Epstein—Barr virus and multiple sclerosis

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ABSTRACT

This review of the considerable evidence linking Epstein—Barr virus (EBV) infection to risk and disease progression in multiple sclerosis (MS) builds on the background to the virus and its interactions with the human host available in the online supplement (see supplement, available online only). The evidence for a similarity in the geographic patterns of occurrence of MS and EBV infection (with infectious mononucleosis or EBV specific serology used as surrogate markers), when reviewed critically, is very limited. There is strong evidence however that people with MS are more likely to report a past history of infectious mononucleosis (thought to represent initial EBV infection at an older age), and higher titres of EBV specific antibodies are associated with an increased risk of developing MS. Elevated levels of the latter are apparent many years before MS onset (compared with non-MS controls) and there is a dose—response relationship between MS risk and antibody titre, with antibodies to the EBV nuclear antigen-1 particularly important. The evidence in relation to EBV DNA load in blood or CSF is conflicting, as is that in relation to T cell responses to EBV. Several hypotheses that have been proposed to explain the links between EBV and MS risk are reviewed and gaps requiring further research are identified.

INTRODUCTION

Evidence supporting a role for Epstein—Barr virus (EBV) infection in multiple sclerosis (MS) comes from ecological studies, observational epidemiological studies, co-occurring pathologies and experimental laboratory based research. But despite a large body of research, definitive evidence that EBV infection is a causative risk factor for MS, rather than the result of a deranged immune system associated with the disease, is not yet available. We build on the background provided in the online supplement (see supplement, available online only) by assessing the current evidence relating EBV infection to MS, and highlight research gaps. This is a comprehensive review of the research evidence to date, sourced by using the search terms 'Epstein Barr virus and Multiple Sclerosis' in PubMed and through searching the reference lists of sourced papers. The original research papers, including meta-analyses, were sought, with published reviews used to provide an additional source of research papers to ensure complete coverage.

ECOLOGICAL PATTERNS

Several reviews[1–4] as well as a recent ecological study[5] suggest that the similar latitudinal distributions of infectious mononucleosis (IM) and MS are evidence of an association. In the latter study, hospitalisation for IM varied across a narrow latitudinal range, but in the studies commonly cited as supporting evidence, it is difficult to separate latitudinal and socioeconomic variation. In their 1980 letter, Warner *et al*[6] noted that classical IM and MS were both uncommon in African, Oriental and Polynesian populations,[6] with the high incidence of IM 'a characteristic of affluent Caucasians'—that is, socioeconomic status was considered the important risk factor. In an earlier study (1974) of US Military Academy cadets,[7] there was considerable geographic variability in EBV seropositivity but this was consistent with socioeconomic status or race (figure 1) and latitude was not considered. More recently, there was no difference in the prevalence of EBV seropositivity in adults in Rome (latitude 42°N) compared with Bogota (4°N) (83.5% and 83%, respectively).[8]

A later age of EBV infection (the EBV variant of the hygiene hypothesis) has been suggested as a key MS risk factor that could explain persisting latitudinal gradients in incidence of CNS demyelination[9] or loss of a previously observed latitude gradient.[10] Certainly, the prevalence of seropositivity in childhood appears to be higher in less developed countries compared with more developed regions (eg, 92.3% of children aged 6–8 years in Bangkok[11] vs 54% in children aged 10–14 years in the UK[12]) but no study has yet shown whether the age specific prevalence of EBV seropositivity varies by latitude after the level of development is considered—that is, within a single country with relative homogeneity of socioeconomic status.

INDIVIDUAL LEVEL ASSOCIATIONS WITH MEASURES OF EBV INFECTION

History of infectious mononucleosis

There is considerable and consistent evidence that people with MS are more likely to report past IM—generally regarded as the hallmark of late EBV infection—than unaffected controls. In the most recent meta-analysis of case control and cohort studies, the combined relative risk (RR) of MS for a past history of IM was 2.17 (95% CI 1.97 to 2.39).[13]

Although some studies have found that other common viral infections[14] were associated with increased MS risk, the most consistent finding is the association with past IM.[15] Increased MS risk was also apparent in those whose sibling had a history of IM (adjusted odds ratio (AOR)=1.41 (95% CI 1.13 to 1.75)),[14] and in relation to a personal history of tonsillectomy (AOR=1.25 (95% CI 1.11 to 1.40)).[14] Recurrent tonsillitis has been separately linked to recurrent EBV infection and reactivation.[16] However, a history of IM is usually determined by interview, with considerable scope for recall error

