What do animal models tell us about the role of EBV in the pathogenesis of multiple sclerosis?

Asma Hassani¹ and Gulfaraz Khan²,3*

¹Dept of Neurology, Division of Movement Disorders, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States, ²Department of Microbiology and Immunology, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates, ³Zayed Center for Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS), marked primarily by demyelination, inflammation, and neurodegeneration. While the prevalence and incidence rates of MS are on the rise, the etiology of the disease remains enigmatic. Nevertheless, it is widely acknowledged that MS develops in persons who are both genetically predisposed and exposed to a certain set of environmental factors. One of the most plausible environmental culprits is Epstein-Barr virus (EBV), a common herpesvirus asymptomatically carried by more than 90% of the adult population. How EBV induces MS pathogenesis remains unknown. A comprehensive understanding of the biology of EBV infection and how it contributes to dysfunction of the immune system and CNS, requires an appreciation of the viral dynamics within the host. Here, we aim to outline the different animal models, including nonhuman primates (NHP), rodents, and rabbits, that have been used to elucidate the link between EBV and MS. This review particularly focuses on how the disruption in virus-immune interaction plays a role in viral pathogenesis and promotes neuroinflammation. We also summarize the effects of virus titers, age of animals, and route of inoculation on the neuroinvasiveness and neuropathogenic potential of the virus. Reviewing the rich data generated from these animal models could provide directions for future studies aimed to understand the mechanism(s) by which EBV induces MS pathology and insights for the development of prophylactic and therapeutic interventions that could ameliorate the disease.

KEYWORDS
Epstein-Barr virus, multiple sclerosis, neuroinflammation, animal models, non-human primates (NHP), rodents, rabbits
**Introduction**

Multiple sclerosis (MS) is a disease that causes demyelination, or damage to myelin sheaths in the brain, spinal cord and optic nerve (1), leading to disability, particularly among young adults (2). Neuroinflammation is believed to be fundamental to MS pathology (3, 4). T and B lymphocytes infiltrate the central nervous system (CNS) during the early stages of MS, and to a much lesser degree during the late stages (5). Yet, our understanding of what causes pathology in MS is relatively limited.

MS development is believed to be greatly influenced by both genetics and environmental factors. The increased susceptibility to MS in Caucasians compared to other ethnic backgrounds reflects the impact of genes on MS risk (6–8). Among the genes discovered to influence MS risk, HLA class II allele HLADRB1*1501 has been shown to have the greatest odds ratio in conferring susceptibility to MS (9–11). However, the concordance rate for MS is only as high as 25% in genetically identical individuals (12, 13). This implies that other factors are also involved in the development of MS (14). Indeed, immigration studies revealed increased MS risk in immigrants from low MS-risk regions to regions with high MS incidence (15–17). This however, is also impacted by the age at which immigration takes place. Moreover, various environmental factors including infectious agents are thought to modulate susceptibility to MS (18–21).

Substantial evidence from seroepidemiological and pathological studies support the role of Epstein-Barr virus (EBV) infection in MS pathogenesis (22–26). This herpesvirus is one of the most successful pathogens persisting silently in as many as 95% of the human population. It targets circulating B lymphocytes to establish a life-long reservoir in the face of a pathological studies support the role of Epstein-Barr virus (EBV) in MS development (22–26). This herpesvirus is one of the most successful pathogens persisting silently in as many as 95% of the human population. It targets circulating B lymphocytes to establish a life-long reservoir in the face of a competent immune surveillance (27, 28). Maintaining a balanced EBV-immune interaction is fundamental to the well-being of the human host. Consequently, several EBV-related pathologies arise in individuals with defects in the immune system (29).

The fact that humans are the only natural host for EBV has made the understanding of how EBV contributes to the development/progression of MS exceptionally challenging. Nevertheless, efforts are continuously made to utilize several animals, including rodents, nonhuman primates (NHP), and rabbits to understand the role of the virus in MS pathogenesis (Table 1). Studies have examined the effect of virus titers, age of animals during primary infection, route of inoculation, the direct use of purified virus versus adoptive transfer of virus infected cells, and the induction of experimental autoimmune encephalitis (EAE) in infected versus naïve hosts on the neuroinvasiveness and neuropathogenic potential of the virus. The rich data generated from these animal models has potentially use to promote autoimmunity and/or demyelination in the CNS. This review outlines some of the lessons we have learnt from the studies examining the link between EBV and MS using different in vivo models.

