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Review article

Epstein–Barr virus and multiple sclerosis: Updating Pender's hypothesis

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ABSTRACT

Substantial epidemiological evidence supports the involvement of the Epstein–Barr virus (EBV) in multiple sclerosis (MS). Mechanisms through which EBV may increase MS risk are reviewed here. Most individuals contract EBV in early childhood yet only develop MS in early adulthood, by which time EBV has been latent for decades. When latent, EBV is confined to a minute subset of memory B cells: about 1000 cells in peripheral blood and 500,000 cells in the lymphoid system, mainly in the mouth. Reactivation of EBV in the central nervous system (CNS) has been proposed as a cause of MS. Alternatively, EBV may enable the recognition of "forbidden" antigens by memory B cells through its presence in this leukocyte type, as first proposed by Pender. Though the requirement for B cells in MS supports both hypotheses, EBV has not been consistently found in MS lesions, as would be expected. EBV episome replication during B cell division is now known to be inefficient, resulting in some descendant B cells becoming EBV-free after a few dozen divisions. EBV-free memory B cells in the CNS may thus have descended from a memory B cell which matured while containing EBV episomes, enabling its B cell receptor to recognize "forbidden" MS-causing antigens in the CNS, even if EBV is absent from this site.

1. Introduction

The majority of patients with multiple sclerosis (MS) experience their first symptom between 20 and 40 years of age, making this disease the most common cause of non-traumatic neurological disability in young adults (Benito-León et al., 1998). MS is a complex heterogeneous, inflammatory disorder characterized by the loss of the myelin sheath surrounding nerve axons in the central nervous system (CNS) (Stadelmann et al., 2011). This damage can cause a wide range of symptoms that may eventually lead to disability (Benito-León et al., 2003). In all forms of MS, inflammation is present when active demyelination and neurodegeneration occur (Stadelmann et al., 2011). Understanding the mechanisms behind the inflammatory process associated with MS appears to be the largest obstacle in primary prevention and in developing effective therapies.

The two strongest established risk factors for MS are Epstein–Barr virus (EBV) seropositivity (Almohmeed et al., 2013; Ascherio and Munger, 2007; Pakpoor et al., 2012) and major histocompatibility complex (MHC) class II gene HLA-DRB1*1501 (Hollenbach and Oksenberg, 2015), each increasing MS risk more than four fold. Possible mechanisms linking EBV and MS have been widely studied and reviewed (Ascherio and Munger, 2010). The three main hypothetical mechanisms linking EBV and MS through B cells involve either the reactivation of EBV within memory B cells in the CNS (Serafini et al., 2007), cross-reactivity of anti-EBV antibodies to human proteins in the CNS (molecular mimicry) (Vaughan et al., 1996), or the facilitation of "forbidden" memory B cells recognizing an antigen in the CNS (Lünemann and Münz, 2009; Pender, 2003). Other hypothetical mechanisms include a hygiene hypothesis where EBV would be acting as a surrogate for a highly hygienic upbringing (Ascherio and Munger, 2010), and the inadvertent recognition of human antigens in the CNS by CD8+ (cytotoxic) T cells targeting EBV antigens (molecular mimicry) (Ascherio and Munger, 2010). No proposed mechanism is fully consistent with observations. In this review, the biology of EBV's lifecycle is examined in relation to MS. Minor modifications to the EBV facilitated "forbidden" memory B cell hypothesis first proposed by Pender (Pender, 2003) can bring this hypothesis back in line with most observations, making it standout as the most plausible mechanism reviewed here.

2. Search strategy and selection criteria

References for this review were identified by searches of PubMed between 1969 and March 2017, and references from relevant articles.

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The search terms “multiple sclerosis”, “Epstein–Barr virus”, “antibodies”, and “B cells” were used. There were no language restrictions. The final reference list was generated on the basis of relevance to the topics covered in this review.