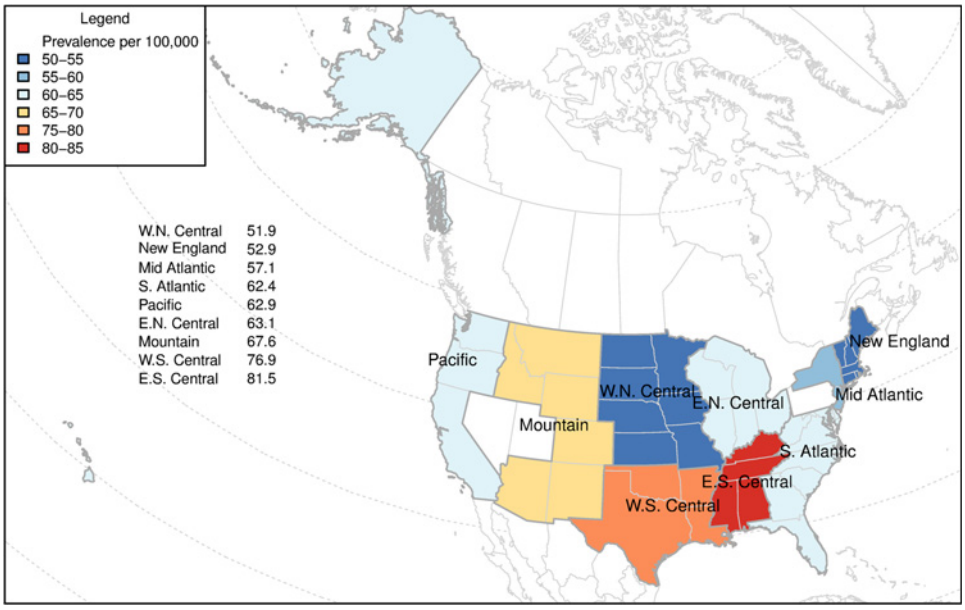

Figure 1 Pictorial representation of the results from Hallee *et al*[7] showing variation in the prevalence of Epstein–Barr virus (EBV) seropositivity in cadets who had resided in the same state for at least 6 years prior to entering the US Military Academy (n=1281) according to their US state of residence.

and disease status misclassification: only 30–40% of late EBV infections are thought to result in the clinical syndrome of IM, and infection by other pathogens may cause an IM-like illness.[7]

Previous hospital admission with IM was associated with a fourfold increase in MS risk compared with a comparison cohort in the Oxford Record Linkage Study,[17] with a mean interval to MS onset of 14 years. Furthermore, in a large (n=25 234) Danish cohort study,[18] IM diagnosed by a positive Paul–Bunnell test (titre >1:32) was associated with increased MS risk (age, sex and period standardised incidence ratio=2.27 (95% CI 1.87 to 2.75)) compared with that expected based on disease patterns in the whole population. Here the increased risk of MS appeared 10 years or more after the diagnosis of IM and persisted for more than 30 years.

Past IM also appears to modify the MS risk associated with the major susceptibility gene HLA-DRB1*15, with a 2.4-fold (95% CI 2.0 to 3.0) increased MS risk associated with DRB1*15 in IM negative individuals, but a sevenfold (95% CI 3.3 to 15.4) increase in risk where both DRB1*15 and IM were positive.[19] This translates into a 10-fold increased risk of MS in persons who are DRB1*15 positive and have a history of IM, compared with persons who are DRB1*15 negative and have no history of IM.

EBV specific serology

Studies dating from 1980[20] consistently show that people with MS are more likely to be EBV seropositive (≥99%) than healthy controls (85–95%). Indeed, EBV seronegativity is rare in MS, as is evidence of a primary EBV infection at MS presentation,[21 22] suggesting that prior EBV infection may be a key component in the development of the disease process. In recent meta-analyses, the summary OR for MS based on being EBV seropositive (vs seronegative, eight studies) was 13.5 (95% CI 6.3 to 31.4),[23] while being EBV seronegative (vs seropositive, 13 studies) was associated with a marked decrease in MS risk (OR (Mantel-Haenszel)=0.06 (95% CI 0.03 to 0.13)).[24]