**EBV and MS in murine models**

Data from Murine γ-herpesvirus 68 infection models

Murine γ-herpesvirus 68 (MHV68, or γHV68) belongs to the gammaherpesvirus subfamily of the Herpesviridae, to which both EBV and human Kapoisi’s sarcoma-associated herpesvirus (KSHV, or HHV-8) belong (38). MHV68 is more comparable to KSHV than to EBV in terms of the genomic structure, as both MHV68 and KSHV are gamma-2 viruses while EBV is a gamma-1 virus (38). Nevertheless, MHV68 and EBV share several key biological characteristics during infection of their natural hosts (58) (Table 1). For instance, MHV68 infection in mice results in life-long latency in B cells and macrophages, chiefly in the spleen and lungs, with intermittent lytic infection (32, 59, 60). During chronic latent infection, various immune-related genes are differentially altered in several organs including the brain (61). Furthermore, primary MHV68 infection is associated with a short-lived surge in the frequency of activated CD8+ T cells and splenomegaly in what resembles human infectious mononucleosis (IM) caused by primary EBV infection (33, 62, 63). As a result, several studies utilized MHV68 as a surrogate virus to understand the neuropathogenic potential of EBV, and thus the role of the virus in the pathogenesis of MS.

To determine whether primary peripheral MHV68 infection could be neuroinvasive, Terry and colleagues inoculated various mice strains with MHV68 intranasally (30). Although asymptomatic viral infection was established in the periphery, there was no evidence of viral infection in the CNS. However, peripheral infection was found to cause CNS infection in mice that were deficient in interferon type I receptor (IFNAR), which suggests the protective function of IFNAR against the spread of MHV68 from the periphery to the CNS (30).

The study also examined the consequences of a different route of viral inoculation. When MHV68 was introduced directly into the brain, viral infection was detected in the meninges, ependymal cells, oligodendrocytes, cerebellar Bergmann cells, and pyramidal neuron of the hippocampus. Infection of the brain was also accompanied with infiltration of inflammatory cells into the infected areas and damage to white matter tracts. Viral infection and neuroinflammation were most pronounced towards the end of week 1 post infection (30). These animals exhibited signs of ailment such as lethargy and body atrophy. Nevertheless, inhibiting MHV68 replication in these animals led to silent viral persistence in the CNS. Similarly, introducing non-productively MHV68-infected cells into the brain produced long-term viral persistence in the CNS (30).
Humanized mice

- EBV-infected humanized mice exhibit some pathologies reminiscent of those seen in EBV infection in humans, including IM-like diseases and LPDs.
- Humanized mice have been used to explore the biology of EBV in the context of the immune system and genetics. For example, EBV infection of mice reconstituted with HLA-DR15 appears to have a poorer control of the virus and are more likely to develop autoimmune.
- Certain types of EBV-infected cells implanted i.v., can traverse the BBB and enter the CNS, leading to neuroinflammation and upregulation of some viral and cellular genes.
- When humanized mice are engrafted with PBMCs from EBV-positive or IRMS patients, then induced to develop EAE, the disease develops earlier with more severe symptoms compared to PBMCs from EBV-negative individuals.

Rhesus monkeys

- Rhesus monkeys are natural hosts for rhLCV. Animals infected with rhLCV reproduce a number of biological features of human EBV infection, including oral shedding and transmission, atypical lymphocytosis, immune response to virus, and long-term persistence.
- Animals inoculated with cells immortalized by HJV, a simian α-herpesvirus related to rhLCV, develop neuroinflammation, but no symptomology typically seen in MS.
- Neuroinvasion of infected cells into the CNS appears to be associated with both viral as well as immunological factors. For example, blockage of CD28-mediated T-cell costimulation protects animals against EAE.

Japanese macaque

- Japanese macaques have been shown to be natural hosts for JMRV, a γ-herpesvirus with close homology to RRV.
- Animals naturally infected with JMRV, spontaneously develop JME, an inflammatory demyelinating disease with clinical and histopathological features resembling MS. A majority of the animals with JME show ataxia, paralysis or paresis of one or more limbs and ocular abnormalities. In most cases the onset of the disease is acute and progresses very rapidly. In contrast to MS, the prevalence of JME is similar in both sexes.
- On histopathology, the brain and spinal cord of animals with JME, show acute and chronic multifocal plaque-like demyelinated lesions. Chronic active lesions predominantly consist of macrophages, activated microglia, astrocytes and infiltrating lymphocytes.