3. Results

3.1. Antibodies against viruses

Antibodies against viruses have been widely studied as risk factors for MS (reviewed by Ascherio and Munger, 2007). According to three recent meta-analyses, EBV seropositivity substantially increases the risk of MS (4.5-fold, 5.5-fold, or 16-fold, depending on the study) (Almohmeed et al., 2013; Ascherio and Munger, 2007; Pakpoor et al., 2012). Among EBV positive individuals, the risk of MS is further increased by high anti-EBV-EBNA antibody titers (Munger et al., 2011), by EBV infections acquired beyond childhood which caused infectious mononucleosis (2.2-fold increase according to the most recent meta-analysis) (Handel et al., 2010), and by co-infections with both EBV types (Santón et al., 2011). Finally, a very large prospective study of EBV as a risk factor for MS in the US military demonstrated that EBV seroconversion in young adults predisposes them to develop MS in the following decade (mean delay of 3.8 years, range 1.7–7 years) (Levin et al., 2010). The high relative risk of MS in EBV carriers (> 4.5-fold) is difficult to explain by EBV acting as a surrogate for other factors, since such factors would have be to extremely correlated with EBV exposure (Ascherio and Munger, 2010). The variety and strength of these studies indicate that EBV is directly involved in most MS cases. However, the vast majority of EBV infections occur in childhood (Balfour et al., 2013), yet the onset of MS is much later, usually in early adulthood (Confavreux and Vukusic, 2006; Ligouri et al., 2000), suggesting other environmental factors occurring later in life may be required for MS (Ascherio and Munger, 2010).

A second virus consistently associated with MS through serology is herpes simplex virus type 2 (HSV-2) (Ascherio and Munger, 2007). The increase in risk observed with HSV-2 seropositivity is much lower than for EBV (1.5–2.0-fold increase in risk) (Catalano, 1972; Ferrante et al., 1987; Hawkes et al., 2006; Wandinger et al., 2000), and could well be due to HSV-2 acting as a surrogate for other environmental factors, as occurred in cervical cancer before the discovery of oncogenic HPV subtypes (Aurelian et al., 1981; Dahlström et al., 2011). In contrast with EBV, the distribution of the age at onset of HSV-2 and MS (Confavreux and Vukusic, 2006; England, 2016; Ligouri et al., 2000) match quite well, and both HSV-2 and MS prevalence is skewed about 2:1 toward females (Ascherio and Munger, 2007; Hawkes et al., 2006; Xu, 2010), which prompted the hypothesis that MS could be directly caused by this virus (Martin, 1981). While EBV infects > 99% of MS cases (Ascherio and Munger, 2007) and thus could be etiologically involved in nearly all cases, HSV-2 infects less than 25% of MS cases in most studies (Catalano, 1972; Hawkes et al., 2006; Wandinger et al., 2000). This means HSV-2 cannot be involved in most MS cases, and if it does play a direct etiological role, the modest increase in risk suggests a minor contribution.

Other viruses such as herpes simplex virus type 1 (HSV-1), cytomegalovirus, human herpesvirus 6 (HHV-6), varicella zoster, measles, mumps and rubella viruses have been either inconsistently or not associated with MS through serology (Ascherio and Munger, 2007). Thus EBV appears to be the only virus strongly associated with MS at this time. A small fraction of pediatric (Alotaibi et al., 2004) and adult (Ascherio and Munger, 2007) MS cases appear to be EBV seronegative, suggesting either EBV is not a *necessary* factor in MS, or multiple distinct mechanisms can cause MS, the most common of which would require EBV.