Although specific serological patterns are described for infection and reactivation of EBV infection,[25 26] the most consistent finding in relation to increased MS risk is elevation of antibodies to the Epstein–Barr nuclear antigen (EBNA) complex, particularly anti-EBNA-1 titres. In a recent meta-analysis (n=30 studies), the summary ORs for MS risk in relation to seropositivity for different EBV specific antibodies were: anti-EBNA complex IgG, OR=5.4 (95% CI 2.9 to 9.8); anti-EBNA-1 IgG, OR=12.1 (95% CI 3.1 to 46.9); antiviral capsid antigen (VCA) IgG, OR=5.5 (95% CI 3.4 to 8.8).[27] In one study however, the strongest effect was for anti-EBNA-2 titres.[28] In several studies MS risk increases monotonically with increasing serum anti-EBNA titres,[28 29] and one study[30] has shown that it is particularly the IgG1 class (rather than IgG2 or IgG4) that is important, consistent with a Th-1 polarisation of anti-EBV immunity.

More than 95% of MS cases of Northern European origin demonstrate oligoclonal IgG bands in the CSF which are not present in serum.[31] Increased intrathecal immunoglobulin synthesis is also seen in viral infections of the CNS, with the antigenic target epitopes predominantly those of the infectious agent itself.[32] In MS, CSF oligoclonal bands are polyspecific and those that are EBV reactive have been found at similar frequency in MS patients and those with other neuroinflammatory conditions.[33] A few studies (but not all[34]) have shown that, compared with non-MS controls, MS patients have higher levels of CSF antibodies that react to EBV antigens, particularly EBNA-1 (85% of MS patients compared with 13% of controls, p<0.001),[35–38] but also peptide sequences derived from the EBV BRRF2 protein[36] and VCA.[37] These findings may indicate a selective intrathecal antibody response to recent infection, or reactivation, in the CNS.

Large cohort studies have provided an opportunity to examine serial blood samples prior to MS onset and provide evidence that the elevation in EBV specific antibody titres precedes disease onset, sometimes by several years.[28 29] In one study, elevations in anti-EBNA complex and anti-EBNA-1 titres in MS cases first occurred between 15 and 20 years before the onset of MS and persisted in a relatively stable pattern thereafter[39] although another found that the heightened risk was greater for sera taken <5 years before MS onset (OR=11, (95% CI 1.5 to 75)) compared with those taken 5 or more years before diagnosis (OR=4.2 (95% CI 1.2 to 14)).[40]

In a recent 3 year follow-up study of children (median age 9.6 years) with acute demyelination, those with serological evidence of past EBV infection were twice as likely to be diagnosed with MS (hazard ratio=2.55 (95% CI 1.26 to 5.18)) than those with negative serology.[41] Nevertheless, evidence from

adult cohort studies suggests that young adulthood is an important developmental window. In a US military cohort (n=69 case control sets with blood samples available prior to disease onset), anti-EBNA complex titres were similar for MS cases and controls when they were <20 years old but in cases there was a sharp and significant increase in antibody titres during early adulthood that was not seen among controls.[29] Thus, by age 25 years or older, anti-EBNA complex titres were 2–3-fold higher among eventual MS cases than controls (p<0.001). Here also, increasing anti-EBNA complex titres between the first and a subsequent serum sample were associated with a marked increase in the risk of developing MS (RR=3.0 (95% CI 1.3 to 6.5) for a fourfold increase in titre), particularly where the first sample was collected at or before age 20 years (RR=18 (95% CI 2.2 to 138), p=0.006).[29] Furthermore, of participants who were EBV seronegative on the first blood sample, 100% of cases seroconverted prior to MS onset compared with only 35.7% of controls (consistent with seroconversion rates in healthy populations of a similar age) over the same period (p<0.0001).[42] The mean interval from the date of the first EBV seropositive serum sample to MS onset was 3.8 years (range 1.7–7.0)—under an assumption that EBV infection occurred, on average, at the midpoint between the last seronegative sample and the first EBV seropositive sample, the mean interval between primary EBV infection and MS onset would be 5.6 years (range 2.3–9.4).[42]

Studies involving cases with clinically isolated syndrome (CIS) generally also show that anti-EBNA IgG titres are higher than in controls[43 44] although one study[44] found no difference. Higher anti-EBNA titres have been associated with lower age of onset (p<0.0001) but not with the clinical MS phenotype.[44]

