Epstein-Barr Virus in Multiple Sclerosis

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Current Neurology and Neuroscience Reports 2009, **9:**405–410
Current Medicine Group LLC ISSN 1528-4042

Recent seroepidemiologic and pathologic evidence suggests that prior infection with Epstein-Barr virus (EBV) may be necessary for the development of multiple sclerosis (MS). 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 and considers the therapeutic implication of this evidence.

Introduction

Epstein-Barr virus (EBV) is a herpesvirus that infects more than 90% of all humans [1]. The virus notoriously infects resting B lymphocytes and transforms them first into proliferating and then into latent memory cells [2]. A relationship between EBV and multiple sclerosis (MS) has long been suspected, and the scientific literature over the past 35 years reflects numerous studies reporting evidence of observed associations therein. Many of these earlier studies had methodologic limitations such as a lack of prospective data or a reporting bias and, as such, the truth or significance of these associations was not definitive. Until recently, there was also a lack of convincing pathologic evidence that demonstrated the presence of EBV in MS lesions within the central nervous system. The publication over the past 8 years of a series of prospective seroepidemiologic studies [3–5] as well as one postmortem pathology [6••] study has strongly implicated EBV in the etiology and pathogenesis of MS.

Infection, Latency, and Persistence of EBV

EBV infection is usually transmitted through contact with oral secretions. The virus infects naive B cells and programs them to differentiate into memory B cells, a process that takes place in the germinal center of lymphoid tissue [1]. This transformation differs from normal memory B-cell differentiation because the process is driven by viral genes rather than by antigen [2]. After a primary infection from EBV has occurred, the virus establishes a latent infection in memory B cells that persists for a lifetime [1]. In healthy EBV carriers, infected B cells that are in the proliferative stage in which viral protein is expressed are restricted to lymph nodes and are denied entry into the peripheral circulation [2]. Latently infected memory B cells, by contrast, express no viral proteins, and it is in this form that the EBV-infected B cells enter the peripheral circulation [2]. By limiting viral gene and protein expression in this fashion within latent circulating B cells, EBV limits its recognition by host cell–mediated immunity. In addition to finding a long-lived niche in resting memory cells, EBV persistence also can be attributed to mechanisms in which the virus inhibits its own degradation and apoptosis [1].

Diseases Associated with EBV

Although most humans infected with EBV remain healthy, some will develop EBV-associated diseases. With regard to neoplastic disease, there is a strong evidence of an association between EBV and Burkitt's lymphoma, nasopharyngeal carcinoma, and B-cell lymphoma [2]. With regard to autoimmune disease, there too is robust evidence of an association with rheumatoid arthritis (RA), systemic lupus erythematosus, and Sjogren's syndrome [7]. For some of these diseases, a mechanistic role of EBV, if any, has yet to be defined, and for many of these diseases, the factors that determine sickness versus health in the face of EBV infection have yet to be identified.

Epidemiologic Evidence

Indirect associations between EBV and MS have been made through observed links between MS and infectious mononucleosis (IM), a disease that typically is the result

of a primary EBV infection that occurs in adolescents and young adults [1]. Primary EBV infection in infants and young children, by contrast, is usually asymptomatic [1]. It was first observed in 1988 that the epidemiology of IM and MS had striking similarities [8]. Both diseases had high incidences in Caucasians, those with high standards of living, and those with good hygiene; both diseases had low incidences among Africans and Asians; and both had similar ages of onset [8]. In 1989, Operskalski and colleagues [9] were the first to report the results of a case-control study that showed that MS patients were significantly more likely to have a history of IM than were healthy controls. In 2006, a meta-analysis of 14 case-control and cohort studies of IM and MS was reported in which the combined RR of MS after IM was estimated to be 2.3 (95% CI, 1.7–3.0) [10]. These observations suggested that the timing of a primary EBV infection was an important factor in the development of MS such that late primary EBV infections (ie, IM) (occurring in adolescence or young adulthood) confer a greater risk of MS than do early primary infections. Further evidence of the importance of the timing of EBV infection was reported by Haahr and colleagues [11], who looked at EBV serology in a Danish region with a high prevalence of MS. More than one third of the population in these areas was still seronegative for EBV at puberty. This finding is interesting in light of the fact that many primary EBV infections occur during infancy and childhood in areas where MS prevalence is low [12].

