to half the disease risk is determined by genetic factors. However, the risk of developing MS cannot be attributed to genetic factors alone, and environmental factors are likely to play a significant role by themselves or in concert with host genetics. Epstein–Barr virus (EBV) infection is the strongest known environmental risk factor for MS. There has been increasing evidence that leaves little doubt that EBV is necessary, but not sufficient, for developing MS. One plausible explanation is EBV may alter the host immune response in the presence of MS risk alleles and this contributes to the pathogenesis of MS. In this review, we discuss recent findings regarding how EBV infection may contribute to MS pathogenesis via interactions with genetic risk loci and discuss possible therapeutic interventions.

Keywords: EBNA2, EBV, GWAS, multiple sclerosis, risk genetic variation, RNA therapeutics targeting EBV

INTRODUCTION

Multiple sclerosis (MS) is a complex autoimmune disorder targeting the central nervous system (CNS) resulting in neuronal loss via demyelination. Ultimately, left untreated, this condition leads to significant neurological disability.[1,2] It has a female preponderance with a 2:1 female-to-male ratio at the global level. This ratio varies demographically and increases up to 4:1 in some regions such as the Middle East.[3–6] Typically, MS develops between the ages of 15 and 50, disproportionally affecting young people, resulting in a variety of permanent disabilities, including sensory, motor, autonomic and cognitive impairment.[7,8] The aetiology and pathogenesis of MS have been extensively reviewed by others.[9–12]

Although the exact cause of MS is still unclear, multiple lines of evidence have implied that it might be caused by a combination of a strong genetic component along with several highly implicated environmental factors including vitamin D deficiency, sun exposure, age, gender, sodium intake, smoking and viral infections, in particular, Epstein–Barr virus (EBV).[1,2,13] In fact, recent studies have demonstrated that EBV is likely to be the strongest environmental risk factor for developing MS.[14,15] Despite various studies exploring the potential role of EBV in the aetiology of MS, with a variety of mechanisms having been proposed to drive MS pathogenesis, there is still a lack of focus on the precise molecular mechanisms that allow EBV to drive MS in some individuals but not others.[16–19] There are few papers that show specific molecular and phenotypic characteristics of EBV-infected B cells and how these could contribute to the development of MS. Obtaining a deeper understanding of the interaction between EBV elements and MS risk loci will be critical for advancing our understanding of such molecular mechanisms and phenotypic traits involved in MS. Furthermore, focusing on the interaction between EBV and MS risk loci might provide direct molecular clues to the pathogenic effect of how some risk alleles associated with MS might stem from their influence on host susceptibility to EBV, which in turn might provide strong direct evidence of a causative role of EBV in developing MS. Ultimately, understanding the molecular mechanisms underpinning how EBV may drive pathogenesis is likely to be crucial in the holy grail of developing a cure or in prevention strategies for this debilitating disease. Thus, this review focuses on how EBV may promote MS through its interaction with host genetic risk factors and the therapeutic opportunities that could arise from this understanding.

EPSTEIN–BARR VIRUS

Epstein–Barr virus, also known as human gammaherpesvirus 4, is one of nine human herpesviruses that infect humans.[20] It is a ubiquitous pathogen with serological studies showing consistently that > 90% of adults have evidence of prior EBV infection.[21,22] It primarily infects epithelial cells of the oropharynx initially before further infecting B cells, its major reservoir,[23–26] although EBV has been shown to infect other cell types such as T cells and natural killer (NK) cells.[27,28]

The EBV genome consists of linear double-stranded DNA, which is covered by an icosahedral nucleocapsid.[29] The EBV outer envelope is spotted with external glycoprotein spikes, and a protein coat exists within the space between the nucleocapsid and envelope.[29] The EBV genome encodes 89 genes, 43 core genes (common to all herpesviruses), and 46 non-core genes, 28 of which are specific to EBV.[30,31]

Epstein–Barr virus, as with other herpes viruses, undergoes both latent (dormant) and lytic (productive) phases during its lifecycle.[32–34] The EBV life cycle consists of four programs for latency phase (0, I, II and III) and three programmes for the lytic phase (early, intermediate and late).[20,35–37] The latent phase of the virus maintains EBV presence in the host asymptomatically, along with the lytic phase, which is responsible for producing virions and spreading the virus throughout the host.[32–34]

In primary infection, EBV first establishes lytic replication within the epithelial cells of the oropharynx. As a result of this process, EBV spreads throughout the lymphatic system, infecting B cells and establishing latency III. Once EBV-infected B cells reach the germinal centre, EBV antigens are suppressed, resulting in the differentiation of latency III infected B cells into a latency 0 (quiescent) phase, thus creating a reservoir of infected memory B cells that are largely protected from the host immune response. Through persistent infection, EBV can replicate itself by proliferating within infected B cells (latent) or by producing virions through lysis (lytic), which can infect new cells. Occasionally, infected B cells are recruited into germinal centres, undergo different latency programs, then re-enter the B-cell reservoir as memory cells or undergo plasma cell differentiation producing and releasing new virions, a process that permits low-level viral shedding in the oropharynx, which may in turn facilitate new latency III infections of either naive or memory B cells.[24]

Type III latency is characterised by the expression of a set of EBV proteins, including EBV nuclear antigen genes (*EBNA1*, *EBNA2*, *EBNA3A*, *EBNA3B*, *EBNA3C* and *EBNA–LP*) and latent membrane proteins (*LMP1*, *LMP-2A* and *LMP-2B*). In addition, two noncoding small RNAs (EBERs) and at 44 microRNAs (miRNAs) are expressed.[20,36,37] The current understanding of why EBV expresses large quantities of miRNAs is that they use these miRNAs to downregulate their own protein expression to minimise exposure to the host cellular response.[38] The establishment of latency III in infected B cells

is regulated under the control of an EBV transcription factor, *EBNA2*. Lytic reactivation is a more complex process and requires the activity of over 80 EBV proteins.[39] A combination of *BZLF1* and *BRLF1*, known as early lytic proteins, initiates the expression of over 30 early lytic genes, which is followed by expression of a set of late lytic genes that drive the production of new viral particles.[39]

