Review

Vitamin D, Epstein-Barr virus, and endogenous retroviruses in multiple sclerosis - facts and hypotheses

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The pathogenesis of multiple sclerosis (MS) remains poorly understood. Presumably, MS is caused by multiple environmental, epigenetic, and genetic factors. Among them, human endogenous retroviruses (HERVs), Epstein-Barr virus (EBV) and vitamin D have been suggested to play a role in the pathogenesis and course of MS. Because vitamin D can affect the immune system and infections, it can be hypothesized that there is a close interplay between vitamins, EBV and ERV in the pathogenesis of MS. Here, we summarize the important data on vitamin D, including polymorphisms in genes related to vitamin D metabolism, EBV and ERV, in the pathogenesis of MS and create hypotheses regarding their interactions. Data indicate that vitamin D has a strong impact on viral infections and interferes with EBV infection, while EBV is capable of activating silent ERVs. We believe that EBV could be the missing link between vitamin D and ERV in MS pathogenesis.

Keywords

Epstein-Barr virus (EBV); Genetic polymorphisms; Human endogenous retroviruses (HERVs); Vitamin D

1. Introduction

Although MS is one of the most common neurological diseases worldwide, its pathogenesis is still largely unknown. Dysregulation of the immune system has been discussed [1], as well as other endogenous and environmental factors, including endogenous retroviruses (ERVs) [2], vitamin D levels [3], herpesviruses [4] such as Epstein-Barr virus (EBV) [5], the gut microbiota [6], short-chain fatty acids [7], smoking [8] and body mass index [9].

Here, we provide a short overview regarding the putative role of ERV, EBV and vitamin D and the interplay between these factors in the pathogenesis of MS. We included data from Mendelian randomization studies on vitamin D that investigated associations of genetic polymorphisms in genes related to vitamin D metabolism and susceptibility to MS. Finally, we aim to shed more light on the interconnected role of ERV, EBV and vitamin D in the pathogenesis of MS.

2. Multiple sclerosis and ERV

During evolution, the infection of germline cells with retroviruses led to accidental stable integration of these viruses into the genome of the infected host. These so-called endogenous retroviruses (ERVs) are present in the genomes of virtually all animals. Approximately 8% of human DNA comprises ERV sequences. Complete ERVs are composed of four major structural genes: *gag* (encoding matrix and retroviral core), *pol* (reverse transcriptase and integrase), *pro* (protease), and *env* (envelope). Indeed, most human ERVs (HERVs) are not capable of replicating due to their high susceptibility to mutations [10]. However, some HERV elements contain intact open reading frames and can thus code for proteins [11]. It is possible that approximately 7% of all HERV sequences are transcriptionally active [12]. HERVs belong to so-called retroelements, which are mobile fragments that use an RNA intermediate. HERVs have regulatory long terminal repeats (LTR). These LTRs can be involved in the expression of ERV-derived sequences or can support the expression of neighboring genes [13, 14]. For example, HERV-W elements affect the transcription of at least 55 genes [15].

ERVs contribute to some important physiological functions, e.g., placental development [16], and they occasionally shelter the host from external viruses [17]. In addition, HERVs seem to be related to certain diseases [18], e.g., diabetes mellitus type I [19], schizophrenia and bipolar disorder [20], or cancer [21].

The first connection between HERV and MS was found in 1989, when the transcriptional activity of retroviruses in MS patients was found [22]. The respective HERV has been referred to as multiple sclerosis-associated retrovirus (MSRV) and is currently classified as HERV-W. In addition, several other HERVs seem to be associated with MS, such as HERVK-18 [23], HRES-1 [24] or HERVFc-1 [25, 26]. It is striking that the number of expressed HERV sequences is higher in MS patients than in healthy subjects [27], and HERV upregulation within MS plaques correlates with dis-

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ease activity [28]. In addition, MS patients show higher antibody reactivity to certain HERV sequences [29]. HERV reactivation can lead to demyelinating plaques by initiating microglial inflammation [30]. For example, MSRV induces the release of the cytokines IL-6 and IL-8 [31].