In 1980, Sumaya and colleagues [13] observed significantly higher serum antibody titers to EBV in MS patients than in controls, which represented the first seroepidemiologic evidence of an association between EBV and MS. In this study, the prevalence of EBV positivity differed such that 98.7% of MS patients, versus 93.8% of controls, had serology positive for EBV. In 2000, a meta-analysis of eight such studies was conducted in which a summary OR of developing MS was estimated to be 13.5 (95% CI, 6.3–31.4) when EBV-seropositive and EBV-seronegative individuals were compared [14]. None of these studies were prospective, however, leading to questions about the nature of the associations. One might have concluded, for example, that the primary immune dysregulation in MS led to the observed EBV dysregulation rather than the contrary.

In 2001, Ascherio and colleagues [4] reported results of the first prospective seroepidemiologic study of EBV and MS. They conducted a nested case-control study and identified 18 cases of MS with blood collected before the onset of MS. Compared with matched controls, individuals with MS had significantly higher titers to EBV well before the onset of any neurologic symptoms. Levin and colleagues [5] conducted a larger, prospective, case-control trial in US military personnel in which the RR of MS for those in the highest EBV titer group was 33.9 ($P < 0.001$) compared with those in the lowest titer group, establishing a clear dose effect of EBV titer on MS risk. In 2006, a case-control trial with long-term follow-up was conducted in which the investigators measured EBV titers in sera collected up to 30 years before the onset of MS symptoms [15••]. Elevations in EBV titers in individuals with MS occurred 15 to 20 years before the onset of symptoms and persisted thereafter, suggesting that EBV titer elevation was likely an early event in the pathogenesis of MS and unlikely the result of MS.

An epidemiologic association between EBV and pediatric MS also has been demonstrated. In 2004, a case-control study found that serologic evidence of remote EBV infection was present in 83% of pediatric MS patients but only 42% of controls ($P < 0.001$) [16]. Similar findings were observed in several subsequent seroepidemiologic investigations, which together represent some of the most robust epidemiologic evidence of the association between EBV and MS [17–19].

Postmortem Evidence

In 2004, ectopic B-cell lymphoid follicles were discovered postmortem in the meninges of the brains of two of three patients with secondary progressive MS (SPMS) [20]. In 2007, Serafini and colleagues [6••] expanded on this finding in an investigation in which 22 postmortem brains of MS patients were examined histologically and screened for several EBV markers. Both SPMS and relapsing-remitting forms of MS (RRMS) were included in the 2007 study, as were two postmortem cases of acute fulminant MS and seven postmortem cases of inflammatory neurologic disease but not MS. The study revealed that intracerebral accumulation of EBV infected B cells is a common feature of MS and that the ectopic B-cell lymphoid follicles in the meninges were the principal site of EBV persistence. Immunohistochemistry showed that the EBV latency proteins were present both in active white matter lesions and in the meninges in most MS patients. EBV lytic protein was commonly identified in the meningeal B-cell follicles, indicating that these ectopic lymphoid structures were the major site of viral reactivation. In the cases of hyperacute and fatal MS, the investigators found limited meningeal involvement but did find EBV latent and lytic proteins throughout the white matter, indicating that involvement of EBV-infected B cells is an early event in MS. A significant relationship between the number of cerebrospinal fluid (CSF) oligoclonal bands and those MS brains with a relatively high degree of EBV involvement was also observed. Significant intracerebral cytotoxic T-lymphocyte (CTL) activity was detected at sites of accumulation of EBV infected cells. Lastly, no evidence of EBV infected B cells in the brains of patients with other inflammatory neurologic disease was found, suggesting that the presence of EBV in the brain is not driven by inflammation but rather is specific to MS.

