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The Role of Epstein-Barr Virus in the Pathogenesis of Multiple Sclerosis

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Abstract

Epstein Barr Virus (EBV) is a ubiquitous human lymphotropic herpesvirus which is also called Gamma herpesvirus and has been involved in several cancers. EBV is the first human tumor virus identified. There has been much epidemiological and mechanistic evidence to prove the causal role of EBV in multiple sclerosis (MS). Multiple Sclerosis is a chronic autoimmune disease of the central nervous system characterized by demyelination and neuroinflammation. For this paper, I aim to provide answers to questions like how a virus, which leads to benign latent infection, can promote cancers and autoimmune disease, how EBV plays an important role in the development of multiple sclerosis, how EBV is involved in the pathogenesis of multiple sclerosis and how this study will help to find the way for future MS treatments. Scientists have long suspected but failed to prove the link between viruses and multiple sclerosis (autoimmune disease). A study led by Stanford Medicine researchers proves that EBV triggers multiple sclerosis by priming the immune system to attack the body's nervous system. This research paper aims to provide an overview of the current understanding of the role of Epstein Barr Virus in the pathogenesis of multiple sclerosis.

Subject Areas

Cell Biology

Keywords

Epstein-Barr Virus, Multiple Sclerosis, Latent, Pathogenesis, Immune System

1. Introduction

1.1. Epstein Barr Virus

First, we should know about EBV, what EBV is, how it causes, its symptoms, and

its diagnosis.

EBV is also called Gamma Herpesvirus, and it belongs to the Herpes virus family. It spreads most commonly through body fluid, primarily "Saliva". It can cause infectious mononucleosis, called **mono** [1]. The symptoms of having EBV infection include fever, fatigue, rash, swollen lever, inflamed throat, enlarged spleen, and swollen lymph nodes. It can occur from childhood (don't show any symptoms) while in adults and teenagers, it shows symptoms but gets better in 2 -4 weeks. Spreads through body fluids, mostly saliva, but it also can spread through blood and semen during sexual contact, blood transfusion, and organ transplantation. It can be transmitted by using objects from an infected person like drinking glasses, toothbrushes, etc. EBV can spread through the infected person for several weeks. If it's in our body, it remains inactive, and then it doesn't transfer during that time [2]. The infection caused by EBV can be challenging because there is no specific treatment for this disease. But if you get infected with it, you can drink plenty of water, and take medication for pain and fever [3].

1.2. Structure

(EBV) has a toroid-shaped protein core that is wrapped with DNA, a nucleocapsid with 162 capsomers, a protein tegument between the nucleocapsid and the envelope and an outer envelope with external glycoprotein spikes. The major EBV capsid proteins are 160, 47, and 28 kDa, similar in size to the major capsid proteins of the herpes simplex virus. The most abundant EBV envelope and tegument proteins are 350/220 and 152 kDa, respectively [4]. The structure of Epstein Barr Virus is shown in Figure 1 below.

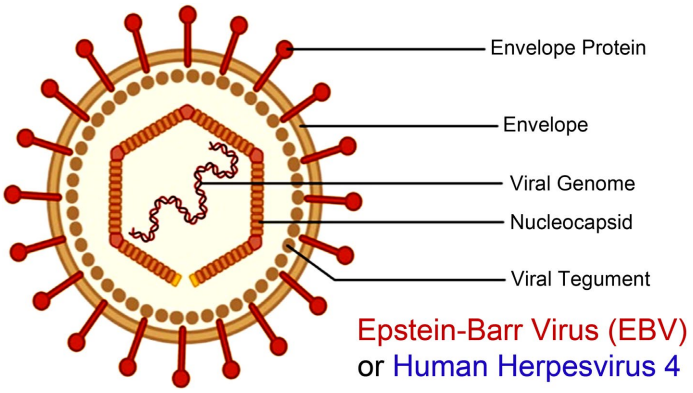

Figure 1. Structure of epstein barr virus.

