British Microbiology Research Journal
6(1): 1-12, 2015, Article no.BMRJ.2015.053
ISSN: 2231-0886

SCIENCEDOMAIN *international*
www.sciencedomain.org

Association of Multiple Sclerosis with Epstein-Barr virus Infection

Masoumeh Ahmadi Jalali Moghadam[1] and Hamidreza Honarmand[2*]

[1]Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran.
[2]Department of Microbiology, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran.

Authors' contributions

This work was carried out in collaboration between both authors. Author MAJM designed the study and wrote the protocol, and author HH managed the literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/12613
Editor(s):
(1) Eggehard Holler, Cedars-Sinai Medical Center, Department of Neurosurgery, Los Angeles,USA and University of Regensburg, Germany.
Reviewers:
(1) Anonymous, University of Thessaly, Larissa, Greece.
(2) Mickael Bonnan, Service de Neurologie, Centre Hospitalier F. Mitterrand, 4 Boulevard Hauterive, Pau 64000, France.
(3) Anonymous, Institute of Experimental Morphology, Bulgaria.
(4) Paola Di Carlo, Department of Sciences for Health Promotion and Mother-Child Care "G. D'Alessandro", University of Palermo, Via del Vespro 127, I- 90127, Palermo, Italy.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=827&id=8&aid=7135

Review Article

Received 10th July 2014
Accepted 19th August 2014
Published 8th December 2014

ABSTRACT

Purpose of Review: There is still controversy whether EBV could be a causative agent as opposed to an innocent bystander in the pathogenesis of multiple sclerosis (MS). We aimed to review main studies which were investigated the subject to reveal whether the presence of a possible latent or active infection with Epstein-Barr Virus (EBV) of people with MS could actually play a role in the development of the disease. This review summarizes current knowledge on the association of EBV and MS.
Summary and Results: Multiple sclerosis (MS) is a demyelinating condition affecting the central nervous system. The etiology and pathogenesis of MS are unknown, but environmental agents and genetic susceptibility are likely to be involved. From the very early days of MS discovery, infections have been proposed to be the underlying causes of disease initiation. This assumption led to the development of the first FDA-approved immune-modulatory treatment for MS, Interferon-beta (IFN-b), known with its antiviral activities. It has been pointed out that a link between delayed infection

**Corresponding author: Email: honarmand.3@gmail.com;*

with EBV and the development of MS is compatible with many unusual epidemiological features of the disease EBV infects more than 90% of all humans, most of whom remain healthy. In contrast, 99% of MS patients have evidence of prior infection with EBV. EBV infects resting B-lymphocytes, immortalizing them into long-lived memory B-cells that survive largely undetected by the immune system in the peripheral circulation. MS patients show elevated titers to EBV years before developing any neurologic symptoms. Postmortem pathologic analysis of brains of patients with MS has revealed diffuse EBV-associated B-cell dysregulation in all forms of MS. Theories of pathogenesis of EBV in MS include antigenic mimicry, immortalization of B-cell clones, and cytotoxic T-cell dysfunction against virally infected B cells. This article reviews the existing evidence of the relationship between EBV and MS.

Keywords: Multiple sclerosis; epstein-barr virus; autoimmunity.

1. INTRODUCTION

An autoimmune disorder is a condition that occurs when by mistake the immune system attacks and destroys healthy body tissue [1]. In patients with an autoimmune disorder, the immune system can't tell the difference between healthy body tissue and antigens. The result is an immune response that destroys normal own body tissues [1]. Auto immune diseases are the third leading reason of morbidity and mortality after heart diseases and cancer in the industrialized world. Researchers are looking into the role of different factors in the development of autoimmune disorders. It seems that some microorganisms and drugs may trigger some of the changes, especially in people who possess genes that make them more likely to develop autoimmune disorders. One theory is that, a combination of genetic, epigenetic, immunological, hormonal and environmental factors, comprising what is known as 'the mosaic of autoimmunity', is required for autoimmune disorders to develop [2]. Among these key elements, the impact of infections on the development of autoimmunity is substantial, and various mechanisms have been suggested to explain this association [2,3].

Post-infection autoimmunity can be induced by multiple mechanisms, such as molecular mimicry, epitope spreading, bystander activation, viral persistence and polyclonal antibody activation. Triggering of autoimmunity is not always a hit and run event, but rather a cumulative process. The immune system is affected by repeated infections from childhood, and in immune-sensitive individuals, a breakthrough point might occur when the infection burden crosses a crucial level. This breakthrough point might be reached when a specific pathogen load, immune load (i.e. antibody titer) or an unique combination of pathogens is established [4].

Multiple sclerosis is an inflammatory disease leading to disseminated lesions of the central nervous system (CNS) resulting in both somato-motor and autonomic disturbances which occur with similar frequency. MS is the most common demyelinating disease of the human central nervous system, which principally affects adults aged 18–50 years. Women are generally affected earlier and more frequently than men. Most patients present with a relapsing disease, progressing over 10–15 years to a chronic phase with increasing difficulty in movement and co-ordination [5].

MS usually begins in early adulthood and is characterized by demyelination and gliosis, with various degrees of axonal pathology and episodic or progressive neurological disability. More than 1 million people worldwide are affected by MS. It is second only to trauma as a cause of acquired disability in young adults in most Caucasian populations [6]. Numerous studies on the genetic epidemiology of MS provide compelling evidence that the susceptibility to the disease is mostly non-genetic, and additional environmental factors might be necessary to trigger it. The disease prevalence of MS varies between 60 and 200 per 100,000 people in North America and Northern Europe and generally follows a north-to-south gradient in the Northern hemisphere and the opposite in the Southern hemisphere, with very low rates or a virtual absence of the disease near the equator [5].