Epstein–Barr virus has been associated with a number of malignancies (particularly in immunosuppressed hosts)[40] but has also been implicated in a range of autoimmune diseases in addition to MS including systemic lupus erythematosus (SLE), rheumatoid arthritis, systemic scleroderma, Sjögren's syndrome and juvenile dermatomyositis.[41–50]

MS GENETIC RISK

Multiple sclerosis was first hypothesised to be caused by genetic factors in the 1890s when familial aggregation was noted among some MS patients.[51] Multiple sclerosis is disproportionately prevalent among different races and ethnicities and is reported to be virtually non-existent among Black Africans, Native Americans and Pacific Islanders.[6,52] It is important to note that the reliability of these reports may be affected by the ability of less established medical systems in accurately diagnosing MS. However, the risk of MS varies significantly with a 47% increase in MS risk noted in African Americans, while in comparison, a 50% decrease risk was found in Hispanics and an 80% decrease risk in Asians, as compared with Caucasian Americans.[52–54] Interestingly, African Americans tend to experience a more severe disease course than people with predominately European ancestry.[55] The chance of a twin sibling of a person with MS going on to also develop MS is higher among monozygotic than dizygotic twins, ~ 24–31% compared to about ~ 2.4–4.7%.[53,56,57] These numbers in twin studies are greater than the global prevalence which is ~ 0.2%[51] and 15–35 times higher than the general population.[51,58] In studies of adoption and half-siblings, those without a genetic connection to MS patients have the same risk of recurrence as the general population, suggesting that genetics play a major role in familial aggregation in MS.[51,58] Taken together, the above accumulative evidence gained from demographic, familial and twin studies suggests the importance of genetic factors in pathogenesis, but suggest that environmental factors also play a significant role.

It is likely that MS follows a non-Mendelian inheritance pattern.[59] The first associated risk genetic variation for MS was discovered in 1972, *HLA-DRB1*1501*. This variant is located in the coding region of the Major Histocompatibility complex (*MHC*) and increases the susceptibility to MS by three times.[60,61] The *MHC* is highly polymorphic and performs a critical role in adaptive immunity. Further insight into the genetic contribution to MS risk has been gained using genome wide association studies (GWAS), described below.

LESSONS FROM MS GWAS

A GWAS is a hypothesis-free approach to search for genetic variations associated with a particular trait (or a disease) among the common genetic variations across the genome (Single Nucleotide Polymorphisms—SNPs). This occurs by analysing the genotype data for a large number of both healthy controls and individuals with the trait of interest.[62] To date, 32 *MHC* and over 200 non-*MHC* loci along with a locus on the X chromosome have been identified as being associated with MS through GWAS, which are estimated to contribute 18.3–48%, depending on the variables included in the models, of the genetic influence on MS susceptibility.[63] It is important to note that GWAS, despite being a powerful tool for studying the links between disease and genetic variation, have some important limitations. These include not determining the precise pathogenic impact of risk loci, not identifying the genes through which risk loci contribute to the pathogenesis, explaining only a small fraction of the heritability and not distinguishing the causal genetic variants from other variants within the same linkage disequilibrium block of the risk locus.[64,65] Thus, GWAS results need to be interpreted in the light of functional analysis to provide insight into the genetic basis of disease pathogenesis.[66–70]

The transcriptomic analysis of MS risk genes (the prioritised set of genes in close proximity to the MS risk SNPs), identified by the most recent GWAS, has strongly implicated multiple well-described immune-related genes, which are present in major immune cell types. Cell-specific gene regulatory network analysis on non-*MHC* risk genes for MS indicate the key role of the immune system in susceptibility to MS, along with B cells.[71] Aligned with this finding, enrichment

analysis of MS risk genes among genes which are expressed predominantly in different cellular contexts also confirmed the importance of immune cells, particularly B cells, in MS pathogenesis.[72] Moreover, the genomewide polygenic enrichment analysis of MS GWAS, utilising linkage disequilibrium score regression, revealed significant associations between GWAS hits and open chromatin regions, highlighting the genetic evidence that links specific cell types to MS and emphasises the involvement of B cells in the development of the disease.[73,74] Furthermore, B cells have recently received great attention in the MS field because of the high efficacy of monoclonal antibodies that target both naïve and memory B cells in treating relapsing and progressive forms of MS.[17]

A combination of the *HLA-DRB1*1501* allele (the strongest genetic risk factor for MS identified by GWAS) and EBV infection increases the risk of developing MS by up to five times compared to either factor alone,[14,75,76] suggesting a synergic link between EBV molecular processes and MS risk genetic background.[17] Considering that B cells are the most common reservoir for EBV and EBV infection is a strong risk factor (32-fold) for the development of MS,[14] it has been postulated that there is likely to be an interaction between EBV molecular processes and those MS risk loci which are active in B cells and drives (causality) or at least contributes to (near causality) MS pathogenesis.[14,77] In fact, the therapeutic benefit of targeting B cells may be a result of reducing the impact of EBV within these cells, although this has not been definitively proven.