1.3. Multiple Sclerosis

MS is a chronic autoimmune disease of the central nervous system characterized by demyelination and neuroinflammation. Most 2.8 million people have MS worldwide.MS is a condition that can affect the brain and spinal cord, causing a wide range of symptoms, including problems with vision, arm or leg movement, sensation, or balance [5]. It's a lifelong condition that can sometimes cause serious

disability. Mostly occurs in people between the ages of 20, 30, and 40, although it can occur at any age. MS is one of the most common causes of disability in younger adults [6]. The symptoms of MS include fatigue, difficulty walking, vision problems (blurred vision), problems controlling the bladder, muscle stiffness and spasms, problems with balance, and problems with thinking, learning, and planning.

1.4. Types of MS

Relapsing-remitting MS and Primary progressive MS

1) Every 8 - 9 people among 10 people with MS are diagnosed with relapsing-remitting type. They show the symptoms known as "Relapses". It lasts for days to weeks to months, then slowly improves over some period. Periods between relapses are known as "Periods of remission". These can last for years, and after many years, people with relapsing-remitting MS develop secondary progressive MS. But that's not the case for all of them. About 2/3rd of patients having relapsing-remitting MS will develop secondary progressive MS [7].

2) Between 1 and 2 among 10 people with MS, start to have worsening symptoms. There are no periods of remission, though people have periods where their condition starts to stabilize.

1.5. Cause of Multiple Sclerosis

It is an autoimmune disease, and it is caused when something goes wrong with the immune system and it mistakenly attacks a healthy part of the body, in this case, a brain or spinal part of the nervous system.

The immune system attacks the layer that surrounds and protects the nerve called "Myelin Sheath".

This damages the sheath, and underlying nerves, causing the message traveling along the nerves to become slowed or disrupted.

1.6. Treatments

There's currently no cure for MS, but several treatments can help control the condition and ease symptoms.

Treating relapses with short courses of steroid medicine speed up recovery.

Treatment to reduce the number of relapses using medicines called **disease-modifying therapies**. (See Table 1)

Table 1. The epidemiological, immunological, and virological evidence.

Evidence	Result	Refs.
Epidemiological Evidence	Low rates of MS in areas with more childhood infections	[8] [PubMed] [CrossRef] [Google Scholar]
	Increased risk of MS with a history of infectious mononucleosis	[9] [PubMed] [CrossRef] [Google Scholar]
	Increased risk of MS with EBV seroconversion	[10] [PubMed] [CrossRef] [Google Scholar]

Continued

Epidemiological Evidence	Decreased risk of MS in seronegative individuals	[11] [PubMed] [CrossRef] [Google Scholar]
Immunological Evidence	Increased level of EBV specific antibodies in MS	[12] [PubMed] [CrossRef] [Google Scholar]
	MS risk alleles enriched for transcription control by EBNA2	[13] [PMC free article] [PubMed] [CrossRef] [Google Scholar]
	Deficient cytotoxic T lymphocyte control of EBV in MS	[14] [PubMed] [CrossRef] [Google Scholar]
	EBV reactive OCBs	[15] [PubMed] [CrossRef] [Google Scholar]
	Molecular mimicry between EBNA1 and CNS antigens	[16] [PubMed] [CrossRef] [Google Scholar] Yea C, et al. [PubMed] [CrossRef] [Google Scholar]
	Increased shedding of EBV in saliva of pediatric patients with MS	[17] [PubMed] [CrossRef] [Google Scholar]
	EBV BZLF1 in MS lesions	[18] [PubMed] [CrossRef] [Google Scholar]
Virological Evidence	Pro survival influence of EBV latency genes on memory B cells	[19] [PubMed] [CrossRef] [Google Scholar]
	EBV loads correlate with T-bet+CXCR3+ memory cells and IFNγ MS B cells production	[20] [PMC free article] [PubMed] [CrossRef] [Google Scholar]

2. A Virus Which Leads to Benign Latent Infection Can Promote Cancers and Autoimmune Diseases

The virus that bears their name, Epstein Barr virus, was discovered exactly 60 years ago, according to pathologists Anthony Epstein and Yvonne Barr. It was the first virus in humans that had been shown to cause cancer. The pathogen, a member of the herpes family, was identified from tumor tissue by Epstein and Barr, who then conducted many tests to examine its potential to cause cancer. The majority of people are EBV carriers: over 90% of adults are infected with the virus, which typically causes no symptoms or sickness. Many people don't get it until puberty, although 50% get it before the age of five [21].