Rabbit

- Rabbits are not natural hosts for EBV but are susceptible to infection upon i.v. inoculation of high doses of the virus. Primary acute infection results in widespread infection, most notable in the spleen.
- Infection of healthy immunocompetent rabbits leads to life-long asymptomatic EBV persistence during which little or no virus is detected in PBMCs.
- Immunosuppression reactivates the virus (type III latency), reminiscent of what has been observed in allograft recipients on immunosuppressive therapy.
- Virus-infected cells can also traverse the BBB and enter the CNS, most probably via the Trojan horse mechanism involving B-lymphocytes - i.e., infected lymphocytes transport the virus into the CNS parenchyma during the cell influx associated with the inflammatory process. Infected CNS shows distinct inflammatory demyelinating aggregates consisting of blood-derived macrophages, microglia, astrocytes and infiltrating lymphocytes.

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- Introduction of high viral dose in the periphery, or directly into the brain of young mice, can lead to CNS infection and associated neuroinflammation which appears to be linked to the expression of viral genes and pro-inflammatory cytokines.
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The ability of MHV68 to establish persistent latent infection in the CNS was also demonstrated by Kang and colleagues (31) following intracerebroventricular inoculation of 9-10wk old BALB/c mice with recombinant MHV68-M3/FL. Viral persistence in the CNS resulted in viral dissemination from the brain to peripheral organs including the spleen and lungs, with the spleen being the main peripheral reservoir for latent MHV68. Immunosuppression with cyclosporin A (CsA) of latently infected mice, after the virus levels had become undetectable in the CNS and periphery, caused virus reactivation, and increased viral load in the brain and spleen (31). While these observations imply that latent infection promote viral persistence in the CNS, the implications of latent virus in the brain of an immunocompetent host are currently unclear.

Additionally, the age of the host can be a crucial factor in determining the outcome of MHV68 infection in the CNS. After intracerebral inoculation, older mice are more likely to survive MHV68 infection than younger mice. Younger mice exhibit a more severe MHV68 infection in the CNS, whereas older mice exhibit a decline in MHV68 load to undetectable levels by the second week of infection (30, 34). MHV68 infection in the CNS has been found to involve increased expression of the proinflammatory cytokines, tumor necrosis factor alpha (TNFα), interleukin 1 beta (IL1β), and IL6 in younger mice (34).

Furthermore, the initial viral load introduced into the periphery influences the neuropathogenic potential of MHV68. In wildtype mice, intranasal inoculation using 2*10^4 plaque-forming units (pfu) of MHV68 was found to be non-neuroinvasive (30), but inoculating animals with 2*10^5 pfu of MHV68 intranasally produced detectable and presumably productive infection in the CNS (35). The infection was associated with infiltration of CD45+ cells and CD3+ cells into different brain areas resulting in meningitis, cerebellitis and perivascular encephalitis. Severe neuroinflammation was more likely to occur in regions of the brain that had increased expression of viral proteins. Over a third of these animals had ataxia, severe dystrophy, or died early (35).

The fact that the risk of MS increases in EBV-infected individuals has raised questions about how a pre-existing infection promotes autoimmune demyelination. Studying EAE in murine models of MHV68 infection revealed that latent virus could be the culprit to exacerbated EAE pathology (36). A study examined myelin oligodendrocyte glycoprotein (MOG)-induced EAE course in C57BL/6 mice 5 weeks post primary infection with MHV68 (i.e., during latent infection) and compared it to EAE course in non-infected animals and animals previously infected with either lymphocytic choriomeningitis virus (LCMV) or murine cytomegalovirus (MCMV) (36). MHV68-infected animals experienced EAE more rapidly, with more severe morbidity and higher risk of mortality than non-infected, LCMV- or MCMV-infected animals. The aggravated disease in MHV68-infected animals appeared to occur in the absence of any signs of productive infection in the CNS (36). This was further corroborated by the observation that mice infected with latency-deficient MHV68 (MHV68 AC-RTA) had an EAE course comparable to that in noninfected controls, indicating that latent infection - rather than acute infection- plays a role in disease progression (64).