INTERACTION BETWEEN EBV AND MS GENETIC RISK LOCI

The genetic variation that increases the risk of MS may indicate molecular pathways controlling EBV and suggests targets for improved therapy. At the genomic level, the role of EBV infection in MS pathogenesis has been implicated by the over-representation of *EBNA2* binding at MS risk loci,[78] especially in conjunction with vitamin D receptor binding sites.[79] Following on from this, using gene expression data derived from lymphoblastoid cell lines (LCL—an *in vitro* model of latency III EBV infected B cells), Afrasiabi *et al*. identified that 25% of MS risk loci display genotype-dependent differences in the expression of risk genes more so in the LCL context than in other immune cells.[80] An over-represented number of these risk loci were located near EBV transcription factor binding sites, particularly so for *EBNA2*, and mapped onto the *LMP1/LMP2* signalling pathway. They also demonstrated a functional consequence of the interaction between EBV infection and MS risk loci where it was shown that inhibition of LCL proliferation by *CD40L* was affected by genotype of the MS risk SNP in cis with *CD40*.[80] Collectively, these findings suggest that the EBV life cycle is affected by MS risk loci and mainly orchestrated through *CD40-LMP1/LMP2* pathways mediated by *EBNA2* in a genotype-dependent manner.[80] To tackle this hypothesis, Keane *et al*. showed that *EBNA2* binding was risk allele dependent in LCLs for five of the six MS risk loci proposed by Afrasiabi *et al*.[81] *EBNA2* was found to bind preferentially to the risk allele of two loci, *TRAF3/RCOR1* (rs1258869) and *CD40* (rs1883832); and to the protective allele of three loci, *TNFAIP8* (rs32658), *TNFRSF1A* (rs180069) and *TBX6* (rs3809627). This provided further supportive evidence of crosstalk between MS risk genetic factors and EBV infection mediated by *EBNA2* (see Figure 1). The key role of *EBNA2* in regulating this interaction network indicates importance of the EBV latency III life cycle phase in the MS pathogenesis. A possible mechanism could be MS risk genetic factors facilitating the transformation of B cells by EBV (poor control of EBV infection) that can then lead to stimulating autoimmune T-cell responses, some of which are cross-reactive to myelin antigens, and thereby drive autoimmunity in the CNS.[82,83]

Despite the large transcriptomic changes that occur in a B cell upon infection with EBV and subsequent immortalisation, MS risk loci have been shown to be over-represented in genes that are differentially expressed in LCLs compared with uninfected B cells.[80] Whilst there are also large epigenetic changes in response to EBV transformation of B cells to LCLs, with over two-third of the genome exhibiting hypomethylation,[84] MS risk loci were in fact shown to be under-represented in the hypomethylated regions.[85] It has been also reported that MS risk genes are highly over-represented among host genes correlated with EBV DNA copy number level, whereas risk genes for diseases and traits not associated with EBV were only slightly or not over-represented.[86] This study also showed that EBV DNA copy number is associated with 13% of MS risk loci in a genotype-dependent manner.[86]

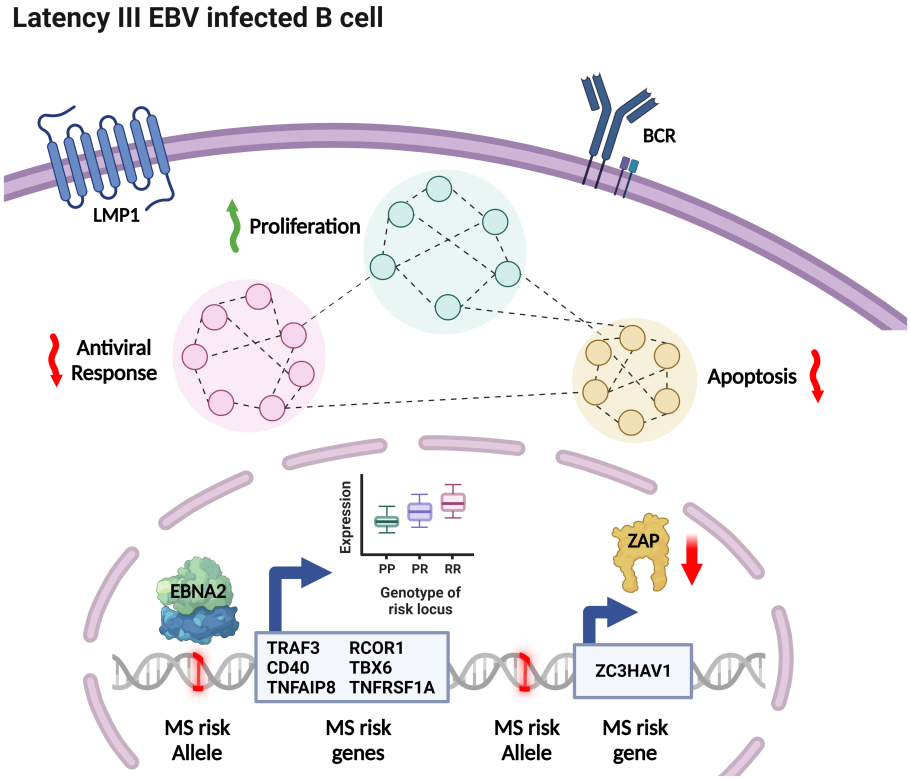

Figure 1. Interaction between EBV and MS risk loci in the latency III infected B cell. Epstein–Barr virus (EBV) dysregulates the B-cell transcriptome, including genes proximal to MS risk loci (MS risk genes), either directly or indirectly, which may contribute to MS pathogenesis.[80] EBNA2, an EBV encoded transactivator protein specific to latency III, regulates the expression of MS risk genes by preferentially binding to the protective or risk alleles at the respective MS risk loci.[81] This preferential binding could alter the effect of risk alleles on gene expression. In the case of ZC3HAV1, the MS risk allele reduces expression.[87] This may happen through EBV elements, or as a consequence of EBV-related molecular processes in the infected B cell context. The net effect of these interactions may alter molecular pathways in B cells resulting in less control of EBV infection via a reduction in anti-viral responses and apoptosis as well as an increase in cell proliferation. Further experimental validation is needed to prove this postulated model at the physiological level. The image was created using BioRender.com, under the agreement number OB24ZS37BU.

Afrasiabi *et al*. also investigated the role of EBV-encoded and host–encoded miRNAs in MS pathogenesis. They provided evidence that EBV infection can dysregulate the miRNA machinery in B cells, including MS risk miRNAs.[87] Here, it was also shown that *has-let-7b-5p* may interact with *ZC3HAV1*, an MS risk gene with antiviral function, differently in LCLs than in B cells. *In vitro* assays indicated that the risk allele decreases *ZC3HAV1* expression in LCLs, but not in B cells (Figure 1). This supports the notion of decreased *ZC3HAV1* leading to a reduced interferon response and less viral mRNA degradation, leading to increased immune evasion by EBV.

