Vitamin D as an immune modulator in multiple sclerosis

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disorder of the central nervous system. The disease is characterised by inflammation with extensive immune infiltration, demyelination, axonal loss and damage of oligodendrocytes, presumably auto-immune in nature. The influence of environmental factors on the development and activity of MS has been known for a long time. Vitamin D and sun exposure are among the most important ones. Both serum vitamin D level and sun exposure independent of vitamin D production are correlated with epidemiological and clinical parameters of MS, and the impact of vitamin D on immune parameters has been clearly confirmed in experimental studies. Nevertheless, the impact on clinical aspects is inconclusive, especially when the influence of supplementation is assessed. In this work we review the state of knowledge regarding the effect of vitamin D on immune cells subsets in relation to experimental and clinical studies.

Key words: multiple sclerosis, vitamin D, supplementation, T cells, B cells, dendritic cells, macrophages
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Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disorder of the central nervous system (CNS). The disease is characterised by inflammation with extensive immune infiltration, demyelination, axonal loss and damage of oligodendrocytes. The precise cause of MS remains unknown, but it is considered to be an autoimmune disease. A key role is played by T-lymphocytes, which after activation outside of the CNS pass through the blood-brain-barrier (BBB). T cells can contribute to CNS damage directly via cell-cell death or via soluble mediators including cytokines or proteases. Many studies suggest that B cells are equally important in MS pathogenesis. The presence of immunoglobulinG oligoclonal bands in the cerebrospinal fluid (CSF) of MS patients is a biomarker for disease and antibodies play a crucial role for demyelination in experimental autoimmune encephalomyelitis (EAE) [1]. The prevalence of MS is greater at higher latitudes, and tends to peak in areas with fewer sunny days. Traditionally, this prevalence was explained by a low exposure to ultraviolet light and/or diet, but several studies have shown that it is mainly the result of a lack or small amount of vitamin D [2, 3]. Vitamin D is an immunomodulator affecting both the innate and the adaptive immune system which is important

in the development and activity of MS. The impact of vitamin D on immune parameters is evident *in vitro*, while the effects on the clinical aspects are inconclusive, especially when the effect of supplementation is assessed. In this work, we review current knowledge on the effect of vitamin D on immune cells subsets in relation to *in vitro* and *in vivo* studies. In this review, the term 'vitamin D' is used in relation to 25(OH)D or 1,25(OH)2D3, unless otherwise stated.

Vitamin D

Vitamin D is a pro-hormone belonging to the category of fat-soluble group of vitamins. It is primarily responsible for maintaining calcium homeostasis by facilitating absorption and utilisation of minerals and acts toward bone formation and homeostasis [4, 5]. This vitamin has also a much broader effect, as evidenced by the presence of its receptor in many organs and tissues [6]. The main sources of vitamin D are sunlight, diet, and supplementation. Vitamin D in the skin is present in the form of pro- vitamin D3 (7-dehydrocholesterol) and is converted to pre-vitamin D3 photochemically by ultraviolet B (UV-B) rays from the sun and subsequently converted to

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vitamin D3 by isomerisation. Foods rich in vitamin D include fatty fish (e.g. salmon, mackerel), cod liver oil, and, in small amounts, eggs. Relative to sun exposure, diet is a poor source of vitamin D. Vitamin D3 from the skin, food, or supplements is transported to the liver by vitamin D–binding proteins, where it is converted to 25(OH)D3 (calcidiol) through the process of hydroxylation. In the kidneys, 25(OH)D3 goes through a second hydroxylation to 1,25(OH)2D3 (calcitriol) which is the active metabolite. 1,25(OH)2D3 mediates its biological effects by binding to intracellular vitamin D receptor (VDR), which is a nuclear receptor that acts as a transcription factor. The binding 1,25(OH)2D3 with VDR leads to its heterodimerisation with the retinoid-X receptor (RXR), resulting in modulation of vitamin D responsive gene expression by translocation of heterodimer complex (1, 25 (OH)2D3-VDR/RXR) to nucleus, and its recruitment on vitamin D response elements (VDRE) of target genes. Depending on the site of recruitment of VDR, 1,25(OH)2D3 may regulate protein expression of target vitamin D-sensitive genes involved in diverse cellular processes. This includes cellular growth, proliferation, differentiation, apoptosis, oxidative stress and membrane transport [4, 6, 7]. A detailed review of the relationship between VDR and immune cells in multiple sclerosis was presented recently by Lu et al [8]. Thus, vitamin D affects the metabolism of various tissues and also has a significant influence both on innate and adaptive immunity. Because all immune cells express VDR, (e.g. macrophages, dendritic cells, T cells and B cells), vitamin D's effect on immunity via VDR leads to a shift in the immune response toward anti-inflammatory [7–9].