This landmark study was the first to directly demonstrate EBV involvement in the brain of MS patients [6••]. It established that EBV-infected B cells are common in all forms of MS and that that the frequency of EBV-infected cells correlates with the degree of brain inflammation. The observation that ectopic B-cell follicles were the

major site of EBV persistence in MS brains was evidence of a direct link between EBV and B-cell dysregulation in MS. The authors also provided direct evidence that EBV reactivation may be related to acute relapses. Lastly, the widespread presence of ectopic EBV-infected B-cell follicles in the brains of SPMS patients suggests that EBV plays a role in the chronic inflammation of MS and possibly in the establishment of sustained autoimmunity.

Theories of Pathogenesis of EBV in MS

Antigenic mimicry

One potential mechanism of EBV's pathogenic role in MS is that of antigenic mimicry. Several studies have demonstrated CTL cross-reactivity between EBV proteins and myelin basic protein [21–23]. This evidence is consistent with the prevailing idea that T-cell autoimmunity to myelin antigens is central to MS pathogenesis. Another example of such antigenic mimicry with myelin lies in the stress protein alpha B-crystallin, a protein that has been found to be a dominant myelin antigen in MS patients but not in healthy controls [24]. This protein is thought to play a protective role against autoimmune demyelination [25] but is also known to be abundant in MS lesions, where it is presented to $CD4^+$ T cells [26]. Such alpha B-crystallin–specific T cells are thought to have a proinflammatory, type 1 helper T-cell (Th1) phenotype [27]. Interestingly, it has also been demonstrated that B cells express alpha B-crystallin after EBV infection, which leads to presentation of the antigen to T cells, suggesting a direct mechanistic link between EBV infection and Th1-mediated CNS demyelination [28].

Immortalization of B-cell clones

The pathologic evidence of highly organized, ectopic, B-cell follicles in the meninges of MS patients challenges the notion that MS is primarily a T-cell disease and suggests that B cells also play a central role in MS pathology. The importance of B cells in MS has been further supported by recent phase 2 evidence that anti–B-cell therapy is effective against RRMS [29]. As early as 1979, a higher rate of spontaneous immortalization of EBV-infected B cells was observed in culture from MS patients than in EBV-infected B cells from controls [30], suggesting EBV-associated dysregulation of B cells in MS. Kuenz and colleagues [31] compared the CSF of MS patients with the CSF of patients with other neurologic diseases. They found no difference in the number of CSF T cells but found significantly more CSF B cells and plasma blasts in MS patients than controls. Furthermore, the fact that more than 90% of MS patients have evidence of intrathecal antibody synthesis in the form of oligoclonal bands also supports the role of B cells in MS. The antigen specificities of most oligoclonal IgG in the CSF of MS patients are uncertain and polyspecific, with the most commonly identified specificities being measles, rubella, and varicella zoster viruses [32]. A clear pathologic role of these antibodies has never been established in MS. Their presence in the CSF in MS may merely represent the by-product of a clonally expanded B-cell population in the brain [33], an idea made even more plausible by the fact that the initial differentiation of EBV-infected B cells into memory B cells is driven not by antigen but by the viral genome [2]. The pathologic role of CNS B cells in MS may have more to do with T-cell interactions and bystander activation causing inflammatory damage to adjacent structures than with the oligoclonal IgG they produce [6••].