There is also evidence of gender differences in the interaction between EBV and the MS genetic risk factors. Using a large LCL gene expression data set, it was shown that two of the MS risk loci, *TRAF3/RCOR1* and *TBX6* (that had earlier displayed allele dependent binding of *EBNA2*), showed sex differences in their associations with gene expression.[88] The expression level of *TRAF3* also showed a female-biased correlation with expression of the oestrogen receptor 2 (*ESR2*) gene. The authors also found that *CD40* and *ZC3HAV1* demonstrated a male-biased response to oestradiol treatment of LCLs. Furthermore, this study showed that oestradiol treatment can alter the EBV latency III characteristics including *EBNA2* level, and cell proliferation rate in a sex-dependent manner. These data indicate that the interplay between MS risk loci and EBV infection may contribute to a sex-biased immune response to EBV infection and explain in part the sex differences in MS susceptibility.

Together these findings support the hypothesis that the EBV-infected B-cell latency III phenotypes,

EBV DNA copy number and proliferation rate, are correlated with the expression of MS risk genes, associated with risk variants, and affected by these in a manner suggesting that targeting EBV viral load would reduce EBV-driven pathogenesis in MS. Also, it suggests that this process is likely regulated by *EBNA2* and may also be influenced by oestrogen. Moreover, it is likely that the interaction of *EBNA2* with MS risk loci can alter MS susceptibility. These data also provide strong support for further studies into targeting the interaction between the most associated host MS risk genes and EBV genes affecting EBV DNA copy number and that this pathogenic process could be inhibited in MS by anti-EBV therapies. They provide strong support for a facilitative role of EBV infection in MS but do not definitively prove it. These findings align with the autoreactive B-cell hypothesis, in the way that MS risk alleles lead to the establishment of more transformed EBV B cells, which can efficiently stimulate autoimmune T-cell responses and some of these responses may be cross-reactive to myelin antigens, leading to the development or progression of MS.[83] They indicate molecular processes important in regulating the EBV life cycle in B cells and suggest molecular targets for control of EBV infection and potentially reducing MS progression through reducing EBV reactivation. In addition, EBV infection and the *HLA-DRB1*1501* MS risk haplotype likely interact in susceptible individuals and contribute to MS pathogenesis, as recently reviewed by Thomas *et al*.[89] Despite the established correlation between *HLA-DRB1*1501* and EBV, there is a lack of specific studies examining the interaction between EBV and the HLA region at a genomic level. Our research, as well as others, has primarily focused on non-MHC risk loci. Interestingly, several EBV miRNAs have been observed to directly or indirectly down-regulate HLA class I and class II, potentially reducing immune surveillance by virus-specific $CD4^+$ and $CD8^+$ T cells.[90–92] However, these findings are yet to be validated in latency III EBV-infected B cells, which are believed to play a more significant role in MS pathogenesis.[78,80] An in-depth understanding of the possible genomic level interaction between EBV and *HLADRB1*1501* within the context of MS pathogenesis is pivotal, as it could shed light on the currently ambiguous role of heightened antibody responses against EBV antigens in the development and progression of MS.

TARGETING EBV THERAPEUTICALLY

Controlling EBV infection as a therapeutic strategy to treat MS is a logical step in the light of the strong association between the virus and pathogenesis of MS. The advent of recent therapeutics that target B cells directly using monoclonal antibodies (such as ocrelizumab) raises the possibility that part of their therapeutic action may be partially related to reducing EBV reservoirs. Whilst B cell depletion is standard treatment for EBV lymphoproliferative disease, it is not entirely clear how B cell depletion reduces MS progression and whether this is directly or indirectly related to EBV being targeted in B cells or by other mechanisms (such as eliminating antigen presentation to T cells). The long latency between EBV infection and the development of autoimmunity, and the fact that most individuals with EBV have no signs of an EBV related autoimmune disease, makes it a particularly challenging area to study.

A number of therapeutic interventions are currently being tested to combat EBV infection, including immunomodulating agents, antiviral agents, chemotherapy, cytotoxic T-cell therapy and stem cell transplantation. These agents are used to treat chronic EBV infection, but their effectiveness has been limited. There have been attempts to treat EBV with existing antiviral medications that are designed specifically for other viruses but these have demonstrated poor clinical efficacy.[93] In addition, vaccines against EBV are currently being developed but are not currently available for routine clinical use.[94]

There remains an unmet need to develop more specific and efficient therapies for targeting EBV to treat MS. According to a recent study by Bjornevik *et al*., targeting EBV at an earlier stage may be a more effective way of treating MS.[14] Among the possible strategies, one could be to target the initial symptomatic EBV infection (IM) in young adults harbouring MS risk alleles. As vaccinating against EBV or filtering EBV-infected B cells are challenging tasks,[17,95] investigating nucleic acid inhibitors against EBV elements may be a beneficial solution. EBV elements (protein coding, non-coding and miRNAs) are especially attractive targets since normal host cellular machinery could be left intact which minimises adverse reactions. If it can be demonstrated that nucleic acid inhibitors alter the expression of EBV life cycle markers, particularly latent phase, EBV

DNA load, EBV-infected B cell proliferation, and the expression of MS risk genes, this would serve as a proof-of-concept that this approach is effective for controlling EBV infection and potentially slowing the progression of MS.