Impact of vitamin D on immunity cells

Dendritic cells (DCs) — Dendritic cells are the main antigen-presenting cells (APCs). Their function is to take up foreign antigens and present them as peptides to T cells on the human leukocyte antigen (HLA) molecules. DCs are predominantly found in an immature state in peripheral tissues. Upon encountering a foreign antigen, they mature and migrate to the lymphoid tissues to stimulate antigen-specific T cells. Depending on the cytokines secreted by the DC, the T cells will differentiate into effector cells with appropriate pro- or anti-inflammatory properties [10]. As 'professional' APCs, DCs play a key role in the pathogenesis of MS and EAE, in both mediating immune responses and inducing immune tolerance [11]. DCs constitutively express VDR and in the presence of 1,25(OH)2D3 remain in an immature-like tolerogenic state. This is characterised by inhibiting upregulation of the expression of MHC class II, CD40, CD80, and CD86 plus decreased production of pro-inflammatory IL-12 and TNFα and increased anti-inflammatory IL-10 production. 1,25(OH)2D3 can only induce this tolerogenic phenotype in DCs when it is added before their maturation [12, 13]. These tolerogenic DCs induce the differentiation of T regulatory (Treg) cells. Furthermore, they specifically induce apoptosis

in autoreactive T cells, while not affecting the proliferation of other T cells [14, 15]. *In vitro* studies have confirmed that vitamin D inhibits DCs differentiation and maturation. However, the mechanism of Treg induction by DCs is not entirely clear. Recent studies have indicated that this depends on the type of DCs and there may be various mechanisms leading to the formation of Treg [16]. In EAE, both *in vivo* administration of vitamin D, and transfer of vitamin D-induced tolerogenic DCs, leads to a significant increase in the percentage of CD4(+) CD25(+) Foxp3(+) regulatory T cells and IL 10 production, as well as a decrease in the number of autoreactive T cells. Moreover, it significantly decreased the incidence of EAE and also reduced its severity [17–19]. Tolerogenic DCs are a very interesting direction of therapeutic research in MS, and currently the use of tolerogenic DCs generated with 1,25(OH)2D3 is being tested [20].

Macrophages — Macrophages are main phagocytic cells and they are also important APCs. In a normal immune response, they produce inflammatory mediators and recruit other immune cells to eradicate the pathogen. Macrophages can be divided into two categories: M1 and M2 macrophages. M1 macrophages produce pro-inflammatory mediators such as nitric oxide, TNFα, IL-23, IL-12, and IL-1β, whereby they kill pathogens and promote the polarisation of T helper (Th) cells to proinflammatory T helper 1 and 17 cells (Th1, Th17). On the other hand, M2 macrophages produce the anti-inflammatory cytokine IL-10 and are important in wound repair and restoring tissue homeostasis. In autoimmune diseases, macrophages are hyperactivated and produce more pro-inflammatory cytokines, suggesting a dysregulated balance between M1 and M2 cells [16, 21]. Vitamin D has dual roles in the differentiation and activation of macrophages. In the early stages of infection, 1,25(OH)2D3 stimulates differentiation of monocytes into macrophages and enhances the antimicrobial activity of human monocytes and macrophages. This pathway is vitamin D dependent and is not induced if the level of vitamin D is not sufficient [21–24]. Vitamin D also promotes the production of anti-inflammatory IL-10 and decreases the production of pro-inflammatory IL-1β, IL-6, TNFα, RANKL, COX-2, and nitric oxide. Finally, 1,25(OH)2D3-treated macrophages have been shown to reduce T cell stimulatory capacity [16, 22, 24]. In EAE, Nashold et al showed that vitamin D decreases macrophage accumulation in the CNS of mice, but flow cytometric analysis detected no significant differences between the groups with or without vitamin D suplementation, with respect to T cells or B cells or macrophages in draining lymph nodes or spinal cords [25].