Interestingly, women have higher levels of MSRV DNA copies than men, both in MS patients and controls [32], and the prevalence of MS is higher in women than in men [33]. When patients suffering from MS undergo antiretroviral therapy, symptoms of the disease can temporarily disappear [34–36], suggesting a strong association between HERV and MS. Indeed, these effects have been reported only in single cases and are, therefore, difficult to interpret in the context of a complex disease such as MS. However, the LTR sequences of some HERVs show polymorphisms that are typical for MS patients, and these polymorphisms might explain the epidemiology of MS in certain populations [37]. Notably, not all individuals have the same set of HERV copies [38]. Such insertional polymorphisms might influence susceptibility to MS development. Moreover, we observed an enrichment of HERV-like sequences near MS-related single-nucleotide polymorphisms (SNPs) [39]. The presence or absence of specific HERV sequences might indicate genetic factors that predispose patients to MS. However, genetic determinants alone cannot explain disease development. This is evident by the low heritability of MS, as only a minority of monozygotic twins are concordant for MS [40]. Therefore, it can be assumed that environmental factors are necessary for disease development. Vitamin D could be one of these factors.

3. Multiple sclerosis and vitamin D

Vitamin D is a group of fat-soluble secosteroids and is mainly synthesized by the skin after sunlight exposure [41]. Vitamin D deficiency is prevalent in many parts of the world [42] and affects approximately one billion people worldwide [43]. Although vitamin D is mainly associated with the regulation of calcium balance, it also has immunomodulatory effects and appears to beneficially impact respiratory infections [44, 45].

Low-vitamin D status is also associated with MS [46, 47]. Moreover, children born in autumn have a lower risk of developing MS than those born in spring [48, 49]. Since vitamin D status is usually higher at the end of summer than at the end of winter, we hypothesize a link between vitamin D and MS risk in children. The higher global prevalence of MS in countries distant from the equator [50] supports the presumed role of vitamin D in the pathogenesis of MS. This assumption is supported by the fact that people emigrating from a country with a high MS risk to a country with a lower MS risk are less affected by MS than their former countrymen [51]. Females have been shown to have lower plasma concentrations of vitamin D than males [52], which could also explain the elevated MS incidence in females.

The role of vitamin D in the immune system has been studied intensively. Vitamin D can promote a shift from T-helper 1 cells to T-helper 2 cells [53]. In addition, vitamin D increases the number of T regulatory cells [54] and influences the expression of the class II MHC molecule HLA-DRB1*1501, which is associated with MS [55]. Therefore, vitamin D can support anti-inflammatory mediators and reduce the levels of different cytokines, such as TNF-α, IL-1β, IL-6, IL-8, and IL-17 [56]. These functions of vitamin D in the regulation of immune cells and cytokines support the hypothesis that vitamin D deficiency may modulate the progression of MS [57]. In addition, adequate plasma concentrations of vitamin D have been shown to be linked to a lower risk of relapse [58], and SNPs associated with lower levels and function of vitamin D are associated with a higher risk of MS [59].

Polymorphisms in genes implicated in vitamin D metabolism and signaling were also observed in MS patients. The genes affected by these polymorphisms include the vitamin D receptor [60], the vitamin D binding protein DBP [61], the 25 hydroxyvitamin D hydroxylase CYP27B1 [61], and the mitochondrial 1,25-dihydroxyvitamin D3 hydroxylase CYP24A1 [62], which degrades bioactive D vitamers. Interestingly, polymorphisms in genes that are not causally linked to vitamin D pathways were related to serum vitamin D levels [63], such as CD40 (which is associated with immune regulation and homeostasis), the interleukin 7 receptor gene (which is essential for survival and proliferation of T cells) and the immune-regulatory lymphocyte activation gene 3.

However, a narrative review summarizing the data from meta-analyses and systematic reviews found no clear evidence that vitamin D may prevent MS [45], and studies including MS patients did not find consistent or convincing effects of vitamin D supplementation on the MS course [64]. As mentioned above, genetic factors are unlikely to explain MS susceptibility. Today, it is not possible to exclude that a combination of vitamin D deficiency and genetic factors influencing vitamin D metabolism might be responsible for MS. The similarity of vitamin D concentrations was shown to be significantly greater in monozygotic twins than in dizygotic twins [65]. However, in this study, vitamin D was not an independent risk factor for MS. A major problem for the interpretation of such data is the fact that it is unclear at which time in life vitamin D deficiency might be required for MS development. An interesting recent study investigated differences in immune cell composition between MS-affected monozygotic twins and their healthy cotwins [66]. This study demonstrated that immune cell composition in twins is highly similar, independent of disease status. Interestingly, the similarity was higher in pairs where the healthy cotwin showed signs of subclinical neuroinflammation. A possible interpretation of this observation is that the interaction of the immune system with exogenous or endogenous antigens has taken place. In this regard, the influence of vitamin D on infections with exogenous viruses seems interesting.