The infection is also thought to be connected to Multiple Sclerosis, an autoimmune disease, due to its canerorganic characteristics. In a subset of memory B-cells, EBV creates a latent infection that persists for life. Despite the important role B-cells play in the pathophysiology of multiple sclerosis, little is known about the EBV-associated mechanisms behind B-cell inflammation and disease

pathogenesis in EBV+B-cells from MS patients. EBV targets the infected cells' metabolism. Researchers under the direction of Professor Christoph Hess have outlined the process by which EBV reprogrammes immune cells, or "B-cells." This "transformation" process is what makes the infection persistent and leads to a number of illnesses, including MS and cancer, autoimmune diseases, and other conditions. The infection causes the virus-infected cell to produce more of the IDO1 enzyme. In the end, this results in increased energy generation by mitochondria, the powerhouse of infected cells. Consequently, the extra energy is required to boost metabolism and the quick division of B-cells that have been reprogrammed by EBV [22].

3. EBV Plays Important Role in the Development of MS

It's critical to understand how EBV differs from other infectious agents in MS. In many aspects, EBV is distinct from other infectious agents. Unlike other infections, which cause MS exacerbations to occur only a few weeks before the original onset of the disease, EBV infection causes the disease to manifest years before it does. Because EBV is the only agent that infects B-cells involved in the disease process, it remains in the host for the duration of the host's life, unlike most other infections that are eliminated after the acute phase. These characteristics of EBV demonstrate its critical role in the development of multiple sclerosis [23].

4. EBV is Involved in the Pathogenesis of MS

Numerous studies have demonstrated that ABs to common viruses such as EBV, mumps, and measles are elevated in MS patients. EBV antibodies, which signify a previous infection, are present in the blood of over 99% of MS patients. Even with this epidemiological correlation, researchers have not been able to establish a connection between MS and EBV. The researchers began by looking at the antibodies made by immune cells in the patients' blood and spinal fluid in an attempt to find a link between them [24]. They discovered protein patterns known as oligoclonal bands, which are discovered during spleen fluid analysis and are included in the MS diagnostic criteria. The oligoclonal band antibodies are analyzed, and the results indicate that the B-cells in the spinal fluid produced them. White blood cells called B-cells are produced in the bone marrow; the Robinson lab invented the technology to sequence these cells in 2015-16. Serum and spinal fluid from MS patients were previously tested on planar arrays and placed on histology slides for observation by researchers. For a long time, scientists have suspected—but not proven—a connection between viruses and multiple sclerosis. According to a study conducted by researchers at Stanford Medicine, the herpes virus, known as EBV causes multiple sclerosis by inciting the immune system to target the body's nervous system. Individuals with multiple sclerosis (MS) have antibodies in their blood that bind tightly to glial cell adhesion molecule, or Glial CAM, a protein produced in the brain and spinal cord, as well as a protein from the EBV called EBNA1. A portion of the EBV protein resembles Glial CAM, a host protein that

is present in the insulating sheath of nerves. This implies that the immune system targets Glial CAM in myelin when it assaults EBV to eradicate the virus. Damage to myelin, which surrounds nerve cells and acts as a protective layer, prevents electrical impulses from traveling from one nerve to another, which causes muscle weakness, numbness, and exhaustion [24].

Mouse Model: The Experimental Autoimmune Encephalomyelitis mouse model of multiple sclerosis was utilized by the researchers. Compared to mice injected with a control protein fragment, the mice that received an injection of a fragment of EBNA1 protein showed more severe paralysis, more immune cells invading the central nervous system, and more damage to the protective coating on their nerve cells. This offered more evidence supporting EBV's connection to MS [25].

5. Methods and Analysis

EBV can be identified using the following techniques. It is possible to determine through laboratory testing whether a person has had an infection in the past or is susceptible to contracting EBV infection. These techniques help us identify the EBV infection and manage an autoimmune condition [26].

5.1. Viral Capsid Antigen (VCA)

Anti-VCA IgM typically vanishes in four to six weeks after the onset of an EBV infection. Anti-VCA IgG first manifests during the acute phase of EBV infection, peaks two to four weeks after onset, then gradually declines before remaining constant for the duration of the infection.

5.2. Early Antigen (EA)

Anti-EA IgG typically reaches undetectable levels three to six months after the acute phase of illness. The presence of an antibody to EA is frequently indicative of active infection. Nonetheless, 20% of healthy individuals may have EA-specific antibodies for years.