Indeed, the neuropathological potential of latent infection was also demonstrated by immunizing mice with latent Epstein-Barr virus nuclear antigen 1 amino acid region 411–426 (EBNA-1.411.426) (65). EBNA-1.411.426 immunized mice developed neurological signs reminiscent of EAE, and MRI-confirmed cortical lesions (65). This region of EBNA-1 was also found to trigger high antibody response in individuals with relapsing-remitting (RRMS) and secondary progressive MS (SPMS), and these antibodies cross-reacted with myelin-basic protein amino acid region 205-224 (MBP_{205-224}). Similarly, mice immunized with EBNA-1.386–405 in combination with proteolipid protein amino acid region 139-151 (PLP_{139-151}) exhibited pronounced EAE course and T helper1 (Th1) phenotype-driven inflammation (66). Notably, immunization with EBNA-1.386–405 triggered an antibody response against the CNS-derived antigen, glial cell adhesion molecule (GliCAM). In MS cases, intrathecal antibody response to EBNA-1.386–405 was found to cross-react with GliCAM, most probably via a molecular mimicry mechanism (66).

EAE pathology in noninfected animals was characterized by infiltration of interferon gamma (IFNγ) and IL17-producing CD4+ T cells into the spinal cord, whereas MHV68-infected mice had elevated expression of IFNγ and increased infiltration of T bet+ CD4 T cells, granzyme B-secreting CD8+T cells and F4/80+ macrophages/microglia in the brain and spinal cord. Some of the CNS-infiltrating CD8+ T cells were MHV68-specific. In contrast to noninfected controls, MHV68-infected animals showed demyelination in the cerebellum and corpus callosum (36). Additionally, MHV68-infected mice displayed noticeably greater levels of the costimulatory marker CD40, which appears to promote enhanced expression of the Th1 signature cytokine, IFNγ, and reduction of the frequency of regulatory T cells (Tregs) (64). These results suggest that latent MHV68 infection skews the immune response during EAE toward a Th1 response, rather than a Th17 response, via CD40-mediated immune modulation.

How MHV68-infected B cells contribute to EAE pathology was also studied. Animals that received splenic CD19+1gD+ B cells derived from MHV68-infected animals, before EAE induction, developed severe EAE pathology, including increased production of IFNγ and infiltration of CD8+ T cells in the brain and spinal cord (37). Antibody-mediated depletion of B cells prior to EAE induction alleviated IFNγ production in the CNS, but was ineffective in eliminating detectable virus in the periphery. On the other hand, B cell depletion prior to primary MHV68 infection led to EAE disease similar to that in control animals that received noninfected B cells (37). This implies that
B cells are fundamental for MHV68 to establish latency, which in turn contributes to the exacerbation of EAE disease. EAE was also observed to be aggravated in mice that received EBV-immortalized B lymphoblastoid cell lines (BLCLs) derived from patients with SPMS, prior to MOG-EAE induction (67). Furthermore, mice that were given these BLCLs and recovered from the initial EAE course, experienced disease relapses. These animals also exhibited marked changes in the makeup of their gut microbiota (67). This may shed insight into potential mechanisms, by which EBV-infected and transformed B cells could aggravate autoimmune by interfering in otherwise balanced gut-brain axis.

Data from humanized mice

Humanized mice have also been utilized to examine EBV involvement in MS (Table 1). Humanized mice, such as NOD SCID IL2Rγ-deficient (NSG) mice, are immunodeficient animals that can be successfully engrafted with human hematopoietic stem cells to rebuild functional parts of the human immune system. Studies in humanized mice can help delineate how strictly human pathogens such as EBV, behave in their natural host. EBV infection in humanized mice can result in acute IM-like disease, latent infection, or lymphoproliferative disorders (LPDs), depending on the dose of the inoculum (39, 68, 69).

A recent study explored the association between EBV infection and HLA-DR15 in NSG mice (41) in light of the observation that individuals with a history of IM and carrying the HLA-DRB1*15:01 allele are more likely to develop MS (70). Mice reconstituted with human HLA-DR15 hematopoietic progenitor cells displayed significantly greater numbers of activated CD8+T cells in the periphery (blood and spleen), at 4-6 weeks post EBV infection, compared to controls. EBV-infected mice reconstituted with either HLA-DR15-negative or HLA-DR4-positive immune compartments served as controls. EBV load was also greater in EBV-infected HLA-DR15-carrying animals than in controls. EBV load correlated positively with frequencies and total numbers of activated CD8+T cells in the periphery. Thus, the genetic risk associated with HLA-DR15 may mediate impaired control over EBV infection, and likely contributes to the pathogenesis of IM (41). The study also demonstrated that CD4+ T cells derived from HLA-DR15-carrying mice can recognize MBP epitopes and allogeneic targets. HLA-DR15-restricted cells were found to respond more robustly and non-specifically to HLA-mismatched targets than to their HLA-matched targets. This cross-reactivity mechanism, in conjunction with the lack of specificity in the T cell response, may explain how EBV and the HLA-DR15 allele synergistically enhance MS risk (41).