Small interfering (si)RNAs have started to enter the therapeutic space in recent years, with five siRNA currently FDA-approved and in clinical use.[96–100] They are highly specific and offer the potential to target viral sequences without affecting host genes. However, there are no established siRNA therapeutics that have entered clinical trials for EBV treatment. Further, targeting just one of the EBV elements may not necessarily be a one-size-fits-all solution for therapeutic purposes. For example, in most EBV-associated cancers, the latency I (Hodgkin lymphoma) or II (Burkitt's lymphoma) programmes are involved,[101] while diseases associated with immune deficiencies or autoimmunity are associated with the latency III programme.[102] According to one proposed model, EBV lytic switching may contribute to the pathogenesis of Systemic Lupus Erythematosus (SLE),[103] contrary to the proposed model for MS,[80] which suggests that latency III plays a greater role in MS development. Since there are some shared risk genetic loci between MS and SLE, particularly those genes that are linked to EBV molecular processes such as *CD40*,[104,105] therapeutic advantage may be gained by focusing on targeting EBV elements that arrest latency III but do not induce lytic activation. An alternative option to address this issue could be a nuanced approach to avoid triggering possible adverse effects through dual targeting of latency and lytic genes simultaneously. Delivering nucleic acid inhibitors to the EBV-infected B cells effectively is one the challenging tasks in targeting EBV elements. The attachment of nucleic acid inhibitors to antibodies or aptamers (reviewed by Kim and Rossi[106]), which could bind to a surface marker specific to EBV-infected B cells such as *LMP1* or *LMP2A* or *LMP2B*, may be a beneficial approach to improve the efficiency of delivering anti-EBV nucleic acid inhibitors.

CONCLUSIONS

Epstein–Barr virus infection has been shown to be an important environmental risk factor for the development of MS. Recent studies suggest that there are important interactions between the virus and host genetics that may explain some elements of MS pathogenesis. The long latency between primary EBV infection and disease outcome makes it challenging to study these interactions. It is unclear whether the pathogenic pathways are directly and/or indirectly linked to EBV infection itself or to an aberrant immune response to the virus. In future, the possibility of vaccinations that prevent infection with EBV in the first place could offer new hope in reducing the incidence of various autoimmune diseases. Therapeutics that target specific elements of EBV such as the transcription factor *EBNA2* could also lead to new avenues to treat MS and other autoimmune diseases. A better understanding of the interplay between MS risk alleles and EBV could potentially lead to a better insight into the disease processes and the development of targeted therapeutics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Ali Afrasiabi: Conceptualization; visualization; writing – original draft; writing – review and editing. **Chantelle Ahlenstiel:** Writing – review and editing. **Sanjay Swaminathan:** Conceptualization; writing – original draft; writing – review and editing. **Grant P Parnell:** Conceptualization; writing – original draft; writing – review and editing.

REFERENCES

1. Compston A, Coles A. Multiple sclerosis. *Lancet* 2008; **372**: 1502–1517.
2. Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. *Nat Rev Neurol* 2017; **13**: 25–36.
3. Koch-Henriksen N, Sorensen PS. The changing demographic pattern of multiple sclerosis epidemiology. *Lancet Neurol* 2010; **9**: 520–532.
4. Orton SM, Herrera BM, Yee IM *et al*. Sex ratio of multiple sclerosis in Canada: a longitudinal study. *Lancet Neurol* 2006; **5**: 932–936.
5. Ramagopalan SV, Byrnes JK, Orton SM *et al*. Sex ratio of multiple sclerosis and clinical phenotype. *Eur J Neurol* 2010; **17**: 634–637.
6. Walton C, King R, Rechtman L *et al*. Rising prevalence of multiple sclerosis worldwide: insights from the atlas of MS, third edition. *Mult Scler* 2020; **26**: 1816–1821.
7. Haahr S, Hollsberg P. Multiple sclerosis is linked to Epstein-Barr virus infection. *Rev Med Virol* 2006; **16**: 297–310.
8. Sospedra M, Martin R. Immunology of multiple sclerosis. *Annu Rev Immunol* 2005; **23**: 683–747.