A persistent synthesis of IgG antibodies in the cerebrospinal fluid (CSF) is an immunological hallmark in MS. In the steady state, only a very low number of B-cells are trafficking through the human brain [7]. Once inflammation has started, however, B-cells, antibodies, and complement

can enter the CNS compartment and B-lymphocytes, plasma cells, and myelin-specific antibodies are detected in late chronic MS plaques as well as in areas of active demyelination in MS patients [8]. Auto-reactive antibodies can cause demyelination by opsonization of myelin for phagocytosis and via complement activation, leading to membrane attack complex deposition and complement-mediated cytolysis [9]. In contrast to the phenotypic composition of B cells in the blood, most of the B-cells in the CSF of patients with MS display a memory phenotype ($CD27^+$) [10]. A receptor analysis of T- and B-cells in the CSF and brain tissue of patients with MS showed clonal expansions in both populations, indicating clonal reactivity to just a few disease-relevant antigens that are yet incompletely defined [11].

2. MS AND INFECTIONS

The cause of multiple sclerosis (MS) is not known, but the environment and the immune responses are accepted as key players in the disease process. Environmental studies suggest that some factor – probably infectious – must be encountered before the age of 15 in order MS develop later in life. Also, epidemiological data and the inflammatory nature of the lesions have driven the research enterprise in search of a pathogen in MS. Several viruses and bacteria, including Epstein-Barr, Chlamydia, pneumonia, measles, canine distemper, and human herpes virus-6 have been or are being studied to determine if they may trigger MS, but none have been definitively proven to do so as of yet. Some data suggest that a common virus may play a role in the etiology of MS. Whether it is a persistent viral infection or an immune reaction caused by a temporary viral infection in the CNS or elsewhere in the body is not clearly known. To satisfy a causal association between MS and an infectious agent, the pathogen should ideally (a) cause a chronic inflammatory disorder of the CNS, (b) preferentially reside within the CNS and undergo periods of activation and quiescence, and (c) cause demyelination.

Current opinion favors the notion that MS is an autoimmune disease directed against self-neural antigens [12]. The auto-antigens that may be responsible for the auto-immunity have remained elusive [13]. Much of the speculation that has driven the notion of autoimmunity in MS is due the similarities between MS in human and the mouse model of experimental auto-immune encephalitis [13].

Several mechanisms by which infections can cause demyelination, have been proposed: direct and indirect [14]. A virus can infect oligodendrocytes leading to its lysis or apoptosis, with consequent demyelination. This is seen in PML, where the infection by the JC virus leads to caspase activation in oligodendrocytes, leading to their apoptosis, and in the TMEV model, where productive viral infection leads to lysis of oligodendrocytes, by activation of cytotoxic T-cells [15,16]. Viral infection can also lead to induction of an autoimmune response by molecular mimicry or bystander activation [4]. In the molecular mimicry model, shared antigenic determinants between putative infectious pathogens and myelin antigens in a genetically susceptible individual lead to the development of autoreactivity and ultimately autoimmune demyelination. In the bystander activation model, microbial infections lead to significant activation of antigen-presenting cells (APCs) such as dendritic cells. These activated APCs could potentially activate pre-pri-medauto-reactive T-cells, which can then initiate autoimmune disease [17].

3. MS AND EBV

From the very early days of MS discovery, infections have been proposed to be one of the underlying causes of disease initiation. This assumption led to the development of the first FDA-approved immuno-modulatory treatment for MS, by application of Interferon-beta (IFN-b), known with its antiviral activities. Epstein-Barr virus (belongs to the herpes virus family) was identified in 1964 by Epstein and colleagues from a lymphoma of the jaw, which had been recognized in Central African children by Burkitt lymphoma. Several years later it was recognized that EBV was the etiologic agent of heterophil-positive infectious mononucleosis(IM). In addition to being the cause of infectious mononucleosis, EBV has also been associated with rheumatoid arthritis and several neoplastic conditions including Burkitt lymphoma, nasopharyngeal carcinoma, hairy cell leuco-plakia, and primary CNS lymphoma [18]. EBV infects naive human B-cells, causing their clonal expansion and subsequent life-long latent infection in mature memory B-cells. In the resting memory B-cell, no proteins are actively expressed, and, hence, antigen cannot be detected on their surface [19].

EBV has also been associated with a variety of CNS complications, including meningo-encephalitis, encephalitis, cerebritis, transverse

myelitis, neuro-psychiatric syndromes, cranial nerve palsies, Guillain-Barré syndrome, and mono-neuropathies. These usually occur 1 to 3 weeks after the onset of IM,but they may also occur at the outset of the disease. The pathogenesis of these complications has not been clarified. Studies suggest that some complications are due to direct viral infection, whereas the reasons for others are due to auto-immune reactions [20].

It has been pointed out that a link between delayed infection with EBV and the development of MS is compatible with many unusual epidemiological features of the disease [20]. In developing countries, where MS is rare, early infection with EBV is almost universal. By contrast, in those areas of the world, in which IM is common and where, by implication, first exposure to EBV is often delayed beyond the early years of childhood (Australia, Canada, many European countries, New Zealand, Scandinavian countries and the United States), the prevalence of MS is high. Furthermore, the age-specific incidence curves for IM and MS are similar; the peak of the curve for IM precedes the peak of the curve for MS by a few years.

Researchers don't yet understand how the EBV causes the body to attack its own central nervous system, as it does in MS. "The mounting evidence that relates EBV infection with other auto-immune diseases, particularly systemic lupus erythematosus (lupus), suggests that EBV may have a broad role in predisposing to auto-immunity or failure of the immune system to recognize the own tissues of the body. A fine understanding of the mechanisms that connect EBV infection to MS is important because as it would provide possibilities about finding of new ways for treatment and control of MS.