Another study used NSG mice to assess the impact of EBV on EAE course. NSG mice were transplanted with PBMCs isolated from either patients with relapsing-remitting MS (RRMS), EBV-seropositive healthy controls, or EBV-seronegative healthy controls (42). Upon induction of EAE, all mice exhibited EAE signs. Nonetheless, mice engrafted with RRMS PBMCs showed a more pronounced disease course than mice engrafted with non-MS PBMCs. Furthermore, mice engrafted with PBMCs from EBV seropositive donors had more rapid onset of EAE than animals reconstituted with PBMCs from EBV seronegative donors (42). Hence, this model emphasizes the fundamental role of EBV in disease progression.

Studies on NSG mice have also helped us understand factors that promote migration of EBV-infected cells from the periphery to the brain. NSG mice implanted intravenously, intracardially or subcutaneously with the MUN14 cell line derived from an EBV+ Burkitt’s lymphoma, showed increased trafficking of EBV-infected cells to the brain (71). As a result, mice developed neurological signs including gait deficits, tremor and seizures. Epigenetic changes in EBV-infected B cells were found to enhance the neuroinvasiveness of infected cells. Increased expression of viral protein EBNA-1, and cellular phosphoprotein-1/osteopontin were suspected to be involved in driving the epigenetic changes leading to the neuroinvasive phenotype (71).

Nonhuman primate model for EBV infection of the brain

Lymphocryptovirus-infected B cells

Nonhuman primates (NHPs) have also served as an appealing model to better understand how EBV modulates the risk of MS (Table 1). A study on adult rhesus monkeys (Macaca mulatta) examined whether EBV-infected and transformed B cells can (1) present myelin antigen to, and activate myelin-specific T cells, and (2) promote inflammation in the brain (47). Herpesvirus papio (HVP), a baboon-tropic virus that shares biological and genetic similarities with EBV, was used to infect and transform B cells isolated from rhesus monkeys and subsequently generate BLCLs. These cells were then loaded with either MOG34-56, citrullinated MOG34-56 or CMV capsid antigen-derived peptides, and infused back into the monkeys. Animals exhibited only weight loss with no signs of neurological deficits. However, they mounted an immune response made up primarily of CD8+ T cells, CD8+CD56+ T cells, and CD3+CD56+NK cells against MOG and viral peptides (47).

Interestingly, myelin-specific T cells reacted against MOG only when its peptides were presented by BLCLs. Moreover, animals, particularly those infused with auto-BLCLs pulsed with citrullinated MOG34-56 exhibited meningeal inflammation and infiltration of T cells, B cells, and macrophages into the brain, with no major signs of myelin destruction (47). These
observations point to the pathogenic role of EBV-infected and transformed B cells in neuroinflammation by serving as efficient APCs and promoting recognition of and response against myelin antigens by myelin-specific T cells. This pathogenic effect of EBV-infected B cells was also demonstrated in another NHP, the marmoset (Callithrix jacchus) (72). Jagessar and coauthors proposed an explanation for this. EBV infection of B cells enables productive processing of myelin peptides for presentation to autoreactive T cells (73). B cells in absence of the influence of EBV infection would instead abort antigen processing and opt for the degradation of self-antigen (MOG34-56) which ultimately prevent the presentation of MOG34-56 to their autoreactive T cells (74).