9. Loma I, Heyman R. Multiple sclerosis: pathogenesis and treatment. *Curr Neuropharmacol* 2011; **9**: 409–416.
10. Baecher-Allan C, Kaskow BJ, Weiner HL. Multiple sclerosis: mechanisms and immunotherapy. *Neuron* 2018; **97**: 742–768.
11. McGinley MP, Goldschmidt CH, Rae-Grant AD. Diagnosis and treatment of multiple sclerosis: a review. *JAMA* 2021; **325**: 765–779.
12. Dobson R, Giovannoni G. Multiple sclerosis – a review. *Eur J Neurol* 2019; **26**: 27–40.
13. Ramagopalan SV, Dobson R, Meier UC, Giovannoni G. Multiple sclerosis: risk factors, prodromes, and potential causal pathways. *Lancet Neurol* 2010; **9**: 727–739.
14. Bjornevik K, Cortese M, Healy BC *et al*. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. *Science* 2022; **375**: 296–301.
15. Bar-Or A, Banwell B, Berger JR, Lieberman PM. Guilty by association: Epstein-Barr virus in multiple sclerosis. *Nat Med* 2022; **28**: 904–906.
16. Laurence M, Benito-Leon J. Epstein-Barr virus and multiple sclerosis: updating Pender's hypothesis. *Mult Scler Relat Disord* 2017; **16**: 8–14.
17. Bar-Or A, Pender MP, Khanna R *et al*. Epstein-Barr virus in multiple sclerosis: theory and emerging immunotherapies. *Trends Mol Med* 2020; **26**: 296–310.
18. Sollid LM. Epstein-Barr virus as a driver of multiple sclerosis. *Sci Immunol* 2022; **7**: eabo7799.
19. Houen G, Trier NH, Frederiksen JL. Epstein-Barr virus and multiple sclerosis. *Front Immunol* 2020; **11**: 587078.
20. Arvin A, Campadelli-Fiume G, Mocarski E *et al*. *Human Herpesviruses: biology, Therapy, and Immunoprophylaxis*. Cambridge: Cambridge University Press; 2007.
21. Gequelin LC, Riediger IN, Nakatani SM, Biondo AW, Bonfim CM. Epstein-Barr virus: general factors, virus-related diseases and measurement of viral load after transplant. *Rev Bras Hematol Hemoter* 2011; **33**: 383–388.
22. Epstein M, Rickinson A. Infectious mononucleosis. In *eLS*. John Wiley & Sons, Ltd; 2010. https://doi.org/10.1002/9780470015902.a0002318.pub2
23. Hutt-Fletcher LM. The long and complicated relationship between Epstein-Barr virus and epithelial cells. *J Virol* 2017; **91**: e01677-16.
24. Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. *Nat Rev Cancer* 2004; **4**: 757–768.
25. Babcock GJ, Hochberg D, Thorley-Lawson AD. The expression pattern of Epstein-Barr virus latent genes *in vivo* is dependent upon the differentiation stage of the infected B cell. *Immunity* 2000; **13**: 497–506.
26. Al Tabaa Y, Tuaillon E, Bollore K *et al*. Functional Epstein-Barr virus reservoir in plasma cells derived from infected peripheral blood memory B cells. *Blood* 2009; **113**: 604–611.
27. Kawaguchi H, Miyashita T, Herbst H *et al*. Epstein-Barr virus-infected T lymphocytes in Epstein-Barr virus-associated hemophagocytic syndrome. *J Clin Invest* 1993; **92**: 1444–1450.
28. Kikuta H, Sakiyama Y, Matsumoto S *et al*. Fatal Epstein-Barr virus-associated hemophagocytic syndrome. *Blood* 1993; **82**: 3259–3264.
29. Thompson MP, Kurzrock R. Epstein-Barr virus and cancer. *Clin Cancer Res* 2004; **10**: 803–821.
30. Calderwood MA, Venkatesan K, Xing L *et al*. Epstein-Barr virus and virus human protein interaction maps. *Proc Natl Acad Sci USA* 2007; **104**: 7606–7611.
31. Fulop A, Torma G, Moldovan N *et al*. Integrative profiling of Epstein-Barr virus transcriptome using a multiplatform approach. *Virol J* 2022; **19**: 7.
32. Cohen JI. Epstein-Barr virus infection. *N Engl J Med* 2000; **343**: 481–492.
33. Moghaddam A, Rosenzweig M, Lee-Parritz D, Annis B, Johnson RP, Wang F. An animal model for acute and persistent Epstein-Barr virus infection. *Science* 1997; **276**: 2030–2033.
34. Dugan JP, Coleman CB, Haverkos B. Opportunities to target the life cycle of Epstein-Barr virus (EBV) in EBV-associated lymphoproliferative disorders. *Front Oncol* 2019; **9**: 127.
35. Tsurumi T, Fujita M, Kudoh A. Latent and lytic Epstein-Barr virus replication strategies. *Rev Med Virol* 2005; **15**: 3–15.
36. Murata T. Encyclopedia of EBV-encoded lytic genes: an update. *Adv Exp Med Biol* 2018; **1045**: 395–412.
37. Kang MS, Kieff E. Epstein-Barr virus latent genes. *Exp Mol Med* 2015; **47**: e131.
38. Forte E, Luftig MA. The role of microRNAs in Epstein-Barr virus latency and lytic reactivation. *Microbes Infect* 2011; **13**: 1156–1167.
39. Ressing ME, van Gent M, Gram AM, Hooykaas MJ, Piersma SJ, Wiertz EJ. Immune evasion by Epstein-Barr virus. *Curr Top Microbiol Immunol* 2015; **391**: 355–381.
40. van Baarle D, Hovenkamp E, Callan MF *et al*. Dysfunctional Epstein-Barr virus (EBV)-specific $CD8^+$ T lymphocytes and increased EBV load in HIV-1 infected individuals progressing to AIDS-related non-Hodgkin lymphoma. *Blood* 2001; **98**: 146–155.
41. Houen G, Trier NH. Epstein-Barr virus and systemic autoimmune diseases. *Front Immunol* 2020; **11**: 587380.
42. Dreyfus DH. Autoimmune disease: a role for new anti-viral therapies? *Autoimmun Rev* 2011; **11**: 88–97.
43. Draborg AH, Duus K, Houen G. Epstein-Barr virus in systemic autoimmune diseases. *Clin Dev Immunol* 2013; **2013**: 535738.
44. Niller HH, Wolf H, Minarovits J. Regulation and dysregulation of Epstein-Barr virus latency: implications for the development of autoimmune diseases. *Autoimmunity* 2008; **41**: 298–328.
45. Guan Y, Jakimovski D, Ramanathan M, Weinstock-Guttman B, Zivadinov R. The role of Epstein-Barr virus in multiple sclerosis: from molecular pathophysiology to *in vivo* imaging. *Neural Regen Res* 2019; **14**: 373–386.
46. Balandraud N, Roudier J. Epstein-Barr virus and rheumatoid arthritis. *Joint Bone Spine* 2018; **85**: 165–170.
47. Nagata Y, Inoue H, Yamada K *et al*. Activation of Epstein-Barr virus by saliva from Sjogren's syndrome patients. *Immunology* 2004; **111**: 223–229.
48. Pflugfelder SC, Crouse CA, Atherton SS. Epstein-Barr virus and the lacrimal gland pathology of Sjogren's syndrome. *Adv Exp Med Biol* 1994; **350**: 641–646.
49. Randone SB, Guiducci S, Cerinic MM. Systemic sclerosis and infections. *Autoimmun Rev* 2008; **8**: 36–40.