Since the early 1980s, remarkable similarities between the epidemiology of MS and IM have been noted [21]. Several epidemiological features of MS, such as the association with higher socio-economic status, occurrence of clusters and epidemics, changes in the prevalence with latitude and changes in the risk of the disease with migration, could possibly be explained by a role for EBV in the pathophysiology of MS [22].

Although the epidemiological evidence linking EBV infection to MS risk is rather compelling, the mechanisms underlying this link remain unclear. One possibility is that EBV-infected B-cells infiltrate the MS brain and elicit a cytotoxic T-lymphocyte response with damage to surrounding tissue. Alternatively, EBV could contribute to MS onset acting outside of the central nervous system. One hypothesis is that of molecular mimicry between EBV and myelin antigens, which could involve antibodies.

4. EBV SERO-PREVALENCE IN PATIENTS WITH MS

A multitude of epidemiological studies, conducted in the past 30 years, revealed that MS patients are almost universally, 99.5%, sero-positive for EBV infection, compared to matched healthy controls that have EBV sero-prevalence of 94.2%. This difference in sero-positivity is even more pronounced in pediatric MS cases which have been shown to carry 83% sero-prevalence compared to only 42% in matched healthy controls, while no significant difference was observed for other viruses such as cytomegalovirus (CMV), parvovirus B19 and *Varicella zoster virus* (VZV).

Earlier studies reported that 100% of the MS patients have serological signs of a previous EBV infection [22] and that a disproportionally high percentage of MS patients have elevated titers of anti-EA antibodies. The presence of these anti-EA antibodies indicates acute or chronic active EBV infection and onset of viral replication [16,17]. There are indications that the primary EBV infection in MS patients has occurred years before the onset of neurological symptoms [22], with a possible risk enhanced role for primary infections occurring relatively later in life (adolescence) [25]. Normally, EA titers decline within weeks or months after a primary infection or a reactivation of EBV.

Many researchers have demonstrated higher titers of EBV antibodies in MS cases compared to controls [23]. Alter et al. [24] compared the data on positive serologic titers to childhood infections in high and low MS frequency areas and generally found a much lower percentage of sero-positives in the areas at high MS risk. EBV sero-positives aged 4±6 years in northern Europe were 41 to 50% whereas at the same age they were 76 to 95% in some developing countries [25]. In a case-control study recall of infectious mononucleosis was associated to a significant relative risk of 1.9 which increased to 2.9 in subjects seropositive for EBV and people reporting IM before age of 18 years had a relative risk of 7.9 [26]. Many studies lend

support to the notion that IM usually indicating a late EBV infection is usually associated with an increased risk for the individual to develop MS. This suggestion confirms previously proposed evidence on an epidemiological relationship between late exposure to childhood diseases and subsequent MS.

A strong positive association for a history of IM was found among MS patients in another case-control study [27]. A combined analysis of case-control studies on this specific topic showed that EBV clinical infection almost doubles the risk of MS. Most epidemiological studies are retrospective: the history of earlier diseases is recalled for cases and controls. Prospective studies, in which exposed people are followed to the possible occurrence of the disease, are relatively rare because of the low population risk of MS and the long follow-up period required. In addition, reliable population MS statistics and follow-up of a large number of unexposed matched controls are necessary. By cross-referencing a cohort of IM cases with a MS register in Sweden, three MS cases were recruited, corresponding to a relative risk of 3.7 for MS to occur after IM [28].

The Danish historical prospective study compared data from the National State Serum Institute with the National MS Registry. Among the people with positive antibodies 16 cases of MS were found. The expected number was 5.7, and the risk ratio - 2.81. Among the negative subjects the expected number of MS cases was found [29].

In 1990s, interesting findings have been reported by many groups, and higher titers have been reported for EBV nuclear antigen (EBNA) antibodies as well [30-46]. During or after puberty, EBV is transmitted to a major proportion of the population in an MS high prevalence area. Haahr et al. [36] demonstrated that recall of diagnosed IM, but not recall of common childhood diseases, is significantly more frequent among MS patients than healthy controls. All MS patients, including those without prior immunosuppressive treatment, were EBV seropositive.

An increase in EBNA antibodies may precede the clinical onset of MS by 5–20 years [34,36,38,41]. DeLorenze et al. [40] who investigated serum samples of MS patients up to 30 years before disease onset and determined a significant increase in EBNA 1-specific IgG titers compared to the matched controls, which occurred 15–20 years prior to disease onset.

MS-associated differences of the humoral immune response to EBV appear to be even more pronounced in pediatric than in adult patients, presumably because the rate of sero-negative individuals is higher in that age group, and potentially also because of the closer proximity to the true onset of the disease [43-45].

In 2000, Ascherio and Munch have performeda systematic review of the case-control studies of EBV infection and MS. Eight published investigations were identified, including a total of 1,005 cases and 1,060 controls. The summary odds ratio of MS comparing EBV seropositive individuals with EBV sero-negative individuals was 13.5 (95% CI = 6.3-31.4) [46]. The strength and consistency of this association, as well as of the high sensitivity and specificity of EBV serology suggest that these results are not readily explained by anon-specific immune activation among MS patients. The consistency of association across the studies supported the likelihood that EBV plays a part in the etiology of MS [47].

A recently done meta-analysis of all published studies on the association of MS and infectious mononucleosis revealed a combined relative MS risk of 2.3 for individuals with IM as compared to EBV positive individuals with a clinically silent primary infection [48]. A longitudinal study including 25,234 Danish patients with IM exactly confirmed these data: 104 patients (4.1‰) developed MS, representing a 2.3-fold increase in MS incidence as compared to the general population. The MS risk increased within 5 years of the IM, and remained elevated for more than 3 decades [49]. Combining the results of the meta analysis with those from other investigations on EBV, a model for the relation between EBV infection and MS has been proposed: the risk for MS is close to zero among EBV-negative individuals, intermediate among those infected with EBV in early childhood and the highest among persons infected in adolescence or later in life [48].