CTL response to EBV-infected B cells

Beyond a role for CTLs in antigenic mimicry in MS, it has been postulated that a dysfunctional CTL response to EBV-infected B cells leads to undeterred B-cell clonal expansion [34]. Recent investigations of EBV-specific CTL function in MS have yielded conflicting results. Pender and colleagues [35] reported a decrease in the number of EBV-specific CTLs that reacted against an EBV-infected B-cell lymphoblastoid cell line in MS compared with that of healthy EBV-infected controls. Similar findings were reported in 1988 by Craig and colleagues [36]. The observation that EBV-infected B cells from MS patients have a higher tendency to spontaneously immortalize in vitro as compared with controls also suggests a deficiency of CTL control of virally infected cells [30]. However, Jilek and colleagues [37] recently demonstrated that EBV-specific CTLs are significantly increased in the blood of patients with a clinically isolated syndrome as compared with RRMS or SPMS. Also, as mentioned above, Serafini and colleagues [6••] demonstrated a positive correlation between the numbers of CTLs and EBV-infected B cells in the brains of MS patients. The exact relationship between CTLs and EBV-infected B cells in MS has yet to be definitively established, but undoubtedly this relationship will be important to a greater understanding of the mechanism of immune dysregulation of EBV in MS.

Hygiene hypothesis

With regard to differential susceptibility to MS after EBV infection, it has been suggested that a disruption in the normal sequence of common viral infections, owing to a late primary infection by EBV, leads to immune dysregulation and ultimately MS in genetically susceptible individuals [38]. Such a disruption might be attributed to hygiene practices associated with modern living conditions in industrialized nations that lead to a delay of common viral infections. Specifically, it has been postulated that infection with human herpesvirus 6, a virus recently associated with MS [39], prior to a late primary EBV infection could lead to lead to such immune dysregulation in MS [38].

Biomarkers of EBV

EBV viral load is not associated with MS risk [40] and does not differ significantly between MS patients and healthy carriers [39]. However, MS patients with high disease activity have serum samples positive for EBV DNA significantly more frequently than do MS patients with

low disease activity [39]. There is evidence that EBV reactivation is associated with clinical disease activity in MS when reactivation is defined as a pattern of increased IgM and IgA levels against EBV [41]. Zivadinov and colleagues [42] investigated the relationship between EBV and MRI parameters in MS. They performed MRIs on 135 patients with MS as well as serum titers for EBV antibodies. There was a significant inverse association between antibody titers to viral capsid antigen (anti-EBV VCA IgG) and MRI markers of gray matter atrophy. This relationship grew stronger after 3 years, suggesting a dose effect of EBV titer on central nervous system damage in MS.

Other MS Risk Factors and EBV

Vitamin D deficiency has been identified in recent years as another important risk factor for the development of MS [43,44]. Vitamin D is considered to be a potent regulator of the immune system, exerting this effect through inhibition of lymphocyte proliferation and antibody synthesis, inhibition of interleukin-2 (IL-2) and IL-12 production, and stimulation of IL-10 [45–49]. Vitamin D receptors are present on B cells, including EBV-infected B cells, and there is speculation that the presence of both vitamin D deficiency and EBV infection in a genetically susceptible individual could lead to B-cell dysregulation and possibly MS [50].

With regard to known genetic risk factors, several recent studies have demonstrated that the presence of the HLA-DRB1*1501 (DR15) haplotype, an established risk factor for MS, is independent from the risk of MS conferred by elevations in EBV titers and that when both DR15 and high EBV titers are present, the risk of MS increases dramatically [51–53]. De Jager and colleagues [51] conducted a prospective case-control study of 148 women with MS and 296 controls. The RR of MS among DR15-positive women who also had elevated (> 1:320) titers of antibodies to the EBV nuclear antigen 1 (anti-EBNA-1) was ninefold higher than that of DR15-negative woman with low (< 1:80) anti-EBNA-1 titers. EBV titers have also been positively associated with a polymorphism in the promoter region of IL-10 in women, but not men, which is interesting given that MS and the IL-10 polymorphism are more frequent in Caucasians, because MS is more common in women than in men, and because EBV depends on IL-10 to survive [54]. A recent genomewide association study has identified an allelic variant of the gene encoding the IL-7 receptor (IL-7R) as a risk factor for MS [55], a fact that may indicate a mechanistic link to EBV given that IL-7R controls proliferation and survival of B cells [6••]. Lastly, human endogenous retrovirus (HERV) K-18 Env is an EBV-associated superantigen, and the gene that encodes it was recently associated with MS in a case-control study in which MS patients were found to be significantly more likely to have a particular single nucleotide polymorphism for this gene than were controls [56].