T cells — T-cells consist of different subgroups such as CD4+ T-helper cells, cytotoxic CD8+ T-cells, regulatory T-cells, natural killer T cells (NKT) and gamma-delta T-cells. Currently, it is hypothesised that an imbalance between pro-inflammatory Th1/Th17 cells and Tregs is the crucial factor in the immunopathogenesis of MS. Vitamin D may act by restoring this balance, thereby restoring immune

homeostasis. This is related to the presence of VDR on Tcells [1, 26].

CD4+ T Cells

Th1 and Th2 - Classically, CD4+ T cells were subdivided into two classes: Th1 cells (with proinflamatory properties) and Th2 cells (with anti-inflammatory properties). 1,25(OH)2D3 inhibits the proliferation and differentiation of Th1 cells and enhances the expression of PD1, PD-L1 and CTLA4, inhibitory markers on CD4+. In addition, it suppresses the production of proinflammatory cytokines mediated by Th1 cells and reduces IL-2, IL-6, IFN-γ, IL-17, and IL-22 secretion [24]. On the other hand, the activity of immune responses mediated by Th2 cells including the secretion of IL-3, IL-4, IL-5, IL-10, IL-13 has been enhanced by vitamin D.

It has been suggested that the impact of vitamin D on immune reactions is by promoting the Th2 cells response and suppressing the Th1 cells immune activity [26–28]. In EAE, vitamin D affects Th1 and Th2 cells and Th1/Th2 cytokine synthesis inhibiting EAE induction and significantly decreases its activity [17, 29–31]. Mayne et al showed that vitamin D acts directly on pathogenic CD4(+) T cells to inhibit EAE through the nuclear VDR. Vitamin D failed to inhibit EAE disease induction in chimeric mice lacking a functional VDR in haematopoietic cells and in T lymphocytes [30]. In another study, 1,25(OH)2D3 treatment significantly reduced the clinical severity of EAE within three days, decreasing chemokines levels and monocyte trafficking [31]. Studies carried out in MS patients have had ambiguous results. Mahon found that vitamin D supplementation significantly increased serum transforming growth factor-beta. Tumour necrosis factor (TNF)-alpha, interferon (IFN)-gamma, and interleukin (IL)-13 were not different following vitamin D supplementation [32]. However, an open-label randomised prospective controlled 52-week trial in patients with MS treated with escalating vitamin D doses up to 40,000 IU/day over 28 weeks showed that T-cell reactivity and proliferation dropped significantly in treated patients over the treatment period, while no change was seen in controls. However, cytokines profiles did not change significantly during the study [33]. A similar result was found in a study with a short term (24 weeks) supplementation of vitamin D in patients with clinically isolated syndrome (CIS) and controls. No significant differences were observed in the concentrations of IL-10, IL-17 and IFN-gamma produced followed stimulation of peripheral blood mononuclear cells (PBMCs) in any treatment arm. Moreover, no significant reduction in the frequency of proinflammatory CD4 T cells was seen [34]. Only Ashtari et al showed that in RRMS patients IL-10 serum level increased significantly after taking high-dose vitamin D3 for three months [35].

Th17 Cells - In most autoimmune diseases, Th17 cells are considered to be important drivers of disease pathogenesis. Th17 cells have proinflammatory properties and are characterised by the production of cytokines such as IL-17A, IL-17F,

TNFα, proinflammatory cytokine granulocyte macrophage colony–stimulating factor (GM-CSF) and expression of the specific transcription factor, retinoid related orphan receptor (ROR). They can also be distinguished based on the expression of the chemokine receptor CCR6, which directs migration toward the chemokine CCL20. Their differentiation can be driven by TGFβ, IL-6, and IL-1β, but they require IL-23 to become pathogenic Th17 cells [36, 37].