A number of factors influence anti-EBV titres in healthy populations. For example, in a non-MS healthy Danish population sample,[45] female gender (β=0.24, p=0.02), current versus never smoking (β=0.36, p=0.002) and number of smoking pack years (β=0.12, p<0.001) were associated with increased anti-VCA titres, with the smoking effect stronger in females (pack years, β=0.24, p<0.0001). In a pooled analysis of three MS case control studies, cases who had ever smoked ('ever smokers') had significantly higher anti-EBNA IgG titres than cases who had never smoked ('never smokers').[46] The increased MS risk associated with higher anti-EBNA titres (in the case control analysis) was stronger among ever smokers than never smokers (p for interaction=0.001); but the increased MS risk associated with smoking was seen *only* among those who had high anti-EBNA titres.[46]

Interactions between markers of EBV infection and HLA-DRB1*15 for MS risk have been examined. In healthy controls, DRB1*15 positivity was unrelated to anti-EBNA-1 titres[47] or associated with higher titres.[48 49] Higher anti-EBNA-1 levels and HLA-DRB1*15 positivity have been shown to be independent risk factors for MS,[47 49] but they interact additively, such that women with high anti-EBNA-1 titres who are also HLA-DRB1*15 positive have a marked (ninefold) increase in risk of MS compared with those with low titres who are HLA-DRB1*15 negative.[47] Studies such as these, which examine the interplay between risk factors, assist our knowledge of mechanisms as they indicate which component factors may operate together.

Several studies have explored the antibody response to specific segments of the EBNA-1 antigen.[50–52] Sera from paediatric onset MS patients recognised a broader range of distinct epitopes within EBNA-1, particularly three unique regions,[51] compared with the restricted EBNA-1 response (directed mainly against the glycine–alanine rich region) seen in their parents or in healthy sibling controls. One of these epitopes (EADYFEYHQE, amino acids 411–420) is contained within a fragment of EBNA-1 (amino acids 385–420), antibodies to which were associated with the highest MS risk in an adult study.[50] Here the combination of being HLA-DRB1*15 positive and having increased antibody reactivity to this segment was associated with a 24-fold increased risk for MS.[50]

EBV DNA load in the peripheral blood or the CNS

In some studies, EBV DNA load in peripheral blood mononuclear cells (PBMC) was higher in patients with a CIS[43] or relapsing–remitting MS[53] (not statistically significant, possibly due to the small sample size) compared with healthy virus carriers but others have found no increase in whole blood, serum or PBMC.[27 54–56] In the Nurses' Health Studies, MS cases who had had blood collected before the onset of symptoms, or before diagnosis, were more likely to have any detectable plasma EBV DNA compared with age matched healthy controls (RR=2.5 (95% CI 0.78 to 7.8), p=0.12), but there was no association between quantitative plasma EBV DNA load and MS risk.[57]

EBV infection of brain infiltrating B cells and plasma cells (based on detection of EBV encoded RNA (EBER) and EBV specific antibodies) was evident in nearly 100% of postmortem brains of patients with MS (but not with other inflammatory CNS diseases) in one study.[58] However, these observations have not been confirmed in subsequent studies[59 60] (possibly due to degradation of EBER by long tissue fixation times in formalin),[61] including in a re-examination of the same tissue used in the earlier study.[58] In one recent study, cell free EBV DNA was found in the CSF of only one MS patient[62] and no EBER was detected in B cells or plasma cells in the CSF of MS patients in another study.[63] Although EBER was occasionally detected in active MS plaques, the authors concluded that there was no evidence of active EBV infection in MS brain tissue or CSF.[63] In one small study, EBV infection of human brain microvascular endothelial cells[64] resulted in upregulation of proinflammatory cytokines and increased adhesion of PBMCs. This process, possibly affecting isolated populations of endothelial cells, could cause local breaches of the blood–brain barrier and allow autoreactive lymphocytes to access the brain.[64]

A small number of studies have examined the specific EBV strains infecting MS cases. Brennan *et al* found marginally different frequencies of several single nucleotide polymorphisms in the EBNA-1 and BRRF2 genes in MS patients compared with non-MS controls but no differences in the frequencies of single nucleotide polymorphisms within the LMP1 gene,[65] the latter in agreement with earlier work.[66]