Actually, many studies advocate the role of EBV in MS. Ramagopalan et al. [49] reported that EBV sero-prevalence was higher in MS patients compared to controls (99% versus 90–95%) and MS showed to have a clear and reproducible clinical relation with IM. Serum and intra-thecalIgG levels to the latency-associated EBNA-

1 are elevated before the onset of MS and they alsocorrelates with disease activity and prognosis [38,48-51]. Contrastingly, IgG to lytic EBV proteins including the viral capsid antigen (VCA) are not changed or they are only marginally increased, suggesting that EBV abnormalities in MS are associated with B-cell responses to latent EBV antigens [48,50]. Serafini et al. [51] reported the presence of EBV-infected B-cells in meninges and perivascular regions of MS lesions. However, these observations as well as the involvement of a local EBV-specific B- and T-cell response are still under debate [48,52–54].

Another meta-analysis of 14 case–control and cohort studies of IM and MS calculated the combined relative risk of MS after IM was 2.3 (95% CI, 1.7–3.0; $p<10^{-8}$) [55]. Based on a recent updated meta-analysis evaluating a total of 18 clinical studies, the combined relative risk for development of MS after IM was estimated at 2.17 (95% CI 1.97–2.39; $P < 10^{-54}$) [56]. Apart from indicating a potential role of EBV in the pathogenesis of MS, these findings also suggest that the timing of primary EBV infection is an important factor in developing MS. Those who are infected in adolescence or young adulthood have a higher risk of developing MS than those infected during childhood [48]. It has been shown that in high prevalence regions for MS, sero-conversion for EBV occurs during or after puberty in a large proportion of the population [57].

A correlation between MS and delayed exposure to EBV could also account for the observation that migrants from areas where multiple sclerosis is uncommon tend to retain a low risk of disease while those moving in early life from areas where the prevalence of multiple sclerosis is high, experience a reduction in risk [25].

5. INTRA-THECALSYNTHESIS OF EBV-SPECIFIC ANTIBODY

There are several reports pointing towards a possibly increased EBV-targeted humoral immune response in the CNS of MS patients. The first of these studies analyzed the CSF-to-serum antibody ratios of EBV and adenovirus in MS patients: 32 of 39 MS patients (82%) showed an increased CSF-to-serum EBV–VCA antibody ratio, whereas only 4 of 20 MS patients (20%) showed an increased adenovirus antibody ratio [31]. An intra-thecal antibody response to the EBV protein BRRF2, and also an oligo-clonal binding pattern of CSF IgG to EBNA-1 and BRRF-2 proteins in MS patients, have been reported. Partoftheir CSF oligo-clonal bands were absorbed by pre-incubation of CSF with EBNA-1 [46]. In a Danish study, more than 25,000 patients with IM observed and more than two-fold increased risk of MS in the IM cohort was seen that is further confirming of the association between IM and MS [57]. The authors also found that the risk of MS development was increased for more than 30 years after IM. These findings can be interpreted as an indication for a raised CNS antibody production against EBV in patients with MS. Similar conclusions were drawn from another survey, in which 10 of 15 MS patients showed intra-thecal IgG antibody synthesis against EBNA-1 protein [57].

EBNA-1 is the only EBV-encoded antigen that is consistently expressed in proliferating EBV-infected memory B-cells, and $CD4^+$ T-lymphocytes are thought to play an important role in the immune control of persistent EBV infection. While healthy EBV-carriers preferentially recognize multiple epitopes within the central part of the immunogenic domain of EBNA-1, MS patients have been shown to have significantly elevated EBNA-1-specific $CD4^+$ T cell frequencies targeting a much larger number of epitopes within this region [58].

Jafari et al. [59] observed no difference in the overall anti-EBV antibody diversity, but in the EBNA-1 reactivity, it was significantly increased in MS patients versus control, according to immune-blot and ELISA ($p < 0.0001$). Epitope analysis on EBNA- 1 revealed one immuno-dominantregion covering residues 394–451($EBNA^{1394–451}$). Anti-$EBNA^{1394–451}$IgG levels in serum and CSF were significantly higher in MS patients compared to controls [59]. However, normalization for total IgG content of paired serum and CSF samples abrogated this disease association. The same authors reported that MS patients have normal overall anti-EBV antibody responses with increased reactivity to $EBNA\text{-}1^{394\text{-}451}$ and not found evidence for intrathecal EBNA1-specific IgG synthesis in MS [59]. They also suggest that antibodies to EBNA-1-specific domains and HLA DRB1*1501 interact as risk factors [59].

Results from another study showed no evidence for intra-thecal anti-EBV IgG synthesis [60].However, whether peripheral infection or immune response plays a pathogenic role, still

remains to be determined. Notably, the MS-associated EBNA-1$^{394–451}$ region identified encompasses several immuno-dominant HLA DR-, including potential HLA DRB1*1501-restricted CD4+ T-cell epitopes [61,62]. Moreover, MS patients have elevated frequencies and broader epitope reactivity of EBNA-1-specific CD4+ T-cells [62], including specific T-cells that cross-reacted with MS-associated myelin proteins [60-67]. According Lunemann et al. [63] EBNA 1-specific CD4$^+$ T-lymphocytes, but not T-cells, specific for other lytic or latent EBV and CMV peptides, were observed to have a higher proliferative capacity and enhanced IFN-γ secretion.

