Vitamin D: a link between Epstein–Barr virus and multiple sclerosis development?

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Giulio Disanto

University of Oxford, Oxford, UK

Ute Meier

Blizard Institute, Queen Mary University of London, Barts and The London School of Medicine and Dentistry, London, UK

Gavin Giovannoni

Blizard Institute, Queen Mary University of London, Barts and The London School of Medicine and Dentistry, London, UK

Sreeram V Ramagopalan

Author for correspondence: University of Oxford, Oxford, UK and Queen Mary University of London, Blizard Institute, Barts and The London School of Medicine and Dentistry, 4 Newark Street, London, E1 2AT, UK
Tel.: +44 207 377 7472
Fax: +44 207 377 7033
s.ramagopalan@qmul.ac.uk

"While it is possible that vitamin D and Epstein–Barr virus may be influencing multiple sclerosis risk through independent mechanisms, another hypothesis is also plausible, which is that these two factors may be biologically interacting to increase the risk of multiple sclerosis."

Multiple sclerosis (MS) is a complex immune-mediated disorder of the CNS, which results from a combination of genetic and environmental factors and their interactions [1]. Although genome-wide association studies have discovered numerous genetic variants predisposing to MS, the environment exerts a greater influence on susceptibility [2]. That nurture is fundamental in MS has been known for decades. Monozygotic twins are, at most, 30% concordant, and familial MS risk is significantly influenced by location and season of birth and the childhood environment [1,2]. Growing evidence lends strong support to vitamin D deficiency and Epstein–Barr virus (EBV) infection as being key environmental risk factors.

Evidence for vitamin D & Epstein–Barr virus in MS

The data supporting a role for vitamin D in the development of MS are overwhelming. The worldwide prevalence of MS positively correlates with latitude [3]. Essential for the production of vitamin D, ultraviolet B (UVB) light radiation is the factor that most likely mediates such a correlation, and this is especially clear in national studies from France and England [2,4]. Vitamin D intake significantly decreases the risk of MS, and vitamin D levels inversely correlate with risk of MS later in life [5,6]. Furthermore, *CYP27B1*, the gene encoding 1α-hydroxylase (the enzyme that activates vitamin D) is associated with MS susceptibility [7]. In addition, vitamin D can also influence MS course. A recent prospective investigation of a large cohort of MS patients has demonstrated that vitamin D status is inversely associated with disease activity over the subsequent 6 months [8].

Functional evidence comes from studies showing that both the vitamin D receptor (*VDR*) and *CYP27B1* genes are expressed in an exceptionally broad range of immune cells, including those that are known to play a central role in MS, such as Th1 and Th17 subsets, $FOXP3^+$ regulatory T cells and B cells [9,10]. This extremely pleiotropic hormone is able to influence cellular function and proliferation through a fine regulation of gene expression. Using chromatin immunoprecipitation followed by massively parallel DNA sequencing (ChIP-seq), our group has demonstrated the presence of 2776 different vitamin D responsive elements (VDREs) through the entire genome bound by the VDR in B cells. Notably, genetic loci associated with MS are strikingly enriched for VDR binding sites [11].

However, vitamin D is not the only environmental factor implicated in MS. EBV is a B-lymphotropic human DNA

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herpes virus associated with lymphoproliferative and immune disorders [12]. Although more than 90% of the general population appears to encounter EBV at some point during their life, several lines of evidence highlight its role in the pathogenesis of MS. Large independent studies have shown that nearly all MS patients have been infected with EBV. Furthermore, both high anti-EBV antibody titers and a history of infectious mononucleosis (IM) increase the risk of developing MS [1,13]. Whether such a strong association between MS and EBV is actually causal has been, and still is, debated. For example, the elevated risk of MS after IM could arise from a common genetic susceptibility to MS and IM. However, this suggestion can be refuted since the *HLA-DRB1*1501* class II allele, the main genetic risk factor for MS, is not associated with the development of IM [14]. Similarly, the hypothesis that good hygiene during childhood may predispose both to MS and to a later contact with EBV, and therefore IM, should be equally rejected given the observation that EBV-negative individuals (likely to be exposed to the highest levels of hygiene) have the lowest risk of MS [13]. Pediatric MS represents a valuable tool for the identification of environmental risk factors. As a consequence of early disease onset, less time is available for potential confounders, and thus any associated factor is more likely to be truly causative. In a recent investigation of a large cohort of pediatric MS patients, both EBV positivity and high EBV antibody titers predicted conversion to MS independently of HLA and vitamin D status, although not all pediatric patients were infected with EBV [15].

Evidence for vitamin D–EBV interaction

While it is possible that vitamin D and EBV may be influencing MS risk through independent mechanisms, another hypothesis is also plausible, which is that these two factors may be biologically interacting to increase the risk of MS. This idea is supported by our recent observation that a statistical interaction between IM prevalence and UVB light radiation could explain 72% of the variance in MS prevalence across England [4]. How this interaction may take place at the molecular level remains largely unknown, but there are a number of potential explanations.