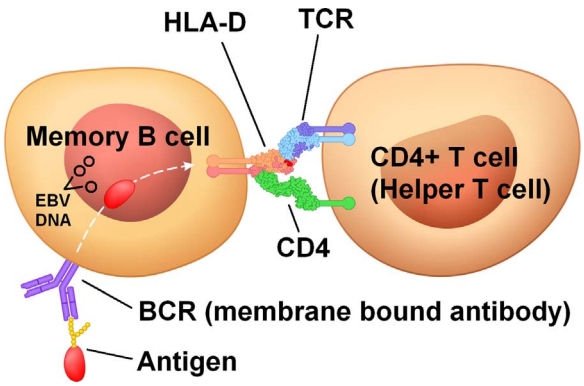

Fig. 1. Standard model of memory B cell activation. If the CD4 + T cell's TCR has a high affinity to the peptide presented by the HLA-D molecule complex (MHC class II receptor), it will signal this through CD154 and CD40 (not shown). Thereafter the memory B cell will differentiate into a plasma cell, which will secrete large amounts of antibodies (not shown). When this occurs to an EBV infected memory B cell, EBV virions are synthesized by the plasma cell, eliciting a strong CD8 + (cytotoxic) T cell response which rapidly clears the infected plasma cell. In this example, the memory B cell was infected with EBV, shown as three circular DNA episomes in the nucleus. EBV is not believed to affect memory B cell activation, as it is in a latent state in memory B cells, expressing only a single protein coding gene (*EBNA1*). The antigen shown in this example is a mannoprotein which binds to the BCR through terminal mannose residues, though many other antigens are recognized by human B cells.

3.2. B cells

According to the standard model of memory B cell activation (Fig. 1), when a memory B cell encounters an antigen cognate with its antibody, this antigen is endocytosed and broken into peptides which are presented to CD4 + (helper) T cells through HLA-D molecules and the T cell receptor (TCR) (Kurosaki et al., 2015). If the CD4 + T cell recognizes this peptide as foreign, a secondary immune response is initiated: the memory B cell differentiates into a plasma cell and synthesizes large quantities of this antibody (Kurosaki et al., 2015).

A number of recent studies have shown that B cells play an important role in MS (reviewed by Disanto et al., 2012), especially since their decimation using monoclonal antibodies targeting CD20 has proven to be a very effective intervention (Disanto et al., 2012). Counterintuitively, secreted antibodies in the CNS do not seem to be directly involved in MS because CD20 depletion only targets B cells, and does not immediately affect plasma cells or secreted antibody levels despite improvements in MS lesions (Disanto et al., 2012). This suggests memory B cells in the CNS are directly involved in MS, possibly through antigen presentation to CD4 + T cells, direct cytokine production or trafficking of EBV into the CNS, but not through differentiation into plasma cells (Disanto et al., 2012).

It is unclear which antigens B cells in the CNS are targeting in MS: antibodies against the most likely candidates, human myelin oligodendrocyte glycoprotein and myelin basic protein, are not associated with MS (Kuhle et al., 2007; Owens et al., 2009). A very recent study suggests oligoclonal antibodies in MS target intracellular human proteins which are not specific to the brain, and may be a consequence of tissue destruction rather than a cause (Brändle et al., 2016).

B cells could also be targeting foreign antigens present in the CNS due to a chronic infection, with the three most obvious candidates being EBV (which is typically latent and would need to become reactivated in the CNS), HHV-6 (a ubiquitous virus which permanently infects leukocytes in the CNS and elsewhere), and *Toxoplasma gondii* (an endemic obligate intracellular protist which permanently infects the CNS). The associations between EBV and HHV-6 infections within the CNS and MS have been variable and inconclusive (Lassmann et al., 2011; Venkatesan and Johnson, 2014). Seropositivity to *Toxoplasma gondii* seems to be protective (Stascheit et al., 2015).

Alternatively, anti-EBV antibodies present in the CNS (Cepok et al.,

2005) could inadvertently have a high affinity to human proteins through molecular mimicry, triggering MS-causing inflammation in this site (Lindsey et al., 2016; Vaughan et al., 1996). However, this simple mechanism fails to explain the multi-year delay between EBV seroconversion and MS onset in young adults, as anti-EBV antibodies precede MS onset by many years (Levin et al., 2010).

3.3. EBV infected memory B cells

EBV is mainly found in memory B cells, in the form of DNA episomes within the nucleus of these cells (Thorley-Lawson, 2015). During infectious mononucleosis, EBV episomes can be found in up to half of all memory B cells, gradually declining to about one in 100,000 memory B cells in healthy carriers (Thorley-Lawson, 2015). Though less studied, the fraction of EBV infected memory B cells following an asymptomatic primary infection is similar to that observed in infectious mononucleosis (Silins et al., 2001). Thereafter, EBV persists at a very low but stable viral load: about half a million memory B cells are infected in the entire human body, approximately one percent of which circulate in the peripheral blood at a concentration of one infected B cell per 5 ml, with remaining cells located mainly in the lymphoid system, especially near the tonsils (Thorley-Lawson, 2015).