Together, Th1 and Th17 effector cells are considered to be the major inflammatory mediators in MS. Notably, MS serum levels of IL-17 correlate with disease severity [38]. Vitamin D inhibits the differentiation and activity of Th17 cells. The presence of 1,25(OH)2D3 inhibits differentiation naïve CD4+ T cells toward the Th17 lineage *in vitro* and the formation of Th17-related cytokines and transcription factors such as IL-17A, IL-17F, RORC, and CCR6 [16, 39]. Similar results have been reported in the EAE model. Mice treated with vitamin D had fewer Th17cells and lower IL-17 production than placebo controls. This was related to a reduction of EAE induction and activity [40, 41]. Recently, vitamin D's impact on the expression of some Th17 cell- related cytokines, chemokines and chemokine receptors was investigated in EAE. In EAE mice, the expression of IL-17, IL-23 P19, IL-23 P40, CCL20, CCL22 and CCR4 in spinal cord and IL-17 and IL-23 serum levels were significantly higher than that in the control group [42]. The results are not conclusive in patients with MS. Da Costa et al evaluated *in vitro* the ability of 1,25(OH)2D in modulating different Th17 cell subsets in MS patients in the remission phase. The 1,25(OH)2D reduced Th17-related cytokines (IL-1β, IL-6, IL-17, IL-22), as well as GM-CSF. Additionally, the proportion of both IL17+/IFNγ+ (CD4+ and CD8+) T cells and IL17+/IFNγ- CD8+ T cells was positively related with neurological symptoms, determined by EDSS score [43]. Also Bhargava et al demonstrated that vitamin D supplementation decreased IL-17 producing CD4+ T cells and effector-memory CD4+ T-cells in MS patients [44]. However, Smolders et al found no correlation between Th17 cells and vitamin D serum level in MS patients [45]. Moreover, a high dose of oral vitamin D3 supplementation did not affect Th17 cells in MS patients [46]. Additionally, a randomised, double-blind, placebo-controlled clinical trial did not show a significant decrease of serum IL 17 level after 12 weeks of vitamin D supplementation. This was a small study (94 patients) and vitamin D was an added therapy to IFN-beta treatment [47].

Similarly, a sub-study of a larger clinical trial exploring high dose (up to 14,000 IU/day) vitamin D supplementation, as an add-on therapy to interferon beta 1a in patients with RRMS, showed no difference in either IL-17 CD4+ or IFN-gamma CD4+ T cells at 48 weeks of observation [48].

Regulatory T cells (Tregs) - suppress the immune response. Tregs express forkhead transcription factor FoxP3 which has been identified as essential in preventing autoimmunity in several animal models. Additionally, T regulatory cells secrete anti-inflammatory cytokines IL-10, IL-35, transforming

growth factor-b (TGF-b), the inhibitory co-receptor CTLA4, and a high level of CD25 [39, 49]. They exert immunomodulatory effects on other immune cells such as macrophages, DCs, CD8+ T cells, and also other CD4+ T cells, thereby maintaining immune homeostasis [49]. In patients with MS, functional defects in FoxP3+CD4+ Treg cells have been described in peripheral blood T cells with a reduction of T cell immune response and a decrease of IL-10 production compared to controls [50, 51]. The impairment correlated with reduced FoxP3 expression in MS patient T cells [52]. In EAE, vitamin D induced the differentiation of Treg cells, while inhibiting Th1 and Th17 cell proliferation. In addition, 1,25(OH)2D3 promoted secretion of the anti-inflammatory cytokine, transforming growth factor beta1 (TGF-β1) but suppressed pro-inflammatory cytokines such as IL-17 [43, 49, 53].

Spanier et al found, interestingly, that vitamin D acts synergistically with oestrogen, causing a decreased EAE risk in a female-biased manner. Moreover, a synergistic impact of vitamin D and oestrogen in VDR-expressing CD4(+) T is essential to induce Helios(+)FoxP3(+) T cells and prevent autoimmune demyelinating disease [54]. This clinical study only partially confirmed the impact of vitamin D on Treg in MS patients.