It is tempting to hypothesize that molecular mimicry enables EBNA-1-specific T-cells to cross-recognize self-auto-antigens that would eventually lead to the initiation and maintenance of autoimmune pathologies. In genetically predisposed individuals, it should be noted thatEBNA-1 expression evokes a neuro-antigen cross-reactive anti-EBNA-1 T-cell response that upon entry into the CNS recognizes and target cells expressing the cognate neuro-antigen [68,69].

In addition, intra-thecal B-cell follicles, which are closely associated with thesub-pial gray matter lesions, have been described in post-mortem brain tissue of subjects with MS [70]. Rand et al. [54] have reported high levels of EBV-infected B-cells in such follicles in 21 of 22 post-mortem MS brain specimens, a finding not seen in neurologic control cases. Fifty percent of these subjects also showed anti-EBV IgG in CSF collected post-mortem. These results corroborate the findings of elevated EBNA-1 IgG in subjects with more active disease. A pool of EBV-infected memory cells may exist both peripherally and centrally that generate or sustain a population of auto-reactive T-cells, thereby mediating the inflammatory response in MS.

Otto et al. [71] found that Intra-thecally produced anti-EBV antibodies are part of the poly-specificintra-thecal immune response in MS and only rarely detectable in patients with MS, both arguing against a direct CNS infection with EBV in patients with MS. According Bray et al. [72] in healthy EBV-sero-positive persons, the EBV-specific, MHC-restricted T-lymphocytes keep the EBV-containing B-lymphocytes locked in the transformed state. However, in the host genetically susceptible to MS, the same population of lymphocytes might recognize and interact with either of the two identified penta-peptides. Nociti et al. [73] found that increased titers of anti-EBV-IgG in serum and cerebro-spinal fluid (CSF)of MS subjects as compared to Chronic Inflammatory Demyelinating Poly-radiculo-neuropathy (CIDP) and Amyotrophic Lateral Sclerosis (ALS) patients thus providing additional evidence for a possible involvement of EBV in MS. Castellazzi et al. [74] found that an intra-thecalIgG production of anti-VCA and anti-EBNA-1 IgG, was present only in a limited number of multiple sclerosis patients and controls (range from 1.3 to 6.3%) which do not support a direct pathogenetic role of EBV-targeted humoral immune response in multiple sclerosis. Villegas et al. [75] found that intra-thecal EBNA-1 specific IgG synthesis was detected in 6.6% MS patients and in 17.3% controls. No EBV DNA was found in plasma or CSF, and our findings showed no evidence of high intra-thecal EBNA-1 specific IgG synthesis or of significant EBV DNA in CSF in MS patients.

It should be noted that technical limits to detect EBV in CNS may strongly interact with the results of these studies, and this is one of the main reasons about the controversial results.

6. OTHER EXPERIMENTS

Several independent groups have analyzed the presence of EBV genome sequencesn in the CSF of MS patients by PCR. The authors have either not detected any EBV DNA at all [76] or demonstrated EBV DNA in only a small percentage of MS patients without significant difference as compared to controls [77,78]. One study of post-mortem brain samples detected EBV in 27% of MS cases as opposed to 38% of controls [78]. A recent study investigating the expression of markers of EBV latent and lytic infection in post-mortem brain specimens showed evidence of EBV infection in brain-infiltrating B cells and plasma cells in 21 of 22 MS patients and none of 11 controls [54].

In other attempts, cell mediated immune mechanisms, involving T and NK cells which have pivotal importance in controlling the proliferation of EBV-infected B cells is investigated. The frequency of EBNA-1 specific CD4+ memory T cells was found to be strikingly elevated in MS patients as compared to healthy EBV carriers. Furthermore, the same T-cells showed increased proliferative capacity and enhanced interferon-gamma production [76]. A strong EBV-specific CD8+ T- cell response in

patients with clinically isolated syndrome has recently been reported [18]. T- cell cross recognition between EBV peptides and myelin proteins including myelin basic protein (MBP) has been demonstrated, supporting a concept of possible autoimmune mechanisms by cross-reactivity towards EBV and auto antigens (molecular mimicry) [79–80]. It should also be noted that EBV–MBP cross-reactive T- cells have been found in similar frequencies in MS patients and healthy controls [80].