50. Fanard L, Van Steenberge A, Demeire X, van der Puyl F. Comparison between propofol and midazolam as sedative agents for surgery under regional anaesthesia. *Anaesthesia* 1988; **43 Suppl**: 87–89.
51. Dyment DA, Ebers GC, Sadovnick AD. Genetics of multiple sclerosis. *Lancet Neurol* 2004; **3**: 104–110.
52. Hollenbach JA, Oksenberg JR. The immunogenetics of multiple sclerosis: a comprehensive review. *J Autoimmun* 2015; **64**: 13–25.
53. Hansen T, Skytthe A, Stenager E, Petersen HC, Bronnum-Hansen H, Kyvik KO. Concordance for multiple sclerosis in Danish twins: an update of a nationwide study. *Mult Scler* 2005; **11**: 504–510.
54. Hansen T, Skytthe A, Stenager E, Petersen HC, Kyvik KO, Bronnum-Hansen H. Risk for multiple sclerosis in dizygotic and monozygotic twins. *Mult Scler* 2005; **11**: 500–503.
55. Okai AF, Howard AM, Williams MJ *et al*. Advancing care and outcomes for African American patients with multiple sclerosis. *Neurology* 2022; **98**: 1015–1020.
56. Ebers GC, Bulman DE, Sadovnick AD *et al*. A population-based study of multiple sclerosis in twins. *N Engl J Med* 1986; **315**: 1638–1642.
57. Sadovnick AD, Armstrong H, Rice GP *et al*. A population-based study of multiple sclerosis in twins: update. *Ann Neurol* 1993; **33**: 281–285.
58. Ramagopalan SV, Sadovnick AD. Epidemiology of multiple sclerosis. *Neurol Clin* 2011; **29**: 207–217.
59. Patsopoulos NA. Genetics of multiple sclerosis: an overview and new directions. *Cold Spring Harb Perspect Med* 2018; **8**: a028951.
60. Naito S, Namerow N, Mickey MR, Terasaki PI. Multiple sclerosis: association with HL-A3. *Tissue Antigens* 1972; **2**: 1–4.
61. Jersild C, Svejgaard A, Fog T. HL-A antigens and multiple sclerosis. *Lancet (London, England)* 1972; **1**: 1240–1241.
62. Uffelmann E, Huang QQ, Munung NS *et al*. Genome-wide association studies. *Nat Rev Methods Primers* 2021; **1**: 59.
63. International Multiple Sclerosis Genetics Consortium. Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. *Science* 2019; **365**: eaav7188.
64. Tam V, Patel N, Turcotte M, Bosse Y, Pare G, Meyre D. Benefits and limitations of genome-wide association studies. *Nat Rev Genet* 2019; **20**: 467–484.
65. Sun S, Dong B, Zou Q. Revisiting genome-wide association studies from statistical modelling to machine learning. *Brief Bioinform* 2021; **22**: bbaa263.
66. Cano-Gamez E, Trynka G. From GWAS to function: using functional genomics to identify the mechanisms underlying complex diseases. *Front Genet* 2020; **11**: 424.
67. Li B, Ritchie MD. From GWAS to gene: transcriptome-wide association studies and other methods to functionally understand GWAS discoveries. *Front Genet* 2021; **12**: 713230.
68. Gallagher MD, Chen-Plotkin AS. The post-GWAS era: from association to function. *Am J Hum Genet* 2018; **102**: 717–730.
69. Afrasiabi A, Keane JT, Heng JI, Palmer EE, Lovell NH, Alinejad-Rokny H. Quantitative neurogenetics: applications in understanding disease. *Biochem Soc Trans* 2021; **49**: 1621–1631.
70. Freedman ML, Monteiro AN, Gayther SA *et al*. Principles for the post-GWAS functional characterization of cancer risk loci. *Nat Genet* 2011; **43**: 513–518.
71. International Multiple Sclerosis Genetics Consortium. A systems biology approach uncovers cell-specific gene regulatory effects of genetic associations in multiple sclerosis. *Nat Commun* 2019; **10**: 2236.
72. Parnell GP, Booth DR. The multiple sclerosis (MS) genetic risk factors indicate both acquired and innate immune cell subsets contribute to MS pathogenesis and identify novel therapeutic opportunities. *Front Immunol* 2017; **8**: 425.
73. Guo MH, Sama P, LaBarre BA *et al*. Dissection of multiple sclerosis genetics identifies B and $CD4^+$ T cells as driver cell subsets. *Genome Biol* 2022; **23**: 127.
74. Yang Y, Musco H, Simpson-Yap S *et al*. Investigating the shared genetic architecture between multiple sclerosis and inflammatory bowel diseases. *Nat Commun* 2021; **12**: 5641.
75. Nielsen TR, Rostgaard K, Askling J *et al*. Effects of infectious mononucleosis and HLA-DRB1*15 in multiple sclerosis. *Mult Scler* 2009; **15**: 431–436.
76. Sundqvist E, Sundstrom P, Linden M *et al*. Epstein-Barr virus and multiple sclerosis: interaction with HLA. *Genes Immun* 2012; **13**: 14–20.
77. Kearns PKA. Prevention of MS requires intervention on the causes of the disease: reconciling genes, epigenetics, and Epstein Barr virus. *Front Neurol* 2022; **13**: 817677.
78. Harley JB, Chen X, Pujato M *et al*. Transcription factors operate across disease loci, with EBNA2 implicated in autoimmunity. *Nat Genet* 2018; **50**: 699–707.
79. Ricigliano VA, Handel AE, Sandve GK *et al*. EBNA2 binds to genomic intervals associated with multiple sclerosis and overlaps with vitamin D receptor occupancy. *PLoS One* 2015; **10**: e0119605.
80. Afrasiabi A, Parnell GP, Fewings N *et al*. Evidence from genome wide association studies implicates reduced control of Epstein-Barr virus infection in multiple sclerosis susceptibility. *Genome Med* 2019; **11**: 26.
81. Keane JT, Afrasiabi A, Schibeci SD, Swaminathan S, Parnell GP, Booth DR. The interaction of Epstein-Barr virus encoded transcription factor EBNA2 with multiple sclerosis risk loci is dependent on the risk genotype. *EBioMedicine* 2021; **71**: 103572.
82. Laderach F, Munz C. Epstein Barr virus exploits genetic susceptibility to increase multiple sclerosis risk. *Microorganisms* 2021; **9**: 2191.
83. Munz C. Kissing genetic MS risk loci to life. *EBioMedicine* 2021; **72**: 103594.
84. Hansen KD, Sabunciyan S, Langmead B *et al*. Large-scale hypomethylated blocks associated with Epstein-Barr virus-induced B-cell immortalization. *Genome Res* 2014; **24**: 177–184.
85. Ong LTC, Parnell GP, Afrasiabi A, Stewart GJ, Swaminathan S, Booth DR. Transcribed B lymphocyte genes and multiple sclerosis risk genes are underrepresented in Epstein-Barr virus hypomethylated regions. *Genes Immun* 2020; **21**: 91–99.