EBV specific T cell responses

Control of EBV infection involves the presentation of viral peptides by MHC class I molecules to CD8 T cells, and by MHC class II molecules to CD4 T cells. Several studies have used synthetic EBV peptides to investigate T cell immunity to EBV in MS, with conflicting results. Studies using panels of HLA class I restricted EBV peptides have found an increased frequency of reactive CD8 T cells in MS patients,[67] in CIS but not established MS,[43] or no increase in either CIS[43] or MS patients.[68] In one study, MS patients had an increased CD4 T cell response to peptides derived from EBNA-1.[53]

However, the use of selected EBV peptides to study T cell immunity does not allow examination of the total T cell response in any subject,[69] including the normal physiological antigen processing.[69] Furthermore, comparisons in the T cell

responses to any given EBV peptide can only be made between subjects with the restricting HLA molecule.[69] Thus addition of exogenous EBV peptide may result in a high frequency of T cells producing interferon-γ, but if the peptide is presented only at low density on the surface of EBV infected B cells, the latter will be poorly recognised by peptide specific T cells.[70]

Recent work has focused on the T cell response to EBV infected B cells in autologous B cell lymphoblastoid cell lines (LCL). This provides a direct measure of the aggregate T cell response to all EBV antigens presented by all HLA molecules on EBV infected B cells, using each person's natural antigen processing mechanisms and viral antigens at normal physiological concentrations.[69] In addition, as a proportion of the cells in LCL are in the lytic phase of infection,[71 72] the method detects responses to both latent and lytic EBV proteins. Using this approach, MS patients had a decreased CD8 T cell response to EBV infected B cells[69] that could potentially allow accumulation of EBV infected autoreactive B cells in the CNS and contribute to MS development.[69] A greater decrease in LCL specific T cell response was associated with an earlier age of onset of MS.[69] However, the finding of *decreased* CD8 T cell reactivity to EBV infected B cells in patients with MS[69] differs from another small study that reported a non-significant *increase* in the frequency of LCL specific CD8 T cells.[36] It is, however, consistent with earlier reports that MS patients have decreased T cell control of immunoglobulin secreting B cells after in vitro infection with EBV[73] and that EBV infected B cells of MS patients have a higher rate of spontaneous immortalisation in vitro than EBV infected B cells of controls.[74]

INTERACTION WITH OTHER VIRUSES

One intriguing hypothesis for the association between EBV infection and subsequent MS is that the pattern of infection with other viruses is also important. A history of exposure to infant siblings (possibly a marker of early exposure to common childhood infections) has been associated with a reduced EBV specific IgG response in control participants (AOR=0.33 (95% CI 0.11 to 0.98)).[75] Lack of such early life exposure (and thus a *greater* EBV specific IgG response to EBV infection) may increase the risk of developing MS[75] but this hypothesis requires further testing. In paediatric onset MS, there is some evidence that MS risk is decreased by exposure to herpes simplex virus (OR=0.14 (95% CI 0.04 to 0.51))[76] but this has not been a consistent finding.[77]

Clinical exacerbations of MS are more likely to occur following acute systemic infection (with a wide range of viruses and bacteria) than at other times.[78–80] Possible mechanisms include cross reactivity between microbial antigens and CNS antigens and a general upregulation of the immune system. Coinfection with other herpes viruses may alter the immune control of the host for the latent EBV, permitting reactivation,[27] although, if this were the dominant mechanism, one would expect to see an elevated EBV DNA load.

IS EBV INFECTION A REQUISITE COMPONENT OF MS PATHOGENESIS?

Up to 5% of MS occurs before the age of 18 years—paediatric onset MS. In this group, over 80% of cases had serological evidence of past EBV infection[76 77] compared with 42–64% of age matched controls and >99% usually seen in adult MS. In one study, 14% of children diagnosed with MS were EBV seronegative, leading to an interpretation that EBV infection may not be an absolute requirement of MS pathogenesis,[77] although diagnostic misclassification between MS and other childhood demyelinating disease[81] or differences between paediatric and adult onset MS have been suggested as alternative explanations. Further confirmation of the exact proportion of individuals who are EBV seropositive at onset of paediatric demyelinating disease and who, after long term follow-up are designated to have classical MS, is required.

POSSIBLE MECHANISMS/HYPOTHESES

The previous sections have detailed the considerable evidence that EBV infection is somehow involved in the development of MS (for summary, see table 1). The question then arises as to whether EBV initiates or perpetuates the disease process, via an immune mechanism, or is an epiphenomenon of a causative immune derangement. This section provides a critical appraisal of the evidence against possible pathogenic pathways. The strongest evidence against the latter probably arises from longitudinal cohort studies that show changes in anti-EBNA antibodies 15–20 years before MS onset and a dose–response of increasing risk with increasing anti-EBNA-1 titres. If EBV is a causal risk factor, a number of pathways are possible.