7. CONCLUSION

There is strong epidemiological evidence linking symptomatic EBV infection with MS development rendering the virus a major candidate for MS initiation and there is a growing body of evidence pointing to EBV as a culprit. The role of this virus is probably as an initiator of the disease process of MS, or as a contributor to its early development, rather than as an activator of latent, existing disease. Further investigations should be designed with adequate controls to better define the role of EBV in MS pathogenesis, but it is also necessary more researches aimed at understanding how EBV interacts with the immune system in MS.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sinha AA, Lopez MT, McDevitt HO. Autoimmune diseases: The failure of self tolerance. Science. 1990;248(4961):1380-88.
2. Shoenfeld Y, Zandman-Goddard G, Stojanovich L, Cutolo M, Amital H, et al. The mosaic of autoimmunity: Hormonal and environ mental factors involved in autoimmune diseases. Isr Med Assoc. 2008;10(1):8-12.
3. Sherbet G. Bacterial infections and the pathogenesis of autoimmune conditions. BJMP. 2009;2(1):6-13.
4. Fujinami RS, von Herrath MG, Christen U, Lindsay Whitton J. Molecular Mimicry, Bystander Activation, or Viral Persistence: Infections and Autoimmune Disease. ClinMicrobiol Rev. 2006;19(1):80–94.
5. Scolding NJ, Zajicek JP, Wood N, Compston DAS. The pathogenesis of demyelinating disease. Progr Neurobiol. 1994;43:143–173.
6. Sospedra M, Martin R. Immunology of multiple sclerosis. Annu RevImmunol. 2005;23:683-747.
7. Antony JM, van Marle G, Opii W, Butterfield D A, Mallet F, Yong VW, J. et al. Human endogenous retrovirus glycoprotein-mediated induction of redox reactants causes oligodendrocyte death and demyelination. Na Neurosci. 2004;7:1088-1095.
8. Ozawa K, Suchanek G, Breitschopf H, Bruck W, Budka H, Jellinger K, Lassmann H. Patterns of oligodendroglia pathology in multiple sclerosis. Brain.1994;117:1311-1322.
9. Storch MK, Piddlesden S, Haltia M, Iivanainen M, Morgan P, Lassmann H. Multiple sclerosis: In situ evidence for antibody- and complement-mediated demyelination. Ann Neurol.1998;43:465-471.
10. Cepok S, Rosche B, Grummel V, Vogel F, Zhou D, Sayn J, et al. Short-lived plasma blasts are the main B cell effector subset during the course of multiple sclerosis. Brain. 2005;128:1667-1676.
11. Hafler DA. Multiple sclerosis. J ClinInvestig. 2004;113:788-794.
12. Compston A, Coles A. Multiple sclerosis. Lancet. 2008;372:1502–17.
13. Sriram S, Steiner I. Experimental allergic encephalomyelitis: A misleading model of multiple sclerosis. Ann Neurol. 2005;58:939–45.
14. Frohman EM, Racke MK, Raine CS. Multiple sclerosis--the plaque and its pathogenesis. N Engl J Med. 2006;354:942–55.
15. Weber T. Progressive multifocal leukoencephalopathy. Neurol Clin. 2008;26:833–54. PMID:18657729.
16. Oleszak EL, Chang JR, Friedman H, Katsetos CD, Platsoucas CD. Theiler's virus infection: A model for multiple sclerosis. Clin Microbiol Rev. 2004;17:174–207.
17. Rickinson AB, Kieff E. Epstein-Barr virus. In: Knipe DM, Howley PM, Griffin DE, Martin MA, Lamb RA editors. Fields

virology. Philadelphia: Lippincott Williams and Wilkins. 2001;2575-2627.

18. Jilek S, Schluep M, Meylan P, Vingerhoets F, Guignard L, Monney A, et al. Strong EBV-specific CD8+ T-cell response in patients with early multiple sclerosis. Brain. 2008;131(Pt 7):1712–1721.
19. Steven NM. Infectious mononucleosis. EBV Reports. 1996;3:91-95.
20. Warner HB, Carp RI, Multiple sclerosis and Epstein-Barr virus. Lancet. 1981;2(8258):1290.
21. Haahr S, Höllsberg P. Multiple sclerosis is linked to Epstein–Barr virus Infection. Rev Med Virol. 2006;16 950:297–310.
22. Munch M, Hvas J, Christensen T, Moller-Larsen A, Haar S. The implications of Epstein-Barr virus in multiple sclerosis ± a review. Acta Neurol Scand. 1997;(Suppl. 169):59-64.
23. Munch M, Riisom K, Christensen T, Moller-Larsen A, Haahr S. The significance of Epstein-Barr virus seropositivity in multiple sclerosis patients. Acta Neurol Scand. 1998;97:171-174.
24. Alter M, Zhen-xin Z, Davanipour Z, Sobel E, Zibulewski J, Schwartz G, Friday G. Multiple sclerosis and childhood infections. Neurology. 1986;36:1386-1389.
25. Martyn CN, Cruddas M, Compston DAS. Symptomatic Epstein-Barr virus infection and multiple sclerosis. J Neurol Neurosurg Psychiatry. 1993;56:167-168.
26. Operskalski EA, Visscher BR, Malmgren RM, Detels R. A case-control study of multiple sclerosis. Neurology. 1989;39:825-829.
27. Lindberg C, Andersen O, Vahlne A, Dalton M, Runmarker B. Epidemiological investigation of the association between infectious mononucleosis and multiple sclerosis. Neuroepidemiology. 1991;62-65.
28. Haahr S, Koch-Henriksen N, Moller-Larsen LS, Andersen HM. Increased risk of multiple sclerosis after late Epstein-Barr virus infection: A historical prospective study. Mult Scler. 1995;1:73-77.
29. Bray PF, Bloomer LC, Salmon VC. Epstein–Barr virus infection and antibody synthesis in patients with multiple sclerosis. Arch Neurol. 1983;40:406–8.
30. Sumaya CV, Myers LW, Ellison GW, Ench Y. Increased prevalence and titer of Epstein–Barr virus antibodies in patients with multiple sclerosis. Ann Neurol. 1985;17:371–7.
31. Larsen PD, Bloomer LC, Bray PF. Epstein–Barr nuclear antigen and viral capsid antigen antibody titers in multiple sclerosis. Neurology. 1985;35:435–8.
32. Shirodaria PV, Haire M, Fleming E, Merrett JD, Hawkins Sa, Roberts SD. Viral antibody titers. Comparison in patients with multiple sclerosis and rheumatoid arthritis. Arch Neurol. 1987;44:1237–41.
33. Ascherio A, Munger KL, Lennette ET, Spiegelman D, Hernán MA, Olek MJ, et al. Epstein–Barr virus antibodies and risk of multiple sclerosis: A prospective study. JAMA. 2001;26:3083–8.
34. Villoslada P, Juste C, Tintore M, Llorenç V, Codina G, Pozo-Rosich P, et al. The immune response against herpesvirus is more prominent in the early stages of MS. Neurology. 2003;60:1944–8.
35. Sundström P, Juto P, Wadell G, Hallmans G, Svenningsson A, Nyström L, et al. An altered immune response to Epstein–Barr virus in multiple sclerosis: A prospective study. Neurology. 2004;62:2277–82.
36. Haahr S, Plesner Am, Vestergaard BF, Höllsberg P. A role of late Epstein–Barr virus infection in multiple sclerosis. Acta Neurol Scand. 2004;109:270–5.
37. Levin LI, Munger KL, Rubertone MV, Peck CA, Lennette ET, Spiegelman D, et al. Temporal relationship between elevation of Epstein–Barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. JAMA. 2005;293:2496–500.
38. Myhr KM, Riise T, Barrett-Connor E, Myrmel H, Vedeler C, Gronning M, et al. Altered antibody pattern to Epstein–Barr virus but not to other herpesviruses in multiple sclerosis: A population based case-control study from western Norway. J Neurol Neurosurg Psychiatry. 1998;64:539–42.
39. Buljevac D, van Doornum GJJ, Flach HZ, Groen J, Osterhaus AD, Hop W, et al. Epstein–Barr virus and disease activity in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2005;76:1377–81.
40. De Lorenze GN, Munger KL, Lennette ET, Orentreich N, Vogelman JH, Ascherio A. Epstein–Barr virus and multiple sclerosis: Evidence of an association from a prospective study with long-term follow-up. Arch Neurol. 2006;63:839–44.