86. Afrasiabi A, Parnell GP, Swaminathan S, Stewart GJ, Booth DR. The interaction of multiple sclerosis risk loci with Epstein-Barr virus phenotypes implicates the virus in pathogenesis. *Sci Rep* 2020; **10**: 193.
87. Afrasiabi A, Fewings NL, Schibeci SD *et al*. The interaction of human and Epstein-Barr virus miRNAs with multiple sclerosis risk loci. *Int J Mol Sci* 2021; **22**: 2927.
88. Keane JT, Afrasiabi A, Schibeci SD *et al*. Gender and the sex hormone estradiol affect multiple sclerosis risk gene expression in Epstein-Barr virus-infected B cells. *Front Immunol* 2021; **12**: 732694.
89. Thomas OG, Rickinson A, Palendira U. Epstein-Barr virus and multiple sclerosis: moving from questions of association to questions of mechanism. *Clin Transl Immunology* 2023; **12**: e1451.
90. Li Q, Cohen JI. Epstein-Barr virus and the human leukocyte antigen complex. *Curr Clin Microbiol Rep* 2019; **6**: 175–181.
91. Masucci MG, Stam NJ, Torsteinsdottir S, Neefjes JJ, Klein G, Ploegh HL. Allele-specific down-regulation of MHC class I antigens in Burkitt lymphoma lines. *Cell Immunol* 1989; **120**: 396–400.
92. Albanese M, Tagawa T, Buschle A, Hammerschmidt W. MicroRNAs of Epstein-Barr virus control innate and adaptive antiviral immunity. *J Virol* 2017; **91**: e01667-16.
93. Pagano JS, Whitehurst CB, Andrei G. Antiviral drugs for EBV. *Cancers (Basel)* 2018; **10**: 97.
94. Wei CJ, Bu W, Nguyen LA *et al*. A bivalent Epstein-Barr virus vaccine induces neutralizing antibodies that block infection and confer immunity in humanized mice. *Sci Transl Med* 2022; **14**: eabf3685.
95. Cohen JI, Fauci AS, Varmus H, Nabel GJ. Epstein-Barr virus: an important vaccine target for cancer prevention. *Sci Transl Med* 2011; **3**: 107fs107.
96. Adams D, Gonzalez-Duarte A, O'Riordan WD *et al*. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N Engl J Med* 2018; **379**: 11–21.
97. Ray KK, Wright RS, Kallend D *et al*. Two phase 3 trials of Inclisiran in patients with elevated LDL cholesterol. *N Engl J Med* 2020; **382**: 1507–1519.
98. Balwani M, Sardh E, Ventura P *et al*. Phase 3 trial of RNAi therapeutic Givosiran for acute intermittent porphyria. *N Engl J Med* 2020; **382**: 2289–2301.
99. Scott LJ, Keam SJ. Lumasiran: first approval. *Drugs* 2021; **81**: 277–282.
100. Adams D, Tournev IL, Taylor MS *et al*. Efficacy and safety of vutrisiran for patients with hereditary transthyretin-mediated amyloidosis with polyneuropathy: a randomized clinical trial. *Amyloid* 2022; **30**: 1–9.
101. Shannon-Lowe C, Rickinson A. The global landscape of EBV-associated tumors. *Front Oncol* 2019; **9**: 713.
102. Auclair H, Ouk-Martin C, Roland L *et al*. EBV latency III-transformed B cells are inducers of conventional and unconventional regulatory T cells in a PD-L1-dependent manner. *J Immunol* 2019; **203**: 1665–1674.
103. Afrasiabi A, Keane JT, Ong LTC *et al*. Genetic and transcriptomic analyses support a switch to lytic phase in Epstein Barr virus infection as an important driver in developing systemic lupus erythematosus. *J Autoimmun* 2022; **127**: 102781.
104. Ramos PS, Criswell LA, Moser KL *et al*. A comprehensive analysis of shared loci between systemic lupus erythematosus (SLE) and sixteen autoimmune diseases reveals limited genetic overlap. *PLoS Genet* 2011; **7**: e1002406.
105. Richard-Miceli C, Criswell LA. Emerging patterns of genetic overlap across autoimmune disorders. *Genome Med* 2012; **4**: 6.
106. Kim DH, Rossi JJ. Strategies for silencing human disease using RNA interference. *Nat Rev Genet* 2007; **8**: 173–184.

Graphical Abstract

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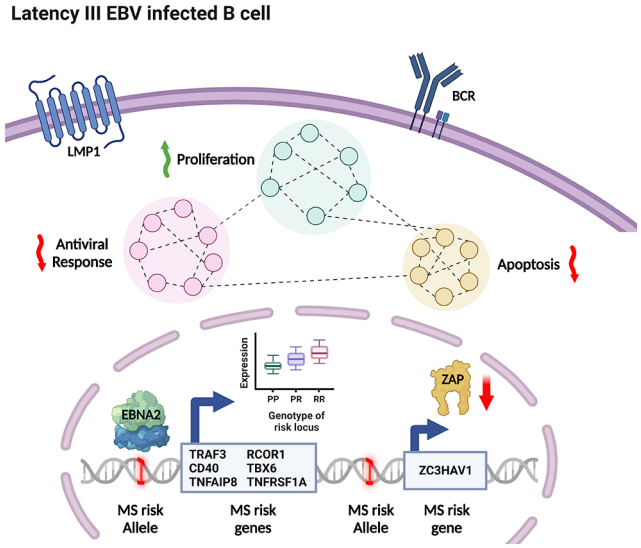

There has been increasing evidence that leaves little doubt that Epstein–Barr virus (EBV) infection is necessary, but not sufficient, for developing multiple sclerosis (MS). In this review, we discuss recent findings regarding how EBV infection may contribute to MS pathogenesis via interactions with genetic risk loci and discuss possible therapeutic interventions.