



Vitamin D status modulates the immune response to Epstein Barr virus: Synergistic effect of risk factors in multiple sclerosis

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Summary MS risk is associated with low vitamin D status prior to disease, and Epstein Barr virus (EBV) infection seems to be a prerequisite for MS. EBV could activate autoreactive T cells by several mechanisms, but it is not clear why this leads to MS. Only a small proportion of those infected with EBV develops MS, whereas autoreactive T cells are present in the normal T cell repertoire. Genetic factors cannot explain this enigma alone, because the genetic predisposition to MS in most cases is quite weak. Vitamin D receptors are expressed on EBV infected B cells, antigen presenting cells and activated lymphocytes, and the bioactive vitamin D metabolite dihydroxyvitamin D₃ suppresses antibody production and T cell proliferation and skews T cells towards a less detrimental Th2 phenotype. EBV infected B cells constitute a constant challenge to the immune system, also during seasonal periods of relative low vitamin D status. I propose that vitamin D modulates the immune response to EBV, and that detrimental activation of autoreactive T cells leading to MS is more likely if the vitamin D status is suboptimal.

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Introduction

Whoever wishes to investigate medicine properly should proceed thus: in the first place to consider the seasons of the year, and what effect each of them produces for they are not at all alike... Hippocrates, approximately 400 years BC.

Environmental factors are probably important in the etiology of MS, but so far no single environmental exposure has been identified as

the causal factor. This could either mean that the contribution from each risk factor is small, or that several risk factors exert additive or permissive effects upon each other. Vitamin D and Epstein Barr virus (EBV) top the list of potential environmental factors associated with MS. Based on the ability of EBV to activate and expand autoreactive T cells and the immunoregulatory effects of the bioactive vitamin D metabolite dihydroxyvitamin D₃, I here suggest that vitamin D protects against MS by modulating the immune response to EBV, and that low vitamin D status facilitates detrimental activation of autoreactive T cells and skews the immune

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response to EBV in a proinflammatory direction. This hypothesis could explain why only a modest impact on MS risk is conferred by EBV and vitamin D status when analyzed alone. Because vitamin D levels are lower during the winter, the validity of this hypothesis could be tested by analyzing whether the increase in MS risk associated with infectious mononucleosis displays seasonal variability.

Epidemiological evidence linking MS to EBV and vitamin D

The epidemiological evidence linking EBV and MS is strong. Virtually all adult and pediatric MS patients have been infected with EBV [1]. MS risk is associated with high serum titers of antibodies against EBV prior to clinical manifestations of MS [2], exacerbations of MS correlates with EBV reactivation [3], and infectious mononucleosis is associated with increased MS risk [4].

Epidemiological evidence also supports a role for vitamin D in MS. Sunshine is essential for vitamin D synthesis in the skin. MS risk is approximately seven times higher in Tasmania at 43° south than in North Queensland at 19° south in latitude [5], and is inversely correlated with past exposure to UV irradiation [6]. Vitamin D supplementation protects against MS [7], and serum 25 hydroxyvitamin D levels prior to disease are negatively associated with MS risk [8]. A recent study suggests a protective role of a vitamin D rich diet in northern areas of Norway, where solar exposure is low [9].

Is the immune response to EBV influenced by vitamin D status?

EBV persists in memory B cells throughout life, and both acute and persistent EBV infection is controlled by a strong T cell mediated immune response. Defective immune control of EBV in MS was early suggested from the observation of increased spontaneous EBV transformation of B cells *in vitro* [10]. The mechanism for this is unknown, but T cell responses to the EBV antigen EBNA-1 is less focused in MS patients than in healthy controls [11], compatible with perturbation of T cell responses to EBV in MS.

Based on the assumption that MS is mediated by autoreactive T cells, several mechanistic links between MS and EBV have been suggested: (i) EBV specific T cells may cross-recognize myelin proteins within the central nervous system [12]. (ii) EBV transactivates superantigens which expands T

cells irrespective of their antigen specificity, and some of these T cells might by chance be autoreactive [13]. (iii) EBV infected B cells may serve as highly efficient antigen presenting cells [14]. (iv) EBV infected B cells express the myelin protein α B-crystalline, and may activate encephalitogenic α B-crystalline-specific T cells [15].