Japanese macaque rhadinovirus and spontaneous onset of encephalomyelitis

NHPs have also emerged as a natural model for herpesvirus-associated autoimmune demyelinating diseases. Japanese macaques (Macacafuscata) housed at the Oregon National Primate Research Center have been found to naturally develop Japanese macaque encephalomyelitis (JME), an MS-like disease, at a rate of 1-3% annually since 1986 (49). In contrast to MS where females are at higher risk than males, JME is believed to develop in both sexes at comparable rates, with a reported median age at disease onset of ~4.3 years. Furthermore, the majority of animals experience acute neurological signs that are almost immediately followed by rapid progression that necessitates euthanasia within a median time of 6 days. Neurological signs commonly include ataxia, paralysis or paresis of at least one limb, and less commonly ocular paresis, body tremors, and head tilt (49). Antemortem MRI examination of the CNS, using post-gadolinium contrast T1-weighted images and T2-weighted axial images of affected animals showed several conspicuous lesions in the white matter of both the brain and spinal cord. Pathologically, these lesions were restricted to the white matter and characterized by demyelination, disrupted axons and marked influx of immune cells in what resembles chronic active MS plaques (49). Unlike MS, pathological changes in the meninges and cortical grey matter are not characteristics of JME (49). JME white matter lesions are characterized by the infiltration of CD163+ cells, some of which are MBP-reactive, the aggregation of CD20+B cells in periventricular areas, and the presence of CD4+T cells, CD8+T cells, and Th17 cells at variable levels (51). Similar to MS patients, JME-affected animals produce intrathecal oligoclonal bands (51).

Interestingly, a newly characterized gamma-2 herpesvirus was recovered from these lesions (48, 49). The virus termed Japanese macaque rhadinovirus (JMRV) had 89.5% and 47.9% sequence homology with rhesus macaque rhadinovirus and KSHV, respectively (49). However, JMRV does not appear to share considerable genetic homology with EBV (48). Subsequent studies described the complete sequence of the viral genome, which revealed both conserved and unique open-reading frames (ORFs), and viral-encoded miRNAs that may be implicated in disease pathogenesis (48, 75).

In spite of the presence of the virus in JME lesions, the intrathecal humoral response does not appear to be directed against the virus (50). T lymphocytes specific for MBP, MOG, and PLP infiltrate the brain and circulate in peripheral blood of infected animals (50).

Rabbit model of EBV infection

Rabbits are susceptible to EBV infection

A number of reports have shown that New Zealand White (NZW) rabbits are susceptible to EBV, and the infection mimics that observed in humans (52–55, 76) (Table 1). During primary EBV infection, rabbits show no symptoms, but can experience temporarily enlarged lymph nodes and spleens, short-term lymphocytosis, elevated titers of anti-EBV viral capsid antigen antibodies, and detectable levels of EBV genome within the first 2 weeks of infection (57). However, EBV levels vary between different rabbits, and fluctuate overtime in a given animal (57). This suggests that different infection dynamics are influenced by differences in rabbit immune responses to EBV, similar to those observed in humans. Similar to humans, the rabbit immune system does not completely eradicate EBV infection, as EBV can persist latently in these animals (52). EBV infection in rabbits produces a robust humoral response, which helps reduce viral loads below detectable levels in the blood, whereas cyclosporin A (CsA)-mediated suppression of T cells can cause reactivation of the latent virus (53).

Peripheral EBV infection of rabbits can lead to CNS infection

A recent study examined if EBV, following primary infection, could enter the rabbit CNS and by what mechanism (56). The study revealed that, at day 14 of infection, the levels of viral DNA were highest in both peripheral and CNS compartments. Although viremia was pronounced at day 14 of infection, EBV load in the brain did not correlate with the levels of free virus in the circulation. There was, however, a positive correlation between the levels of cell-associated virus (i.e., infected cells) in the periphery and EBV levels in the brain. Thus, we posit that migrating infected cells, presumably B lymphocytes, could be the primary source of EBV infection in the brain (77). Indeed, some brain infiltrating B lymphocytes were infected with EBV, in addition to a few infected astrocytes and microglia.
EBV infection of the CNS induces the formation of inflammatory cellular aggregates

Primary EBV infection has been investigated in healthy rabbits and rabbits immunosuppressed with cyclosporine A (CsA). In line with previous data, EBV load increased significantly when the immune system was suppressed, and this raised the likelihood of virus-infected cells breaching CNS barriers. Despite the absence of overt signs of neurological impairments, peripheral EBV infection induced neuroinflammatory cellular aggregates in some animals within 2 weeks of infection (Figure 1). These animals displayed focal CNS cellular aggregates composed of densely clustered blood-derived macrophages surrounded by activated microglia and astrocytes, neutrophils, CD8⁺ T lymphocytes, dispersed EBI2⁺ cells and PCNA⁺, IgM⁺, and IgG⁺ B lymphocytes (Figure 1). Remarkably, the cell aggregates showed myelin damage in the center (Figure 1) (56). This may model the early stages of lesions that progress into MS smoldering active plaques (78). Of interest, some of the immunosuppressed noninfected controls also developed cell aggregates in the CNS, which was probably brought on by the reactivation of opportunistic intrinsic pathogens. This scenario mimics the development of CNS pathology seen in immunosuppressed individuals who experience progressive multifocal leukoencephalopathy as a result of JC virus reactivation (79). Nonetheless, the distinctions between