EBV remains mostly latent during the rest of an infected individual's lifetime, expressing a single gene (*EBNA1*) within memory B cells which allows its episome to be copied when the cells divide (Thorley-Lawson, 2015). The EBNA1 protein is not well recognized by CD8+ (cytotoxic) T cells, allowing infected memory B cells to mostly escape detection (Thorley-Lawson, 2015). EBV infected memory B cells periodically reactivate in the mouth and genital tract (Israele et al., 1991; Sixbey et al., 1986), differentiating into plasma cells and shedding virions to infect other hosts and to maintain a stable infected memory B cell pool in the same host (Thorley-Lawson, 2015). Plasma cells synthesizing EBV virions are rapidly cleared by CD8+ (cytotoxic) T cells (Thorley-Lawson, 2015).

3.4. EBV infected memory B cells in the CNS

B cells and EBV both seem to be necessary factors in MS, suggesting their interaction may be important (Márquez and Horwitz, 2015). The presence of EBV infected memory B cells within the CNS is controversial (Lassmann et al., 2011; Owens and Bennett, 2012). If the EBV infected memory B cell concentration in the CNS is similar to that found in peripheral blood (0.001% of memory B cells infected), such a low load should not elicit an immune response since EBV is very effective at evading CD8+ (cytotoxic) T cells while latent (Thorley-Lawson, 2015). Were EBV infected memory B cells in the CNS to reactivate, expand and differentiate into plasma cells, viral synthesis would occur, eliciting a strong CD8+ (cytotoxic) T cell immune response (Hislop and Taylor, 2015). In principle, even a single acute EBV reactivation event in the CNS could lead to a permanent loss of immune tolerance to CNS antigens, though such a hit-and-run mechanism would be difficult to prove (Opsahl and Kennedy, 2007).

The EBV load in the CNS of MS patients is currently unclear. The first positive study of EBV in the CNS found EBV infected B cells in 21 of 22 cases analyzed using *in situ* hybridization of EBV-encoded small RNA (EBER) transcripts, and reactivation markers EBV nuclear antigen 2 (EBNA2) and EBV latent membrane protein 1 (LMP1) in respectively 13 and 16 of 18 cases analyzed using immunohistochemistry (Serafini et al., 2007). However, these findings could not be replicated by several groups (Opsahl and Kennedy, 2007; Peferoen et al., 2010; Sargsyan et al., 2010; Torkildsen et al., 2010; Willis et al., 2009), suggesting either important methodological differences occurred between studies or no more than a trace amount of EBV is typically present in the CNS of MS patients (Lassmann et al., 2011; Owens and Bennett, 2012). In addition to *in situ* techniques, many of these replication studies used highly sensitive and specific reverse transcription PCR (RT-PCR) assays to detect both EBER transcripts (which are abundantly present in all EBV infected B cells including those where EBV is latent) and messenger RNA transcripts (which indicate EBV reactivation). These replication studies analyzed more than 100 cases, including many cases from the first positive study. Thereafter three studies, including one from an independent group, were published with methods and results similar to the first positive study (Magliozzi et al., 2013; Serafini et al., 2013; Tzartos et al., 2012). Finally two more studies with mixed result were published using PCR and RT-PCR based assays, which found EBV DNA in 9 of 26 MS cases' CNS (Hassani and Khan, 2015) and EBV RNA in 5 of 31 MS cases' cerebrospinal fluid (CSF) (Veroni et al., 2015).

EBV reactivation in the CNS is consistent both with Pender's hypothesis, where reactivation occurs through the recognition of a "forbidden" antigen cognate with the BCR of an EBV infected memory B cell, and with memory B cell reactivation independent of the BCR. However, currently available evidence supporting EBV reactivation in the CNS of MS patients is weak, and the EBV reactivation hypothesis cannot easily account for the multi-year delay observed between EBV acquisition in adults and the onset of MS (Levin et al., 2010). This delay would be expected to be short because the EBV load is ten thousand times higher during primary EBV infections as compared to established EBV infections, transitioning from a high to low viral load over approximately one year (Silins et al., 2001; Thorley-Lawson, 2015).