Molecular mimicry between EBV gene products and MS autoantigens

The EBV cross reactivity hypothesis postulates that T cells primed by exposure to EBV antigens cross react with and attack CNS antigens.[83] In support of this, 3–4% of EBNA-1 specific CD4 T cells in healthy subjects and MS patients react with peptides derived from myelin proteins[30] but this is unlikely to represent the main role of EBV in MS pathogenesis.[84] Against this hypothesis, other infectious agents also have the potential to induce cross reactivity with CNS antigens but EBV appears to have a unique and almost obligatory role in the development of MS. This hypothesis would also not require or explain the

Table 1 Summary of the level of evidence in relation to measures of Epstein–Barr virus infection and multiple sclerosis risk

Measure of EBV infection (and relevant references)	Level of evidence for an association with MS risk*
Latitudinal gradient in EBV infection[5–8]	+
History of infectious mononucleosis[13–15 17–19]	+++
EBV antibodies in serum[21–24 27–30 39 40 42 50] (others see text)	+++
EBV DNA load in the peripheral blood[27 43 53–57]	+
EBV DNA in the CNS[58–60 62–64]	+
EBV specific T cell responses[36 43 53 67–69]	++
Interaction with other viruses[76 77]	+

*Level of evidence is based on the quantity and quality of the research evidence currently available, including strength of associations, consistency across studies and locations, temporality of exposure and outcome, and a dose–response effect (where relevant).[82]
EBV, Epstein–Barr virus; MS, multiple sclerosis.

presence of EBV infected B cells in the brain[58] because cross reactivity is initiated by exposure of T cells to EBV in lymphoid tissue outside the CNS.

αB-crystallin or 'mistaken self' hypothesis

αB-crystallin is a small heat shock protein expressed by lymphoid cells following exposure to infectious agents.[85] Unlike other heat shock proteins, αB-crystallin is found in a very restricted range of human tissues, and is absent from healthy lymphoid tissue. The αB-crystallin or 'mistaken self' hypothesis proposes that immune tolerance to the protein does not develop due to its limited expression in tissues, including the thymus. Expression of the protein following infection thus generates a CD4 T cell response which attacks αB-crystallin derived from oligodendrocytes, with resultant inflammatory demyelination.[86] A key requirement of the hypothesis is that infection of the CNS by a microbial agent, not necessarily the same as that inducing αB-crystallin in lymphoid cells, upregulates the expression of αB-crystallin in oligodendrocytes and provides other 'danger' signals in the CNS, encouraging inflammation to develop. Although the hypothesis is not EBV specific, EBV is a candidate because it induces the expression of αB-crystallin in B cells, which present the protein to CD4 T cells in a HLA-DR restricted manner.[85] This hypothesis by itself cannot account for the initial development and subsequent persistence of inflammation in the CNS but may explain how CD4 T cells target oligodendrocytes and myelin after inflammation has been initiated in the CNS.

Common pathways through interleukin 10 and interactions with vitamin D

EBV infection results in the production of viral interleukin 10 (IL-10). This homologue of human IL-10 produced by lymphocytes may compete with human IL-10 for binding sites but fail to perform some essential IL-10 functions. Hayes and Acheson have proposed that both EBV infection and low vitamin D status have their adverse effect on MS risk through changes in the level or function of human IL-10.[87] This hypothesis also fits with the observed protective effect of infection with intestinal parasites,[88] which results in induction of IL-10 and B regulatory cells.[89 90]

Holmoy *et al* proposed that an interaction between vitamin D status and EBV infection modulates MS risk, with low vitamin D status—for example, during winter—facilitating the activation of autoreactive T cells and skewing the immune response to EBV in a proinflammatory direction.[91] In a recent case control study,[92] paediatric onset MS cases who were vitamin D sufficient only (≥75 nmol/l) had higher anti-EBNA-1 levels (p=0.04) than controls (recruited from the same clinic and being treated for a range of non-MS illnesses). These data are difficult to interpret: the control group was non-representative and the interaction was not seen when vitamin D was considered as a continuous variable. Furthermore, it is not clear whether vitamin D status at the time of primary infection would be important or ongoing maintenance of vitamin D status to avoid activation of T cells. Another possible EBV related mechanism whereby sunlight/vitamin D protects against MS is a sunlight induced increase in the number of CD8 T cells available to control EBV infection.[81]