Future Implications

Pathologic and seroepidemiologic evidence suggests that EBV infection is necessary, but not sufficient, to develop MS. As such, it has been suggested that "prevention of EBV infection would result in a marked, probably tenfold, decline in MS incidence" [57]. There have been several attempts to develop an EBV vaccine, but none has been completely successful [58]. Considerable caution must be afforded this effort because most people who become infected with EBV develop no disease whatsoever. If a childhood EBV vaccine is partially effective with a limited duration of protection, it may serve to delay primary EBV infection and paradoxically increase the risk of IM and MS [57,59]. One phase 2 trial of a vaccine to prevent late primary EBV infection has shown promise in that it prevented IM in EBV-seronegative subjects, but it did not prevent asymptomatic EBV infection [60]. Other preventive strategies would include efforts to influence the timing of EBV infection such that the incidence of late primary EBV infections in the form of IM would decrease and primary EBV infections in early childhood would increase. A greater understanding of the epidemiologic factors that lead to late primary EBV infection might lead to public health measures aimed at ensuring that this ubiquitous human virus gets transmitted as early as possible in childhood.

With regard to treatment, the evidence that EBV has a role in MS suggests that therapeutic strategies targeting B cells will become increasingly important. As mentioned, anti–B-cell therapy with rituximab has proven to be effective in a phase 2 trial of RRMS [29]. Interestingly, rituximab has US Food and Drug Administration approval as therapy for two other EBV-associated diseases, rheumatoid arthritis and B-cell lymphoma, a fact that further suggests that B-cell dysfunction is common to EBV-associated diseases. These considerations support the need for additional clinical trials of anti–B-cell monoclonal antibodies in MS.

The recent pathologic discovery of ectopic B-cell follicles that express EBV protein and are located exclusively in the meninges of SPMS patients also highlights the importance of developing MS therapies that cross the blood–brain barrier. The recognition that there is significant ongoing inflammation in the form of these EBV-dysregulated B-cell follicles in SPMS challenges the theory that SPMS is predominantly a neurodegenerative (noninflammatory) phase of MS [61] and should prompt a renewed and vigorous effort to develop novel treatments for SPMS that target the inflammation. Because there is no in vivo method of identifying these CNS ectopic lymphoid follicles with conventional MS laboratory tests such as MRI or CSF analyses, it will be important to develop such biomarkers to serve as outcome measures in clinical trials of novel treatments. Lastly, it is still unclear why EBV-infected B cells are attracted to the CNS in MS to begin with, and research on this question will be important to our larger understanding of EBV dysregulation of the immune system in MS.

Although viral load does not appear to differ between MS patients and healthy EBV-infected controls, the presence of EBV DNA has been detected more frequently in serial samples of MS patients with high disease activity than those with low disease activity [39]. Only a handful of placebo-controlled clinical trials of antiviral agents have been performed in MS, and they have all shown only a modest effect at best [62,63]; however, the studies were small, and larger, definitive studies of antiviral treatments in MS may be warranted [57].

Conclusions

Epidemiologic investigation of other MS risk factors that influence EBV's role in the development of MS, along with further examination of the pathophysiologic differences in the immune response to EBV between MS patients and healthy virus carriers, will be critical to a greater understanding of the factors that lead to the EBV-driven autoimmunity in MS. Such investigation will lead to advances not only in the development of novel treatments for all forms of MS but also in the prevention of MS.

Disclosure

Dr. Bagert receives compensation from BioMS Medical for serving on a data safety monitoring board.

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