One study showed that the capacity of the CD25+CD4+ Treg cells was correlated with serum 25-OHD levels in MS patients [45], although this association could not be substantiated upon vitamin D3 supplementation [46].

CD8+ Cytotoxic T Cells

CD8+ T cells contribute to the immune response by inducing apoptosis in abnormal cells in cases of infection or cancer, and CD8+ T cells have a higher expression of VDR than CD4+ T cells [9, 51]. In MS, CD8 T cells are the most frequent T cell subset in acute and chronic plaques. Moreover, oligoclonal expansion of CD8 cells were observed in CSF, and the blood of MS patients [55, 56]. Nevertheless, in EAE, Nashold et al detected no significant differences between the groups with or without vitamin D suplemention, with respect to CD4+ or CD8+ cells in draining lymph nodes or spinal cord [25]. In MS patients, Lysandropulos found that vitamin D can act directly on CD8+ T cells [50], and with 1,25(OH)2D3, CD8+ cells secreted less IFN-gamma and TNF-alpha and more IL-5 and TGF-beta [57].

B Cells

B cells play a crucial role in the immune response by the production of autoreactive antibodies, modulating antigen presentation reaction and cytokine secretion. Human B cells are able to respond to vitamin D. They constitutively express low levels of the VDR, which is up-regulated upon B-cell stimulation [58].

In MS immunopathogenesis, B cells are important, something underlined by the presence of IgG oligoclonal bands in the CSF of patients with MS, complement deposition in type II lesions in CNS and B cell follicular structures in the meninges. Moreover, they can play several different roles: production of autoantibodies, formation of B-cell follicles with germinal centre activity, antigen presentation, production of pro-inflammatory cytokines, and anti-inflammatory immune regulation (Breg cells) [1, 58].

The effect of vitamin D on B cells is still not completely clear. Several studies have suggested that vitamin D reduces the proliferation of B cells, induces their apoptosis, and decreases antibody production. Moreover, it has been shown that vitamin D promotes IL-10 production in human B cells [59–61]. Currently, the effect of vitamin D on the proliferation and function of B cells in MS is being discussed. It has been shown that *in vitro* vitamin D has several effects on B cells, which may be beneficial in MS: the inhibition of plasma cell generation, the inhibition of T-cell co-stimulation, and the enhancement of Breg cell activity [62]. Unfortunately, the inhibitory effects of vitamin D have not been confirmed *in vivo*. Despite a 12-week high-dose vitamin D supplementation and a significant increase in serum vitamin D level, a shift in B cell differentiation was not found. IgG levels in serum and CSF of patients with MS did not correlate with 25(OH) D concentrations [63]. Also, after 12 weeks of high dose vitamin D supplementation, patients with RRMS did not show differences in total plasma IgG and IgM levels [64]. Likewise, in a cohort of healthy controls and RRMS patients, there was no correlation between IL-10 producing B cells and serum vitamin D level [65].

Impact of vitamin D on clinical activity of MS

Vitamin D's impact on immunity in MS patients has been confirmed in many *in vitro* studies.

Also studies in EAE strongly suggest the protective role of vitamin D [16]. However, the results of a small number of clinical trials exploring various immunological outcomes in response to vitamin D supplementation in MS have been contradictory. On the basis of current data, it is difficult to determine the molecular immunological mechanism of vitamin D supplementation. It is not clear why human data are conflicting, but vitamin D3 status at enrollment, vitamin D3 dose, dose frequency, use of disease-modifying drugs, and the timing of sampling (season, oestrogen cycling in women) are all potential confounding factors. This is an interesting problem, because a similar situation was encountered when the assessment of MS clinical activity after vitamin D supplementation was evaluated. Several studies have consistently shown an inverse relationship between vitamin D levels and the frequency of relapses, disability progression, and occurrence of new brain MRI lesions [39].