MS AND EBV INFECTION SHARE COMMON GENETIC DETERMINANTS

Several studies suggest that genetic factors may be important in the risk of MS in association with EBV infection. Firstly, the association between EBV infection and MS may be explained by common genetic determinants of EBV infection and MS—that is, a specific genotype may increase the risk of both MS and EBV infection.[24] The major genetic risk factor for MS is within the HLA class II, and the EBV glycoprotein g42 uses the HLA class II receptor when infecting B cells. It is plausible that allelic variation in this receptor could affect EBV infection of B cells.[50] It is not clear, however, that this hypothesis would explain the observed epidemiological findings, including the association not just with EBV infection per se but with late infection with EBV.

Interaction with other viruses

It may not be the EBV infection per se that increases the risk of MS but the nature of the host response where the sequence of both prior and subsequent viral infections may be important. Here EBV infection without the protective benefit of earlier infection with another virus—for example, herpes simplex virus[93]—may increase MS risk[4 67] while subsequent infection—for example, with HHV-6 variant A—may result in reactivation of latent EBV infection thereby increasing MS risk.[28] In the 'EBV variant of the hygiene hypothesis',[1] the increased risk of MS among individuals raised in a more hygienic environment is manifest only after EBV infection. Here the lack of early life exposure to infections due to a more hygienic environment predisposes to late EBV infection and possibly a lack of specificity in the resulting immune response.[4 75]

EBV infection can transactivate human endogenous retroviruses (HERVs) in in vitro models.[94] Some studies have implicated specific HERVs in MS risk[95] but have not explored possible interactions between prior EBV infection and HERV reactivation in vivo.

DeLorenze and Munger suggest that the observed increase in anti-EBNA complex and anti-EBNA-1 antibodies in early adulthood[39] could be the result of activation of EBV specific memory T cells, triggered by infection with another micro-organism or reinfection with a different EBV strain. The finding in some studies that the combination of both higher anti-EBNA titres and higher anti-VCA titres (a marker of recent EBV infection/reactivation) has a particularly adverse effect on MS risk[75] might be considered supporting evidence for the latter.

EBV infected autoreactive B cell hypothesis

This hypothesis for the more general development of autoimmune diseases proposes that, in genetically susceptible individuals, EBV infected autoreactive B cells seed a target organ. Within the organ they produce pathogenic autoantibodies and act as professional antigen presenting cells, providing costimulatory survival signals to autoreactive T cells which would otherwise die by activation induced apoptosis.[84] It is important to note that the probability of EBV infecting naïve autoreactive B cells is not low because at least 20% of human naïve B cells are autoreactive.[96] The hypothesis makes several predictions, some of which have been verified in MS—namely, the presence of EBV infected B cells in the CNS (but see earlier discussion of conflicting findings); a beneficial response to B cell depletion with rituximab[97]; and decreased CD8 T cell immunity to EBV infected B cells.[69]

CONCLUSION

There is strong evidence that EBV infection precedes MS onset and there is a dose dependent relationship between MS risk and the level of EBV specific antibodies, particularly EBNA-1 IgG titres. A key emerging feature is that it is important to examine not only the antibody titre but also the specificity of the

humoral immune response to EBV and better understand what determines this. The evidence in relation to the presence or amount of EBV DNA in blood, CSF or brain is less clear, with studies presenting directly conflicting results. CD8 cytotoxic T cells maintain immunosurveillance and there is some evidence that there is dysfunction of these cells in MS. Such dysfunction should be accompanied by evidence of reactivation or higher EBV DNA load in cellular compartments but this has not been consistently shown. Further work using more sensitive quantitative assays may resolve these discrepancies. More work on the role of CD4 cells is warranted, particularly in view of additive interactions between markers of EBV infection and HLA class II genes (that are particularly involved in antigen presentation to CD4 T cells). We are left with a consistent finding of high anti-EBNA IgG titres without consistent evidence of increased copies of the EBV genome in blood or serological evidence of reactivation. The task is now to achieve some consistency in the findings, using sufficiently large sample sizes to have statistical power, well characterised MS and control populations, and sophisticated laboratory techniques, in order to progress our understanding.

Competing interests None.

Contributors RML undertook the literature review with input of all of the other authors to the review and critical appraisal of the findings.

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