Though EBV reactivation in the CNS is the simplest hypothetical mechanism liking EBV to MS, difficulties in replicating supporting studies and the multi-year lag between EBV acquisition and MS onset suggest other mechanisms should be considered. In particular, EBV may be directly affecting memory B cell populations, which in turn affect MS risk (Pender, 2003; Tracy et al., 2012).

3.5. EBV infected memory B cell receptor

A consensus view as to how EBV affects memory B cell populations is lacking (Tracy et al., 2012). The germinal center model (Thorley-Lawson, 2001), a leading hypothesis, suggests EBV infects naive B cells, then uses its *LMP1* gene to increase the probability of producing long-lived memory B cells by emulating the CD4+ (follicular helper) T cell conjugation phase within B cell follicles of lymph nodes (Tracy et al., 2012). Under normal circumstances, a naive B cell can only mature into a memory B cell after binding to an epitope approximately matching its BCR, endocytosing the entire antigen, breaking it into peptides, presenting some peptides on HLA-D receptors, and finally having some peptides recognized by the TCR of CD4+ T cells (Fig. 2, reviewed by Kurosaki et al., 2015). When EBV infected, naive B cells can mature into memory B cells without going through all these steps (Caldwell et al., 1998).

In peripheral blood, memory B cells harboring EBV have undergone somatic hypermutation and express a normal BCR, suggesting they have gone through most or all maturation steps (Thorley-Lawson, 2015). However, some of these memory B cells are suspected of having escaped the germinal center of lymph nodes through the expression of EBV's *LMP1* gene (rather than through follicular helper T cells conjugation), resulting in memory B cell's BCR recognizing normally "forbidden" epitopes (Fig. 3 – panels 3 and 4) (Tracy et al., 2012). Based on this hypothesis, EBV could influence the epitopes recognized by memory B cells of various isotypes, for example enabling IgG memory B cells to recognize either self epitopes or commensal microbe epitopes, thus causing undesirable chronic inflammation where these epitopes are found in the body (Pender, 2003; Tracy et al., 2012). Note that EBV may not be strictly necessary to produce memory B cells recognizing "forbidden" epitopes, which could explain EBV seronegative MS cases.

If clonally expanded memory B cells in the CNS matured while EBV infected, one would expect them to contain EBV episomal DNA and EBV EBER transcripts readily detectable using PCR and RT-PCR, yet the detection of EBV in the CNS has been inconsistent, and its presence remains controversial (Lassmann et al., 2011; Owens and Bennett,

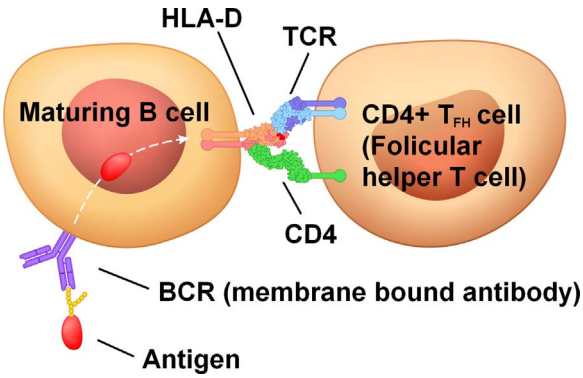

Fig. 2. Standard model of naive B cell maturation into memory B cell (for a more complete description, see Fig. 1 in Kurosaki et al., 2015). An antigen's epitope binds to a maturing B cell's BCR, followed by endocytosis of the antigen, reduction into short peptides, and presentation of these peptides on HLA-D molecule complexes (MHC class II receptors) to the TCR of CD4+ (follicular helper) T cell. If maturing B cell antigen uptake is efficient (indicating high BCR-antigen affinity) and the T cell's TCR has a high affinity to the peptide presented by the HLA-D molecule complex (shown in red), the T cell recognizes the peptide as foreign and signals to the maturing B cell that it can continue on its path to becoming memory B cell. This signaling is done in large part by CD154 on the T cell binding to CD40 on the B cell (not shown). EBV's *LMP1* gene can emulate CD154/CD40 signaling, allowing B cells to mature into the memory compartment while skipping the CD4+ (follicular helper) T cell checkpoint. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2012). Since EBV transmission to daughter cells during memory B cell division is not very reliable (Fig. 3 – panel 5) (Nanbo et al., 2007), some EBV infected memory B cells will eventually become EBV free: clonally expanded memory B cells in the CNS could thus have matured from an EBV infected naive B cell, and target a "forbidden" MS-causing epitope. In light of this, Pender's hypothesis can be extended to include memory B cells which were *previously* infected with EBV, eliminating the requirement of finding EBV in the CNS.