41. De Jager PL, Simon KC, Munger KL, Rioux JD, Hafler DA, Ascherio A. Integrating risk factors: HLA-DRB1 1501 and Epstein–Barr virus in multiple sclerosis. Neurology. 2008;70:1113–8.
42. Banwell B, Krupp L, Kennedy J, Tellier R, Tenembaum S, Ness J, et al. Clinical features and viral serologies in children with multiple sclerosis: A multinational observational study. Lancet Neurol. 2007;6:773–81.
43. Pohl D, Krone B, Rostasy K, Kahler E, Brunner E, Lehnert M, et al. High seroprevalence of Epstein–Barr virus in children with multiple sclerosis. Neurology. 2006;67:2063–5.
44. Alotaibi S, Kennedy J, Tellier R, Stephens D, Banwell B. Epstein–Barr virus in pediatric multiple sclerosis. JAMA. 2004;291:1875–9.
45. Ascherio A, Munch M. Epstein-Barr virus and multiple sclerosis. Epidemiology. 2000;11:220–4.
46. Cepok S, Zhou D, Srivastava R, Nessler S, Stei S, Büssow K, et al. Identification of Epstein–Barr virus proteins as putative targets of the immune response in multiple sclerosis. J Clin Invest. 2005;115:1352–60.
47. Thacker EL, Mirzaei F, Ascherio A. Infectious mononucleosis and risk for multiple sclerosis: A meta-analysis. Ann Neurol. 2006;59:499–503.
48. Lünemann JD, Münz C. EBV in MS: Guilty by association? Trends Immunol. 2009;30:243–8.
49. Ramagopalan SV, Valdar W, Dyment DA, DeLuca GC, Yee IM, Giovannoni G, et al. Association of infectious mononucleosis with multiple sclerosis. A population-based study. Neuroepidemiology. 2009;32:257–62.
50. Farrell RA, Antony D, Wall GR, Clark DA, Fisniku L, Swanton J, et al. Humoral immune response to EBV in multiple sclerosis is associated with disease activity on MRI. Neurology. 2009;73:32–8.
51. Serafini B, Rosicarelli B, Franciotta D, Magliozzi R, Reynolds R, Cinque P, et al. Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. J Exp Med. 2007;204:2899–912.
52. Willis SN, Stadelmann C, Rodig SJ, Caron T, Gattenloehner S, Mallozzi SS, et al. Epstein-Barr virus infection is not a characteristic feature of multiple sclerosis brain. Brain. 2009;132:3318–28.
53. Peferoen LA, Lamers F, Lodder LN, Gerritsen WH, Huitinga I, Melief J, et al. Epstein Barr virus is not a characteristic feature in the central nervous system in established multiple sclerosis. Brain. 2010;133:e137.
54. Rand KH, Houck H, Denslow ND, Heilman KM. Epstein-Barr virus nuclear antigen-1 (EBNA-1) associated oligoclonal bands in patients with multiple sclerosis. J Neurol Sci. 2000;173:32–9.
55. Nielsen TR, Rostgaard K, Nielsen NM, Frisch M, Hjalgrim H. Multiple sclerosis after infectious mononucleosis. Arch Neurol. 2007;64(1):72–75.
56. Haahr S, Plesner AM, Vestergaard BF, Höllsberg P. A role of late Epstein-Barr virus infection in multiple sclerosis. Acta. Neurol Scand. 2004;109(4):270–275.
57. Jafari NA, van Nieropa GP, M.G.M. Verjansb G, D.M.E. Osterhausb A, Middeldorpc JM, Hintzena RQ. No evidence for intrathecal IgG synthesis to Epstein Barr virus nuclear antigen-1 in multiple sclerosis. Journal of Clinical Virology. 2010;49:26–31
58. Sundström P, Nyström M, Ruuth K, Lundgren E. Antibodies to specific EBNA-1 domains and HLA DRB11501 interact as risk factors for multiple sclerosis. J Neuroimmunol. 2009;215:102–7.
59. Sargsyan SA, Shearer AJ, Ritchie AM, Burgoon MP, Anderson S, Hemmer B, et al. Absence of Epstein-Barr virus in the brain and CSF of patients with multiple sclerosis. Neurology. 2010;74:1127–35.
60. Kusunoki Y, Huang H, Fukuda Y, Ozaki K, Saito M, Hirai Y, et al. A positive correlation between the precursor frequency of cytotoxic lymphocytes to autologous Epstein-Barr virus-transformed B cells and antibody titer level against Epstein-Barr virus-associated nuclear antigen in healthy seroposit iveindividuals. Microbiol Immunol. 1993;37:461–9.
61. Heller KN, Upshaw J, Seyoum B, Zebroski H, Munz C. Distinct memory CD4+ Tcell subsets mediate immune recognition of Epstein Barr virus nuclear antigen 1 in healthy virus carriers. Blood. 2007;109:1138–46.
62. Lünemann JD, Edwards N, Muraro PA, Hayashi S, Cohen JI, Munz C, et al.