![Figure 1](image-url)

Figure 1: Inflammatory cellular aggregates in brain of EBV-infected rabbits. Brain sections were stained for: (A) H&E, (B) EBV latent nuclear protein, EBNA-1, (C) Blood-derived macrophage marker, RAM11, (D) Microglia marker, Iba1, (E) Neutrophil marker, RPN3/57, (F) B-cell marker, CD79a, (G) pan-T-cell marker, CD3, (H) Cytotoxic T-cell marker, CD8, (I) Astrocyte marker, GFAP, (J) Myelin marker, MBP, (K) Counter stain, DAPI, (L) MBP/DAPI merge showing areas of demyelination [adapted from (56)]. The figure shows non-serial sections from the same block. (Scale bar= 200µm in A, 20µm in B–I, and 50µm in J–L).
neuroinflammation induced by EBV infection and that attributed to immunosuppression warrants further investigation. Similarly, further investigations are required to understand how the cellular aggregates seen in the rabbits correlates with ectopic lymphoid follicles reported in the brain of MS patients (4, 80, 81).

Expression of EBV latent transcripts correlates with proinflammatory cytokines

Importantly, EBV latent transcripts, most notably EBV-encoded RNAs (EBERs) correlate positively with the proinflammatory cytokines II1β and II6 in the brain and spleen (56). EBER2 expression in vitro is associated with high levels of II-6 produced by infected B cells (82). This proinflammatory cytokine appears to be instrumental for the activation and expansion of EBV-infected B cells (82, 83). Additionally, proinflammatory IL-6 is markedly elevated in rabbits developing aggregates in the CNS. Thus, it is possible that this cytokine is a major player in the pathogenesis of EBV-associated neuroinflammation.

The mRNA levels of IL-1β, IFN-γ and TNF-α have also been observed to be significantly elevated at day 28 of infection in the spleen, brain and the spinal cord of rabbits. The expression of these cytokines in the CNS is associated with impairment of the brain-blood barrier (BBB) (84–88). CNS viral infections can trigger the production of these inflammatory cytokines, which compromises the integrity of BBB, for example by altering the expression of brain endothelia tight junction proteins (89–92). Thus, BBB breakdown could be both a pre-requisite and a consequence of CNS viral infections (90, 91, 93). One could argue that increased mRNA levels of these cytokines in EBV-infected rabbits may induce BBB leakage and recurrent influx of immune cells into the CNS. Whether EBV infection disrupts BBB integrity warrants further investigation.

Concluding remarks

In spite of substantial efforts over the last 6 decades in studying EBV, there are still many important gaps in our understanding of the details of viral pathogenesis and key aspects of the virus life cycle. There is a pressing need to understand how the virus behaves in the host and how that affects various organ systems. Studies on the link between EBV infection and the pathogenesis of CNS diseases, including MS is enormously expanding. There is now substantial and credible evidence that EBV is involved in the pathogenesis of MS (26, 66, 94, 95). Importantly, a number of studies have demonstrated the presence of EBV-infected cells directly in the white matter lesions in MS tissues (96–99). However, the mechanism by which EBV induces MS remains unclear. To characterize viral dynamics and virus interaction with the immune system, host genetic background and environmental cofactors, it is necessary to develop an in vivo model that captures both EBV biology and MS pathology. This review highlights the lessons we have learnt from several animal models used to understand the link between EBV and MS. While none of the models discussed here is a perfect representation of “EBV-induced MS”, utilizing them has provided insight into a number of potential factors, such as viral latent cycle and viral latent proteins, EBV-infected and transformed B cells, HLA-DR15, and epigenetic regulation of EBV-infected B cells, that may contribute to virus-induced CNS pathology. Exploring the various in vivo models for EBV would not only aid in the early identification of at-risk populations but also provide promising possibilities for therapeutic and prophylactic options for this incurable disease.

Author contributions

Conceptualization, writing the initial draft, and revision was performed by AH; conceptualization, writing part of the first draft and revision was performed by GK. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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