3.6. EBV reactivation in the mouth

An EBV-positive memory B cell which never reactivates into a plasma cell *in the mouth* is a dead end for the virus, because shedding virions in this site is required for transmission. In the absence of EBV, memory B cells' BCR must bind to a cognate antigen which is also recognized by a CD4+ (helper) T cell to reactivate into a plasma cell (Fig. 1). The mechanism through which EBV-positive memory B cells reactivate has not been established, though it is suspected of being identical to that of uninfected memory B cells: within memory B cells, EBV only expresses a single protein (EBNA1) which is not known to be involved in reactivation (Thorley-Lawson, 2015). Alternative reactivation mechanisms which are strongly skewed toward the mouth appear implausible.

If EBV infected memory B cell reactivation occurs using the cognate antigen recognition mechanism described in the previous paragraph, then the targeted antigens must be from commensal microbes in the mouth. If the targeted antigens were human, reactivation would not be possible due to a lack of CD4+ (helper) T cell recognition of human proteins. This is consistent with the recent finding that the vast majority of EBV-positive memory B cells do not recognize human proteins (Tracy et al., 2012).

The main objection to cognate antigen recognition as a homing method to the mouth is that EBV is thought to infect resting naive B cells which have a "random" BCR, rather than a BCR which recognizes an antigen present in the mouth (Thorley-Lawson, 2015). However, the combined presence of a functional BCR and somatic hypermutations in EBV-positive memory B cells suggest their BCR is not "random", but rather has been honed within a germinal center to recognize an epitope (Thorley-Lawson, 2015). To reconcile these observations, either EBV must infect antigen experienced proliferating B cell blasts (though this seems unlikely because activated B cells in proliferating B cell blasts don't express CD21, which is required for virion attachment), or initially "random" BCRs of antigen naive B cells must converge toward an antigen present in the lymph node during affinity maturation (Fig. 3 – panel 3).

3.7. EBV and host fitness

Since EBV infects a host for life, it is expected to have evolved mechanisms which limit host morbidity and mortality (Thorley-Lawson, 2005). The increase in risk of MS caused by EBV seems at odds with its own fitness: why hasn't EBV evolved to avoid causing MS? For example, if inadvertent molecular mimicry between EBV antigens and human antigens were causing MS, EBV would be expected to evolve non-cross-reactive antigens (if possible).

To resolve this apparent contradiction, we speculate that EBV requires B cells in the memory compartment to have a high affinity to commensal microbes in the mouth, in order to reactivate in this site and complete its lifecycle. In contrast, optimal host fitness requires filling the memory compartment with B cells specific to pathogens, while avoiding B cells with a high affinity to commensal microbes, as this could result in undesirable chronic inflammation. Thus, EBV would be using its *LMP* genes not to bypass normal B cell maturation altogether, but rather to allow B cells with a high affinity to mouth commensal microbes to enter the memory compartment. MS would be an unfortunate side-effect of this mechanism which is essential for EBV fitness, but harmful for host fitness.

This mechanism would require "random" BCR B cells to proliferate in the germinal center despite initially poor antigen affinity, until somatic hypermutations have had time to increase BCR affinity toward *any* microbial antigen abundantly present in the lymph node. Thereafter, the normal B cell maturation process would produce high affinity memory B cells which recognize an abundant microbial antigen present the mouth, ideal for EBV's lifecycle.

While most EBV-positive memory B cells are not autoreactive (Tracy et al., 2012), it is difficult to exclude the presence of a minute subset of autoreactive EBV-positive memory B cells which could inadvertently recognize a human epitope in the CNS—though these cells would be of no use to complete EBV's lifecycle, as they would not reactivate in the mouth.