5.3. EBV Nuclear Antigen (EBNA)

According to a standard immunofluorescent test, an antibody to EBNA is not visible during the acute phase of EBV infection but instead develops gradually two to four months after symptoms start and lasts the entirety of an individual's life. False positive results from other EBNA enzyme immunoassays are possible.

5.4. Monospot test

Conditions other than infectious mononucleosis can cause the antibodies that the Monospot test detects. Furthermore, research has demonstrated that the Monospot generates false negative as well as false positive results. For instance, children with infectious mononucleosis frequently lack the heterophile antibodies identified by Monospot. The Monospot test, at most, might reveal that a person has a typical case

of infectious mononucleosis but does not confirm the presence of EBV infection (See Table 2).

Table 2. Biomarker, Detective method, and examples.

Biomarker	Detective Method	Examples	Tissue
Antibodies to VCA, EBNA-1, CF/S antigens, neutralizing anti-g350, EA	Serologic, indirect IFA, EIA	Detected in healthy EBV carriers, EA detected in some carriers: higher titers with Burkitt lymphoma	Serum
EBV DNA	PCR	Detection in healthy carriers	WBCs
Cell free EBV DNA	Quantitative PCR	EBV associated diseases: healthy carriers are negative	Serum
EBV DNA	Quantitative PCR	Determination of viral load per cell	Tumor cells, white blood cells
EBER-1, EBER-2 RNAs	Reverse transcription-PCR	EBV infection: highly sensitive for Hodgkin disease detection	Tumor cells, tissue biopsy
EBNA-1 and BARF1 RNA	Reverse transcription-PCR	NPC detection	NPC biopsy or brushing

6. Pathways involved in MS pathogenesis

Because of several characteristics, EBV can infect B memory cells latently and avoid detection by the immune system. EBV accomplishes this by inducing these infected B cells to change from activated B cell blasts to latently (latency I and II) infected resting memory B cells through several unique latency transcription programs that take advantage of normal B cell differentiation pathways. A number of theories have been put forth to explain how EBV infection might contribute to the pathophysiology of MS (Table 3). EBV may function both within the central nervous system (CNS) and in peripheral immune responses that lead to relapses. Relapses may be caused by peripheral EBV-mediated CNS-reactive immune cell activation and trafficking. The molecular mimicry theory explains how T cells primed by exposure to EBV antigens cross-react to identify and attack CNS antigens. A different theory proposes that αB-crystallin could be mistakenly identified as a self-protein. According to the αB-crystallin hypothesis, peripheral B cell infection with EBV can cause αB-crystallin to be expressed in lymphoid cells. This, in turn, can cause a CD4+ T cell response to αB-crystallin, which is also expressed in oligodendrocytes. A different theory known as the Pender hypothesis explains how EBV may preferentially induce proinflammatory B cell cytokine responses, such as those that result in the expression of tumor necrosis factor (TNF), lymphotoxin (LT), IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF), and disrupt IL-10's ability to be downregulated. The ability of EBV to induce expression of EBI2/GPR183, a theory known as the autoreactive T-cells hypothesis, partially explains the migration of autoreactive T cells and EBV-infected B cells into the central nervous system [27].

Table 3. EBV hypotheses, evidence, and references.