4. Vitamin D and viruses

Vitamin D seems to have a great impact on viral infections. In epidemiological studies, lower serum vitamin D concentrations are associated with higher rates of infection with respiratory syncytial virus [67], polyomavirus [68], human papillomavirus [69], cytomegalovirus [70], and herpes simplex virus [71]. In addition, lower serum vitamin D concentrations are associated with EBV [72] and hepatitis C virus [73]. Additionally, vitamin D deficiency can induce higher hepatitis B virus levels [74] or a shorter survival time in patients with human immunodeficiency virus [75].

Vitamin D receptor polymorphisms are related to hepatitis B virus [76], hepatitis C virus [73], and the presence of respiratory syncytial virus infections [77]. In addition, in cell culture studies, vitamin D supplementation suppresses replication of human immunodeficiency virus in T-cells [78] and replication of rhinovirus in cells from patients with cystic fibrosis [79]. *In vivo*, vitamin D supplementation suppresses the replication of influenza virus in mice [80].

Interestingly, some of these vitamin D-affected viruses are able to activate silent HERVs. Such viruses include herpes simplex virus [81], influenza virus [82] and EBV [83]. In addition, the involvement of EBV infection in MS has been discussed for a long time, as the disease frequently develops shortly after infection with EBV [84].

5. Hypothesis on the interplay between vitamin D, ERV and EBV

To date, it remains unclear whether there is an association between vitamin D and HERV in MS pathogenesis. Recent data show a negative association between HERV and circulating vitamin D in MS patients [85]. Vitamin D downregulated ERV3 in a leukemia model [86]. In addition, ERVK LTRs have several intact and conserved binding sites for VDR receptors [87].

Interestingly, vitamin D levels are inversely correlated with EBV load in MS patients [88]. As mentioned above, EBV has been shown to be able to transactivate HERV, with potential superantigen activity [83]. EBV is the causative virus for infectious mononucleosis, and patients with infectious mononucleosis have lower levels of vitamin D [89]. EBV infects B cells and immortalizes these cells into so-called lymphoblastoid cell lines. *In vivo*, a strong immune response against EBV inhibits the proliferation of lymphoblastoid cell lines in immunocompetent hosts. Recently, it was shown that humanized mice carrying the major MS risk allele HLA-DRB1*15:01 were not able to adequately control EBV infection [90]. This gives a direct link between the exogenous factor EBV and an immunologically relevant MS-associated polymorphism. EBV is usually acquired early in life and persists throughout the lifespan in infected individuals. Interestingly, EBV-encoded nuclear antigen 2, a master regulator of EBV-driven B cell immortalization, has overlapping DNA binding sites with the vitamin D receptor [91]. It is likely that at high vitamin D levels, the vitamin D receptor outcompetes EBNA2 for DNA binding, which can explain the inverse correlation between vitamin D levels and EBV. This also reduces the expression of HERV, which is transactivated by EBV. In addition to EBNA2, the vitamin D receptor can bind EBNA3 (reviewed in [92]). This binding inhibits binding of the vitamin D receptor to target genes. Consequently, at high levels of EBV nuclear antigen expression and low vitamin D, EBV target genes, including transactivated HERV, can be activated, whereas at higher vitamin D levels, this transactivation is inhibited. The reduced anti-EBNA-1 antibody levels in MS patients after vitamin D supplementation could be due to the general anti-inflammatory effect of vitamin D [93, 94]. EBV is polymorphic [95], and it has not been elucidated whether all EBV variants have the same transactivation activity for HERV. The vast majority of adult individuals worldwide are latently infected with EBV. Polymorphisms in EBV, variable activity of EBV-interfering pathways such as the vitamin D pathway, and polymorphisms in ERV and ERV-like genetic elements might explain the observation that only a minority of EBV-infected individuals develop MS.

6. Conclusions

Several risk factors for MS have been described, including polymorphisms in immunologically relevant genes, as well as environmental factors such as EBV and vitamin D. In addition, endogenous retrovirus activation has been linked to MS. Recent observations suggest that all these factors are linked together. A possible model implies that the balance between EBV nuclear antigens and activated vitamin D receptors can be shifted towards activation of HERV (low vitamin D, high EBV antigen expression) or inhibition of HERV activation (high vitamin D, low or absent EBV antigens). Direct HERV-mediated toxicity or aberrant immune activation by HERV components can then induce MS. This model suggests multiple therapeutic targets (EBV, HERV, vitamin D metabolism), and elucidation of the exact interplay between these factors might lead to new treatment strategies for MS.

Author contributions

All authors wrote the paper with input from all authors. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors have declared no conflict of interest.

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