4. Conclusion

Epidemiological evidence strongly supports a role for EBV in MS, yet no proposed mechanism has been able to convincingly explain this link so far (Ascherio and Munger, 2010). EBV is present in > 90% of adults, most often as a result of childhood exposure (Balfour et al., 2013), and is restricted to a minute subset of memory B cells (Thorley-Lawson, 2015). The requirement for B cells in MS suggest these cells play a key role, possibly due to being EBV infected. The many links between EBV and MS described here suggest peripheral memory B cells must reach the CNS: deep sequencing studies of the IgG heavy chain of B cells in the CSF and peripheral blood confirm this (Michel et al., 2015; von Büdingen et al., 2012).

Two important aspects linking EBV and MS are puzzling. First, EBV is present at extremely low levels in the body (Thorley-Lawson, 2015), meaning a direct immune response against EBV appears to be an unlikely cause of MS. Second, MS is rare in children (Confavreux and Vukusic, 2006; Ligouri et al., 2000), suggesting EBV infections are insufficient to cause MS on their own. A partial explanation for the first puzzling aspect has been proposed: EBV may rescue "forbidden" memory B cells which recognize an epitope in the CNS (Pender, 2003; Tracy et al., 2012). However, inconsistent detection of EBV in the CNS is somewhat at odds with this hypothesis (Lassmann et al., 2011). The unreliable transmission of EBV during memory B cell division, first reported by Nanbo and colleagues in 2007, can explain