Increased frequency and broadened specificity of latent EBV nuclear antigen-1-specific T cells in multiple sclerosis. Brain. 2006;129:1493–506.

63. Lang D, Vornhagen R, Rothe M, Hinderer W, Sonneborn HH, Plachter B. Cross-reactivity of Epstein-Barr virus-specific immunoglobulin M antibodies with cytomegalo virus antigens containing glycine homopolymers. Clin Diagn Lab Immunol. 2001;8:747–56.
64. Fox R, Sportsman R, Rhodes G, Luka J, Pearson G, Vaughan J. Rheumatoid arthritis synovial membrane contains a 62,000-molecular-weight protein that shares an antigenic epitope with the Epstein-Barr virus-encoded associated nuclear antigen. J Clin Invest. 1986;77:1539–47.
65. Hennard C, Pfuhl T, Buettner M, Becker KF, Knöfel T, Middeldorp J, Kremmer E, Niedobitek G, Grässer F. The antibody 2B4 directed against the Epstein-Barr virus (EBV)-encoded nuclear antigen 1 (EBNA1) detects MAGE-4: Implications for studies on the EBV association of human cancers. J Pathol. 2006;209:430–5.
66. Lünemann JD, Jelci ´c I, Roberts S, Lutterotti A, Tackenberg B, Martin R, et al. EBNA1-specific T cells from patients with multiple sclerosis cross react with myelin antigens and co-produce IFN-gamma and IL-2. J Exp Med. 2008;205:1763–73.
67. Leen A, Meij P, Redchenko I, Middeldorp JM, Bloemena E, Rickinson A, et al. Differential immunogenicity of Epstein-Barr virus latent-cycle proteins for human CD4(+) T-helper 1 responses. J Virol. 2001;75:8649–59.
68. Magliozzi R, Howell O, Vora A, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain. 2007;130:1089–1104.
69. Martin C, Enbom M, Söderström M, Fredrikson S, Dahl H, Lycke J, et al. Absence of seven human herpesviruses, including HHV-6, by polymerase chain reaction in CSF and blood from patients with multiple sclerosis and optic neuritis. Acta Neurol Scand. 1997;95:280-3.
70. Mancuso R, Delbue S, Borghi E, Pagani E, Calvo MG, Caputo D, et al. Increased prevalence of varicella zoster virus DNA in cerebrospinal fluid from patients with multiple sclerosis. J Med Viro. 2007;79:192–9.
71. Otto C, Oltmann A, Stein A, Frenzel K, Schroeter J, Habbel P, et al. Intrathecal EBV antibodies are part of the polyspecific immune response in multiple sclerosis. Neurology. 2011;76(15):1316-21.
72. Bray PF, Luka J, Bray PF, Culp KW, Schlight JP. Antibodies against Epstein-Barr nuclear antigen (EBNA) in multiple sclerosis CSF, and two pentapeptide sequence identities between EBNA and myelin basic protein. Neurology. 1992;42(9):1798-804
73. Nociti V, Frisullo G, Marti A, Luigetti M, Iorio R, Patanella AK, et al. Epstein-Barr virus antibodies in serum and cerebrospinal fluid from multiple sclerosis, chronic inflammatory demyelinating. Polyradiculo-neuropathy and amyotrophic lateral sclerosis. J Neuroimmunol. 2010;225(1-2):149-52.
74. Castellazzi M, Tamborino C, Cani A, Negri E, Baldi E, Seraceni S, et al. Epstein-Barr virus-specific antibody response in cerebrospinal fluid and serum of patients with multiple sclerosis. Mult Scler. 2010;16(7):883-7.
75. Villegas E, Santiago O, Carrillo JA, Sorlózano A, Guerrero M, Fernández O, Gutiérrez J. Low intrathecal immune response of anti-EBNA-1 antibodies and EBV DNA from multiple sclerosis patients. Diagn Microbiol Infect Dis. 2011;70(1):85-90.
76. Alvarez-Lafuente R, García-Montojo M, De Las Heras V, Domínguez-Mozo MI, Bartolome M, Benito-Martin MS, et al. Herpesviruses and human endogenous retroviral sequences in the cerebrospinal fluid of multiple sclerosis patients. Mult Scler. 2008;14:595–601.
77. Sanders VJ, Felisan S, Waddell A, Tourtellotte WW. Detection of *herpesviridae* in postmortem multiple sclerosis brain tissue and controls by polymerase chain reaction. J Neurovirol. 1996;2:249-58.
78. Holmoy T, Kvale EO, Vartdal F. Cerebrospinal fluid CD4+ T cells from a multiple sclerosis patient cross-recognize Epstein–Barr virus and myelin basic protein. J Neurovirology. 2004;10:278-83.

79. Wucherpfennig KW, Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: Viral peptides activate human T cell clones specific for myelin basic protein. Cell. 1995;80:695-705.

80. Lang HLE, Jacobsen H, Ikemizu S, Andersson C, Harlos K, Madsen L, et al. A functional and structural basis for TCR cross-reactivity in multiple sclerosis. Nat Immuno. 2002;3:940–3.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=827&id=8&aid=7135