Hypothesis	Evidence	References
EBV-infected auto-reactive B-cells (Pender Hypothesis)	i) EBV-infected B and plasma cells can be found in the MS brain.	[28] [PubMed] [Google Scholar]
	ii) EBV-infected auto-reactive plasma cells can be found in the synovium in rheumatoid arthritis and in the salivary glands in Sjogren's syndrome.	[29] [PubMed] [Google Scholar]
	iii) MS patients are more likely to be EBV seropositive than controls.	[30] [PMC free article] [PubMed] [Google Scholar]
	iv) Beneficial effects of B-cell depletion in MS.	[31] [PubMed] [Google Scholar]
	v) There is no MS in the absence of EBV serology.	[32] [PubMed] [Google Scholar]
	vi) High levels of IgGs antibodies against EBNA1 enhance the likelihood of getting MS. (Blood samples from US military army)	[33] [PubMed] [Google Scholar]
EBV bystander damage	i) Bystander T-cells contribute to pathogenesis.	[34] [PubMed] [Google Scholar]
	ii) EBV virus infections can lead to significant activation of APSs such as dendritic cells which could activate auto-reactive T-cells, thus initiating autoimmune disease.	[35] [PubMed] [Google Scholar]
αB-Crystallin (Mistaken self)	i) *α*B-crystallin is reported to be an immunodominant antigen in the CNS.	[36] [PubMed] [Google Scholar]
	ii) *α*B-crystallin is the dominant myelin-associated activator of humans T-cells and accumulates in oligodendrocytes.	
	iii) EBV induces the expression of αB-crystallin in B-cells which present the protein to CD4+ T-cells in an HLA-DR-restricted manner.	
Molecular mimicry: cross reactivity	i) 3% - 4% of EBNA1-specific CD4+T-cells in healthy subjects and MS patients react with peptides derived from myelin proteins.	[37] [PubMed] [Google Scholar]
	ii) IgG recognizing peptides from EBV, and MBP85-98 are elevated in MS patients.	[38] [PubMed] [Google Scholar]
EBV-induced B-cell cytokine response	i) EBV infection may interfere with the down-regulatory function of innate IL-10, potentially through the production of vIL-10.	[39] [PubMed] [Google Scholar]
	ii) Pro-inflammatory cytokines play a key role in MS pathology.	[40] [PubMed] [Google Scholar]
	iii) Cytokine-secreting B-cells in the periphery may influence new disease activity and play a role in disease activity in the CNS.	[41] [PubMed] [Google Scholar]

Genetic Susceptibility

The observed aggregation of MS implies that hereditary factors contribute to an individual's greater susceptibility to MS, in addition to environmental influences such as EBV [42]. Moreover, several investigations have revealed that both non-

HLA genes and the same single nucleotide polymorphisms within the human leukocyte antigen (HLA) gene can raise the anti-EBV antibody response [43]. EBV genome genetic variations, such as the EBNA-2 specific 1.2 allele, are linked to an increased risk of multiple sclerosis (MS), but not to clinical or magnetic resonance imaging characteristics [44]. Recombining binding protein suppressor of hairless (RBPJ), an EBNA-2 cofactor, and human transcription factors may form a complex with EBNA-2 protein to occupy autoimmune-associated genetic risk loci and modify gene expression (Harley et al., 2018). According to their findings, 44 of the 109 MS risk loci are occupied by the EBNA-2 protein, suggesting that a possible gene-environment interaction underlies the pathophysiology of the disease (Harley et al., 2018). Additionally, 26 out of 53 systemic lupus erythematosus risk genes showed EBV-associated gene interaction [13].

7. This Study Will Help to Find the Way for Future MS Treatments

Effective control of EBV infection has been developed as a means to prevent or cure autoimmune diseases. In MS, controlling EBV infection could be achieved by B cell depletion, antiviral drugs, boosting immunity, or improving immune responses.

7.1. Antiviral Drugs

Several antiviral compounds, including raltegravir, abacavir, zidovudine, stavudine, and famciclovir, have been studied as potential MS therapies [45]. Because antiviral drugs help treat HIV/AIDS, HPVs have been linked to multiple sclerosis, and they have anti-EBV properties that include inhibiting EBV DNA replication, there has been a push for their usage. Acyclovir and famciclovir have not been tested as treatments for multiple sclerosis (MS), however, they are useful in treating shingles, chickenpox, genital herpes, and herpes zoster. Acyclovir, penciclovir, and ganciclovir—antiherpesviral nucleoside analogs—have all drawn interest. Regretfully, research on MS has not demonstrated any impact on the course of the condition. According to additional investigations, human IFN-β, a powerful antiviral protein, is still ranked among the top five MS therapeutic choices [46].

7.2. Vaccination

There are numerous instances where human disease has been eradicated through vaccination or other methods of limiting viral spread. However, as of right now, no vaccine can guard against EBV infection [47]. Targeting gp350 or other viral proteins might be one way to go about creating such a vaccine. In MS, a short experiment assessing a vaccination against EBV produced mixed outcomes. Studies on immunization in multiple sclerosis may benefit from recent developments in vaccination, particularly in the area of genomic vaccines, which can transmit numerous protein-coding sequences [48].