However, the evaluation of these parameters after vitamin D supplementation has yielded conflicting results. These
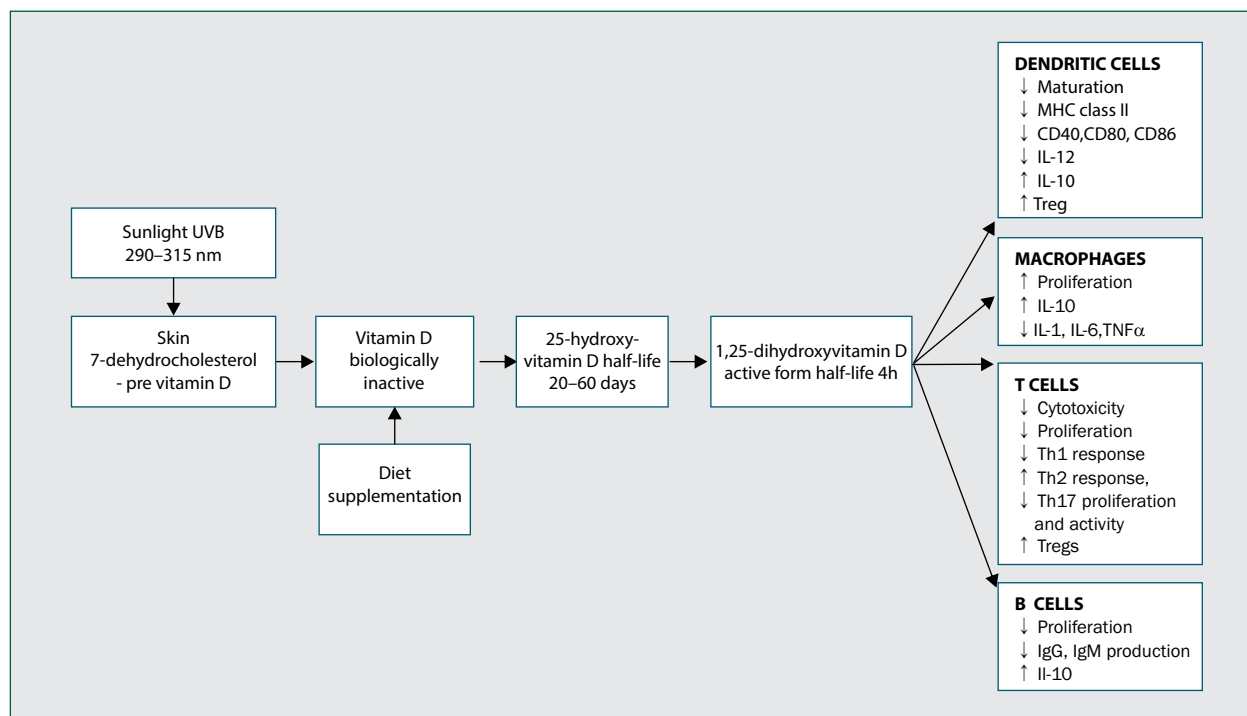
Figure 1. Sources, metabolism and potential immunomodulatory impact of vitamin D

results are difficult to compare because studies evaluating the effect of vitamin D supplementation on clinical parameters of MS have been generally small and with differing patterns. There have been studies with vitamin D alone, or with vitamin D as an add-on to a disease-modifying therapy, and highly varying doses of vitamin D have been used.

Because of that, in this review we arediscussing only double blind, placebo-controlled, randomised trials.

Stein et al investigated vitamin D's effect in RRMS patients. In this six-month, double blind, placebo-controlled, randomised trial, participants received either 1,000 IU vitamin D daily (low-dose) or 6,000IU (high dose). The results from this study did not show any effect of vitamin D in terms of clinical and MRI parameters [66]. Also Kampman et al found that suplementation of vitamin D did not result in beneficial effects on annualised relapse rates in a similar one-year, double-blind, placebo-controlled, randomised study in 66 MS patients [67].