EBV has a great growth-transforming potential, and EBV infected B cells must be constantly surveilled by the immune system throughout life. Even transient perturbation of the immune response to EBV at any time during or after primary infection may therefore be relevant for induction of autoimmunity. Accordingly, an age-related increase in titers of anti-EBNA antibodies after the late teens in people who later developed MS, supports a dysregulation of the immune response also EBV after the primary infection [16].

Dihydroxyvitamin D₃ is a potent regulator of immune responses. Vitamin D₃ receptors are expressed on dendritic cells, monocytes and activated T cells and B cells [17,18], and dihydroxyvitamin D₃ inhibit lymphocyte proliferation, interleukin (IL)2 production and immunoglobulin synthesis [19,20]. Human monocytes and dendritic cells exposed to dihydroxyvitamin D₃ downregulate HLA class II and other costimulatory molecules important for T cell activation, and produce more interleukin IL10 and less IL12, resulting in decreased T cell activation and induction of regulatory T cells [21].

Dihydroxyvitamin D₃ prevents induction and progression of experimental autoimmune encephalomyelitis (EAE) [22]. The mechanism seems to involve suppression of Th1 cell activation [23], which is a common step in the proposed links between EBV and MS. Although the impact of vitamin D status on the immunological control of EBV has not been directly studied, the immune response to EBV involves antigen processing and presentation, as well as activation and expansion of T cells, which both are modulated by dihydroxyvitamin D₃ *in vitro* and *in vivo*.

The pattern of increase in anti-EBV IgG antibodies, with specific increase in anti-EBNA-1 and anti-EBNA complex, is unique to MS [8]. This may reflect an altered T cell response to EBV transformed B cells in MS, because anti-EBNA-1 titers are positively correlated with the precursor frequency of T cells recognizing autologous EBV transformed B cells [24]. In accordance with a deviating immune response to EBV infected B cells in MS, T cell lines recognizing autologous EBV transformed B cells could be generated from the CSF of all MS patients, but only a proportion of the controls [25].

Testing the hypothesis

Several airway infections, most striking influenza type A, display a marked and recurrent seasonal variability with incidence peaks during the winter, which may be attributable to seasonal variation in vitamin D status [26]. If the vitamin D status during primary EBV infection is important for subsequent MS risk, it would be expected that acquiring EBV infection in the winter is associated with higher MS risk than acquiring infection in the summer. Primary EBV infection is usually clinically silent, and the effect of seasonal variation on MS risk can therefore only be assessed if large numbers of healthy people undergo repeated serological EBV testing. However, infectious mononucleosis caused by delayed primary EBV infection can be recognized clinically and serologically throughout the year. Recently, it was reported that a cohort of 25,234 cases with infectious mononucleosis was followed for the occurrence of MS [4]. A synergistic effect of vitamin D and infectious mononucleosis on MS risk could be tested by comparing vitamin D status at the time of infectious mononucleosis between those who developed MS and those who did not. Due to the seasonal variation in vitamin D status, even comparing the seasonal distribution of infectious mononucleosis among those who developed MS and those who did not would be informative.

A large-scale placebo controlled study of primary prophylaxis of MS with vitamin D supplementation would offer another opportunity to test the hypothesis. This setting would allow comparison of the protective effect of vitamin D supplementation between those who contract primary EBV infection during the study period and those who were already EBV seropositive from the beginning. A more pronounced protective effect of vitamin D supplementation in those who contract EBV infection would point towards a combined effect of vitamin D status and primary EBV infection.

These approaches do not address the potential effect of vitamin D status on the continuous immune surveillance of latent EBV infection and reactivation. The immunological effect of vitamin D status on immunological surveillance of latent EBV infection could be studied by analyzing the effect of vitamin D supplementation on the pattern of EBV antibody titers and cellular immune responses to EBV antigens and EBV transformed B cells.

EAE, the most commonly used animal model for MS, does not address the impact of infection in the etiology of MS. However, demyelinating disease can also be induced in mice by Theiler's murine

encephalomyelitis virus (TMEV). Demyelinating disease evolves as T cells triggered by the virus become progressively more responsive to myelin epitopes [27]. Thus, TMEV infection models events which are relevant for the interplay between microbes and the immune system in MS. Studies of vitamin D supplementation or deprivation in the TMEV model could therefore add molecular insight to the interplay between immune responses to viruses and vitamin D in MS. So far, no such studies have been published.

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