While creating a preventative vaccination to stop or inhibit acute EBV infection

is an intriguing idea, there are significant obstacles in the way of realizing this goal to stop MS from developing. The nearly impossible goal of achieving sterile immunity against any herpes virus presents a hurdle in the development of an EBV vaccine. Ideally, a vaccine that can stop acute IM might be enough to lower the chance of getting MS. Promising results from recent trials on alternative herpesvirus vaccines have supported the idea that it would be possible to create a vaccination that prevents illness rather than infection [49].

7.3. Anti-EBV Antibodies and Viral Pathway Targeting

Immunity against EBV would be strengthened by antibodies that recognize EBV proteins expressed during latency, such as EBNA1, LMP1, and LMP2a [50]. Numerous genome-wide association studies (GWAS) have revealed over 250 variations that relate to the susceptibility to MS disease, which may provide information about other viral targets. Furthermore, given that EBV genetic variations are linked to multiple sclerosis, the increasing amount of human genetic data categorized as viral interactomes or transcriptomes of B cells and EBV-infected B cells may be helpful when choosing novel targets. Reducing EBV reactivation may also be accomplished by targeting B cell pathways (such as BAFF and BHRF1) or by using siRNAs that target EBV genes (such as LMP1, LMP2a, and EBNA1) to downregulate expression and cause death in EBV-infected cells [51].

7.4. Cell-Based Immunotherapies

Immunotherapies based on cells, particularly those that target transformed cells infected with EBV, have demonstrated effectiveness. Treating autoimmune disease (s) like multiple sclerosis (MS) with cell-based immunotherapies that aim to lower EBV reactivation may have a similar impact, particularly if those immunotherapies target cell types that are known to be involved in disease pathogenesis. Cell-based therapies include oligodendrocyte progenitor cell transplantation, autologous hematopoietic stem cell transplantation, mesenchymal and related stem cell transplantation, immunoablation to deplete the immune system, and introduction of endogenous stem cells followed by an increase in their reparative capacity [52].

An alternate cell-based strategy is offered by the new use of autologous or allogeneic T cell therapy that targets EBV-infected B cells utilizing cytotoxic CD8+ T cell therapy that is specific to EBV. A case in point of therapy effects likely to occur in the central nervous system (CNS) involves a patient with secondary progressive MS (SPMS). In this case, infusion of EBV-specific CD8+ cytotoxic T cells did not result in any negative consequences, and the patient showed clinical improvement with lower disease activity on magnetic resonance imaging (MRI) and decreased intrathecal immunoglobulin production. The percentage of circulating LMP and B-lymphoblastic cell line (LCL)-reactive effector CD8+ memory cell populations increased concurrently with this [53]. A Phase I clinical trial of autologous EBV-specific T-cell treatment for progressive multiple sclerosis was

initiated as a result of the study's success. No significant side effects, such as a worsening of the condition, were reported by the authors. Additionally, they stated that a clinical improvement was seen by seven out of the ten treated patients. A Phase I trial is being conducted to assess the safety of allogeneic EBV-specific cytotoxic T-cell treatment in patients with progressive MS and RRMS. Targeting EBV-infected cells with a cell-based strategy has the advantage of having less off-target effects and the ability to target infected B cells in the peripheral and central nervous system, which may help with both relapse and non-relapse MS [54].

8. Conclusion

The evidence supporting the involvement of EBV in the pathogenesis of MS highlights the virus as a potential trigger for the autoimmune processes underlying the disease. 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 time between the EBV infection and the disease makes it difficult to study this interaction. It is unclear whether the pathogenic pathways are directly or indirectly linked to EBV infection or the immune response of the virus.

Vaccines against EBV could perhaps eventually eradicate MS. We need to be extra careful in selecting which antigens to incorporate into an EBV vaccine because choosing antigens like EBNA can cause autoimmunity. There are two promising technologies, one involving adverse vaccines using DNA plasmids and another using RNA technology.

EBV causes MS in genetically susceptible individuals by infecting autoreactive B cells, which seed the CNS, where they produce pathogenic autoantibodies and provide survival signals to autoreactive T cells that would otherwise die in the CNS by apoptosis. Infection with EBV increases the risk more than 30-fold, indicating that EBV is a leading cause of MS.

Conflicts of Interest

The author declares no conflicts of interest.

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