Soilu-Hänninen et al did not find a reduction in the annual relapse rate although there was a tendency toward reduced disability accumulation as measured by EDSS and toward improved timed tandem walk. The authors stated that vitamin D add-on treatment to IFNbeta reduces MRI disease activity because patients in the vitamin D group showed fewer new T2 lesions and a significantly lower number of T1 enhancing lesions [68]. SOLAR was a randomised, double-blind, placebo-controlled, multicentre, phase 2 study. In this study, 229 patients were randomly assigned to vitamin D at a dose of 14,000 IU per day or a placebo as an add-on therapy to

subcutaneous IFNbeta-1a. The percentage of patients with 'disease activity free' status (defined as no relapses, no EDSS progression, and no new Gd+ or T2 MRI lesions) was introduced as the primary endpoint. After 48 weeks of the study, no differences were found between the groups according to 'disease activity free' status, annual relapse rate, or EDSS score. Only the MRI parameters showed a significant reduction of the number of new, combined, unique active lesions in the vitamin D group [69]. Likewise, only the MRI parameters showed a significant reduction of the new or enlarged T1 and T2 lesions after vitamin D supplementation in another placebo-controlled study. In this study, 129 patients received 100,000 IU of vitamin D twice a month in addition to IFNbeta-1a over a 24-month period and no effect was found for clinical parameters [70]. Shaygannejad et al studied 50 patients in a 12-month, randomised, double-blind, placebo-controlled study and the patients received either vitamin D 8,000IU/day or a placebo with a disease-modifying agent. Mosayebi et al evaluated the effects of vitamin D3 supplementation at a dose of 300,000 IU/month *vs* placebo in 62 patients over a six-month period [71, 72]. The authors did not find any beneficial effect of vitamin D supplementation either on the clinical or the MRI outcome in patients with RRMS [73].

Because the evidence for vitamin D as a treatment for MS is inconclusive, it is not surprising that a meta-analysis did not find a significant association between vitamin D supplementation and the clinical outcome [74, 75]. However, it is worth noting that the tendency towards a favourable clinical response

Table 1. Overview of randomised controlled trials with vitamin D supplementation and clinical outcome in multiple sclerosis

Study	Study design	Supplementation dosage	Other medication	25(OH)D3 in treated group (nmol/L)	Clinical effect
Burton et al. (2010) [33]	Open, 52 weeks, Randomised 49 MS pts (N = 25 cholecalciferol, N = 24 placebo)	Escalating vitamin D3; 4,000–40,000 IU/day (p.o) Control 4,000 IU vitamin D3/day	calcium 1,200 mg/day, continuation of DMT	Mean 413	No effect on EDSS Trend toward reduced relapse rate
Stein M et al. (2011) [66]	RCT, 6 months 23 RRMS	High dose (initial 6,000 + 1,000 IU/day) vs low dose (1,000 IU/day) vitamin D2.	INF beta, GA	Median High dose 120 Low dose 69	EDSS and relapses in high dose group. No effect on T1Gd+ or T2 lesions
Mosayebi G et al. (2011) [72]	RCT, 6 months 62 MS pts, N = 28 cholecalciferol, N = 34 placebo	Vitamin D3 300,000 IU/month (i.m)	INF beta 1a	Mean ≈ 150	No effect on EDSS or Gd+ lesions
Soilu-Hänninen M et al. (2012) [68]	RCT, 12 months 66 RRMS pts, (N = 34 cholecalciferol, N = 32 placebo)	Vitamin D3 20,000 IU/week (p.o)	INF beta 1b	Mean 110 range (67–163)	T1Gd+ lesions No effect on T2 lesions, EDSS or relapse
Kampman M et al. (2012) [67]	RCT, 96 weeks 68 RRMS pts, (N = 35 cholecalciferol, N = 33 placebo)	Vitamin D3 20,000 IU/week (p.o)	Calcium 500 mg/day IFNbeta — 46% pts, GA 3% pts, natalizumab — 3% pts.	Mean 123 (113–133)	No effect on relapse rate, EDSS, MSFC and fatigue
Shaygannejad V et al. (2012) [71]	RCT, 12 months 50 RRMS pts, (N-25 calcitriol, 25 — placebo)	Escalating calcitriol: 0.25–0.5 µg/day (p.o)	Continuation of DMT: IFN beta 86.0% pts, statins 10.0% pts, immunosuppressive drugs 4.0% pts.		No effect on EDSS or relapses
Golan et al. (2013) [73]	RCT, 12 months 45 RRMS pts, ((N = 24 cholecalciferol, N = 21 placebo)	Vitamin D3 4,370 IU/day, controls 800 IU/day	INF beta	Mean High dose ≈ 120 Low dose ≈ 50	No effect on EDSS or relapses