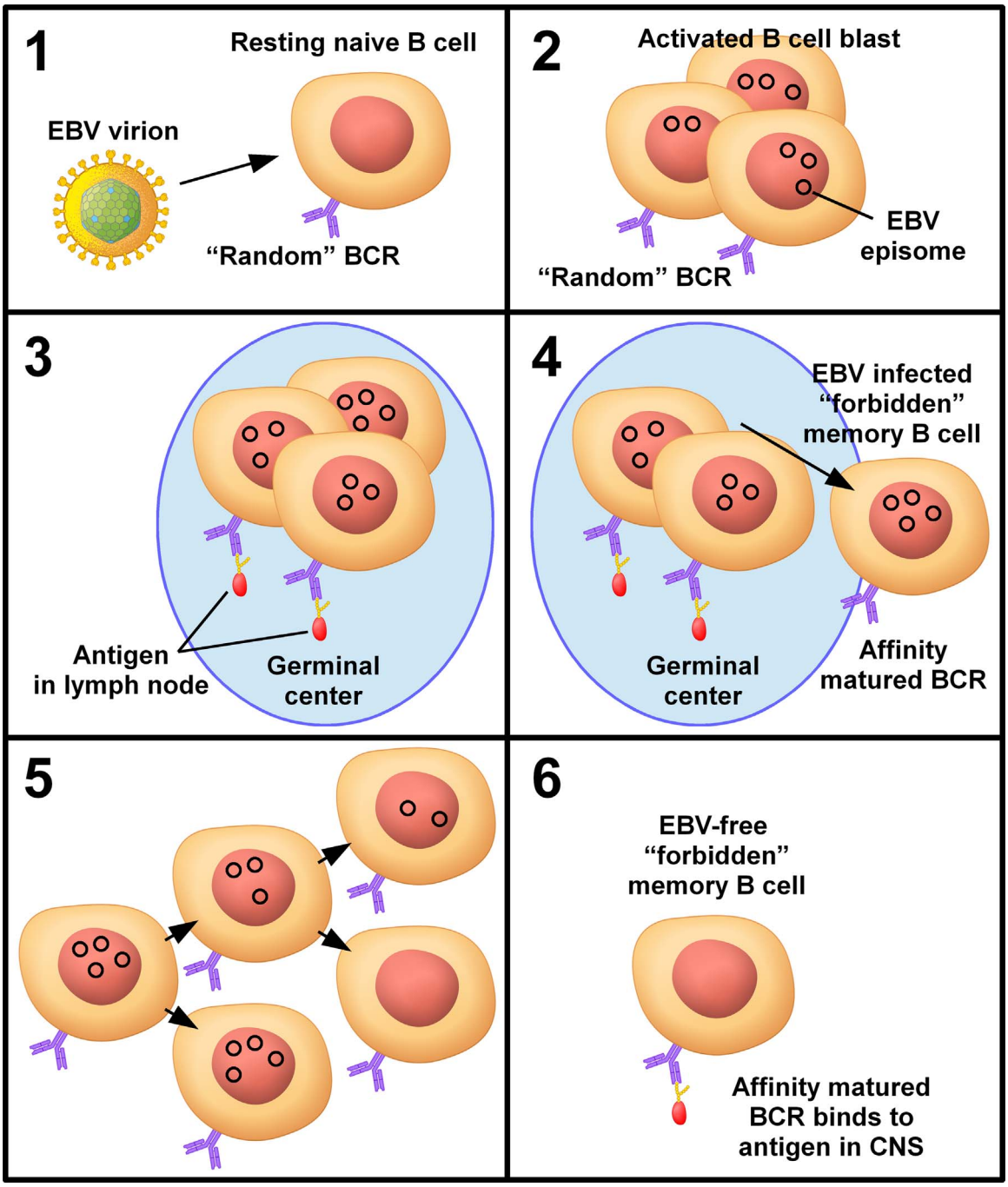

Fig. 3. Proposed sequence of events leading to memory B cell recognition of epitopes in the CNS. (1) EBV infects a resting naive B cell with a "random" BCR in near mucosal surfaces (mouth / genitals). (2) EBV activates naive B cell, which divides and forms a blast. (3) EBV infected B cell blast improves BCR affinity through somatic hypermutation in germinal center, skewing the BCR toward antigens present in the lymph node at that time. (4) "Forbidden" B cell escapes germinal center and enters memory compartment. (5) EBV replication and segregation during memory B cell division is not efficient, allowing some memory B cell descendants to become EBV-free after a few dozen generations; since memory B cells divide infrequently, this process is expected to be long (years). (6) Recognition of an epitope present in the CNS by a *previously* EBV infected memory B cell. The epitope must be similar to that present in the germinal center during affinity maturation, though the antigen itself may be completely different: for example, the epitope can be a ubiquitous terminal glycan of a glycoprotein, with the protein portion being completely different between the mouth and CNS.

the absence of EBV in the CNS while still allowing "forbidden" memory B cells to reach this site (Nanbo et al., 2007). This can also explain the delay of a few years between EBV seroconversion in young adults and MS onset (Levin et al., 2010), which other mechanisms reviewed here do not account for. However, no mechanism reviewed here can explain the multi-decade delay between EBV infections in early childhood and MS in early adulthood. This second puzzling aspect may require additional environmental factors which mainly affect teens and adults.

Both EBV infected memory B cells and memory B cells present in the CNS in MS have a functional BCR and have undergone somatic hypermutation (Qin et al., 1998; Thorley-Lawson, 2015), though antigens cognate to this BCR are not known in either case. EBV's lifecycle suggests this antigen should be present where EBV infects naive B cells (e.g. in the mouth), allowing EBV infected memory B cells to preferentially reactivate and produce virions in this site, completing its lifecycle (Thorley-Lawson, 2015). It appears these cognate antigens are generally not human proteins (Tracy et al., 2012). To our knowledge, no study has investigated the possibility that EBV infected

memory B cells recognize commensal microbe antigens present in the mouth: this seems to be the simplest scenario.

Based on the evidence reviewed here, we slightly modify Pender's hypothesis as follows. EBV infected memory B cells recognizing a commensal microbe epitope eventually become EBV-free through memory B cell division. Once EBV free, these memory B cells can proliferate and recognize a cognate epitope in the CNS, causing inflammation and MS. The nature of the antigen in the CNS on which this cognate epitope is found remains unclear: it could either be a human antigen or an antigen belonging to a microbe present in the CNS (such as *Toxoplasma gondii*). Under EBV-free circumstances, B cells recognizing this "forbidden" epitope are generally not allowed to enter the memory compartment, greatly reducing the risk of MS in EBV negative individuals. When EBV infected, the *LMP1* gene simulates CD4+ (follicular helper) T cell B cell conjugation in the germinal center, allowing a B cell recognizing a "forbidden" epitope to enter the memory compartment.

Disclosures

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JBL reports no disclosures.

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