> "...a statistical interaction between infectious mononucleosis prevalence and ultraviolet B light radiation could explain 72% of the variance in multiple sclerosis prevalence across England..."

First, it is plausible that vitamin D deficiency influences the immune response to EBV. Vitamin D increases the production of the anti-microbial peptide cathelicidin, which may protect against EBV [10,16]. Furthermore, serum levels of 25-hydroxyvitamin D correlate positively with the ability of Tregs to suppress T-cell proliferation [17], and it has been suggested that Tregs are important in controlling primary EBV infection to a subclinical level in most cases and that IM represents a failure of this protective mechanism [18]. This idea is somewhat supported by the epidemiological evidence indicating that early vitamin D deficiency influences MS risk prior to EBV infection (i.e., place and month of birth precedes IM) [19] and the finding that individuals with a high serum 25-hydroxyvitamin D were less likely to have viral (EBV) shedding in saliva [20]. However, as vitamin D deficiency affects all ages there needs to be an explanation as to why the peak age of IM is in the early teens/late 20s. Furthermore, given the variable nature of vitamin D status, it is not clear how this interaction could predispose to MS over the long term if EBV is suppressed when vitamin D levels are high. It is possible that this could be related to an 'immunological imprint' that is left as a result of vitamin D deficiency during a critical time period of development or after infection with EBV.

> "...a substantial proportion of the genes regulated by the viral protein EBNA-3 are likely to be regulated by vitamin D, so it may be that Epstein–Barr virus potentiates vitamin D deficiency by blocking the effect of vitamin D."

Another theory comes from the demonstration that the EBV protein EBNA-3 is able to stop the expression of the vitamin D-regulated genes *C-FOS*, *CYCLIN C*, *CYP24A1*, *GADD45A* and *P21* by blocking VDR activity [21]. A recent study identified the expression profiles of lymphoblastoid cell lines obtained by infecting primary B cells with either wild-type EBV or an EBV mutant strain lacking the *EBNA-3* gene [22]. To explore to what extent EBNA-3 may influence the expression of vitamin D responsive genes we calculated how many of the putative EBNA-3 regulated genes are characterized by the presence of a VDRE using data from our VDR ChIP-seq map. Interestingly, of the 296 genes that were differentially expressed between *EBNA-3* positive and *EBNA-3* negative lymphoblastoid cell lines, 82 (27.7%) were characterized by the presence of a VDRE, which was much greater than expected by chance ($p = 0.003$). A gene ontology analysis demonstrated that the genes with both an EBNA-3 and VDR influence were involved in cell proliferation, apoptosis and immune response processes. This analysis suggests that a substantial proportion of the genes regulated by the viral protein EBNA-3 are likely to be regulated by vitamin D, so it may be that EBV potentiates vitamin D deficiency by blocking the effect of vitamin D. The extent to which *EBNA-3* is expressed may be dependent on the host immune response, which may include MS susceptibility genes. From an evolutionary perspective, EBV has evolved several mechanisms to evade immunological attack [23] and one of these mechanisms may be EBNA-3's interaction with vitamin D, which has well-described antiviral effects [24].

Vitamin D & EBV: causal cascade & disease prevention

Understanding how risk factors for MS integrate to lead to MS development is critical for disease prevention. The proposition that low vitamin D levels may be influencing the immune response against EBV is not surprising since vitamin D is a potent modulator of the adaptive immune system and low levels are known to increase the risk of other viral infections, such as influenza [25,26]. However, further work is needed to address

this question. In particular, future prospective studies should investigate whether the risk of IM and EBV antibody titers are influenced by vitamin D status at the time of infection.

If confirmed, this notion would open up the question as to whether the association between EBV and MS is merely a consequence of vitamin D deficiency predisposing to both MS and EBV infection. Although this possibility cannot currently be ruled out, it appears highly unlikely given the amount of evidence (mentioned above) supporting EBV as being specifically involved in MS causation. It is particularly intriguing to suppose that vitamin D-deficient individuals may be more likely to develop IM after EBV infection, and MS risk is increased in some of these individuals who have higher expression of *EBNA-3*, which blocks vitamin D action in B cells, which are gaining increasing recognition as being key players in the pathogenesis of MS [27,28]. As EBV has coevolved with us over millions of years it may be of interest to study EBV–vitamin D interactions and responses between ancestral populations from Africa and Caucasians.

Great strides are being made in cataloguing the pleiotropic effects of vitamin D in the immune and nervous systems [16]. A major facet to understanding better the interaction between vitamin D and EBV in MS is to better understand how EBV predisposes to MS – that is, is it through molecular mimicry, bystander activation or another mechanism [29]? More basic research is needed to fully understand the environmental risk factors in MS, as this will provide new strategies for both disease prevention and treatment. Longitudinal studies of at-risk populations will be extremely useful.

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