DMT — disease-modifying therapy; EDSS — Expanded Disability Status Scale; GA — glatiramer acetate; Gd+ — gadolinium contrast enhancing lesions; IFNbeta — interferon beta; IU — International Units; MSCFC — Multiple Sclerosis Functional Composite; RCT — randomised controlled trial; RRMS — relapsing-remitting multiple sclerosis

was associated with a higher level of vitamin D. This indicates the need to determine the level of vitamin D during treatment. This is especially important because Bhargava et al found that MS patients have a diminished serologic response to vitamin D supplementation compared to healthy controls and have a lower increase in vitamin D levels with supplementation [76].

The status of vitamin D as the main factor affecting the development and activity of MS is unproven. It is one of the multi-factorial impacts of the environment, which has recently been emphasised. A high importance has been attributed to UV radiation.

It has been confirmed by epidemiological study that vitamin D and sun exposure are additive independent risk factors for MS development.

Further, the direct effects of sun exposure on MRI measures of neurodegeneration in MS, independently of vitamin D, have been reported [77, 78]. Studies in humans and mice suggest that UV radiation has an immunoregulatory property and this pathway is both vitamin D-dependent and vitamin D-independent [39, 79, 80]. The vitamin D-dependent pathway

is considered the more important because humans obtain up to 80% of their vitamin D through sun exposure. UVB photons initiate a pathway of vitamin D production, from 7-dehydrocholesterol to the active form of vitamin D, as described previously. Several reviews have covered the immunomodulatory properties of vitamin D, particularly with reference to the regulation of cells proposed as being important to the development of MS [1, 2, 16]. However, additional factors should be taken into account. Firstly, the immunomodulatory properties of 1,25(OH)2D are dependent on genetic variation of the vitamin D regulating genes, CYP27B1 and CYP24A1 [81]. Moreover, immune cells have the enzymes to directly convert provitamin D or 25(OH)D to 1,25(OH)2D, and local concentrations, at the level of the cells, may be much higher than systemic levels [2]. Also vitamin D may regulate VDR-expressing nonimmune cells in the CNS. In one study of CIS patients, each 25 nmol/L increase in vitamin level was significantly associated with 7.8 mL higher grey matter volume, and there was a trend for an inverse relationship over 12 months between 25(OH)D levels and new brain lesions

and clinical relapses [82]. Vitamin D-independent pathways can be modulated by several molecules: *trans*-urocanic acid in the stratum corneum, DNA, RNA, lipids and tryptophan of keratinocytes, and antigen-presenting cells. All may initiate pathways involved in signalling from skin to immune cells in draining lymph nodes and tissues beyond. It has been shown that sub-erythemal amounts of UVR, as in narrowband UV phototherapy, can suppress both local and systemic immunity, measured functionally by reduced cell-mediated immune responses [79, 80].

In sub-erythemal UV-irradiated skin there are produced cathelicidin LL-37 and the α- and β-defensins in the absence of any inflammation. These peptides have modest anti-microbial activity but rather pleiotropic immunoregulatory properties and β-defensins via induction of T-regulatory cells can prevent and mitigate EAE [83–85]. Other studies have also demonstrated the effect of UV irradiation on an increase of Tregs and tolerogenic DCs both in EAE and in MS patients and in the maintenance of a pool of B-regulatory cells in the periphery [86, 87]. Recently, Breuer et al published a comprehensive review summarising the current knowledge regarding vitamin D and UVB light and concerning the clinical aspects of MS in epidemiological studies and clinical trials. The authors stressed that low vitamin D levels are associated with MS susceptibility and progression, although UVB light is involved in MS aetiology and progression independent of vitamin D [88].

In conclusion, numerous studies have suggested that vitamin D supplementation, and sun exposure independent of vitamin D production, may be protective against MS.

However, none of these can be treated alone as an active and sufficient treatment in MS. Further research is needed to establish how and when individuals with CIS or MS should be supplemented and to elucidate the beneficial mechanism of actions of UV exposure. This could help us to identify new targets that could offer wholly new avenues of MS therapy.

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