MINIREVIEW

Mounting Evidence for Vitamin D as an Environmental Factor Affecting Autoimmune Disease Prevalence

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Low vitamin D status has been implicated in the etiology of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, insulin-dependent diabetes mellitus, and inflammatory bowel disease. The optimal level of vitamin D intake required to support optimal immune function is not known but is likely to be at least that required for healthy bones. Experimentally, vitamin D deficiency results in the increased incidence of autoimmune disease. Mechanistically, the data point to a role for vitamin D in the development of self-tolerance. The vitamin D hormone (1,25-dihydroxy vitamin D_3) regulates T helper cell (Th1) and dendritic cell function while inducing regulatory T-cell function. The net result is a decrease in the Th1-driven autoimmune response and decreased severity of symptoms. This review discusses the accumulating evidence pointing to a link between vitamin D and autoimmunity. Increased vitamin D intakes might decrease the incidence and severity of autoimmune diseases and the rate of bone fracture. Exp Biol Med 229:1136–1142, 2004

Key words: vitamin D; autoimmunity; multiple sclerosis; arthritis; inflammatory bowel disease; insulin-dependent diabetes mellitus

Introduction

Autoimmune diseases are characterized by the targeted destruction of self-tissue by the immune system. More than 80 known autoimmune disorders exist; as a whole, they represent a leading cause of death of young to middle-aged women in the United States today (1). Despite their relatively high prevalence rate, the etiology and pathogenesis of most autoimmune disorders remain unknown, and cures remain elusive. To cure an autoimmune disorder, one would need to eradicate either the self-antigen or the immune cells responsible for the pathology. Eradication of the self-antigen is impossible; therefore, treatment options include various strategies aimed at regulating the autoimmune response.

The evidence linking vitamin D status as a potential environmental factor affecting autoimmune disease prevalence continues to accumulate. The data link vitamin D and insulin-dependent diabetes mellitus (IDDM), multiple sclerosis (MS), inflammatory bowel diseases (IBD), and rheumatoid arthritis (RA) (2). Autoimmunity is driven by T helper cells (Th1), which attack various self-tissues in the body. It is clear that both genetic and environmental factors affect disease prevalence. The fact that vitamin D has been implicated as a factor in several different autoimmune diseases suggests that vitamin D might be an environmental factor that normally participates in the control of self-tolerance. In addition, there may be a higher vitamin D requirement for patients at risk for developing and those that already have an autoimmune disease. The optimal amount of vitamin D to support the immune response may be different from the amount required to prevent vitamin D deficiency or to maintain calcium homeostasis. The current recommended intake levels for vitamin D are too low to support bone mineralization, which is already a problem in patients with autoimmunity. New evidence from human, animal, and *in vitro* mechanistic experiments suggest that vitamin D may play a role in the etiology of autoimmunity.

Vitamin D Requirements

The diet is an unreliable source of vitamin D because most foods contain insignificant amounts of vitamin D. The most cost-effective approach to obtain adequate vitamin D levels is through sunlight exposure of the skin. However, concerns about skin cancer and premature aging reduce the enthusiasm of the health-care professionals for advocating this approach. Vitamin D deficiency diseases (rickets in children and osteomalacia in adults) are relatively uncommon mostly because of the low levels of vitamin D required for their elimination. More common is the increased incidence of bone fracture associated with low intakes of vitamin D. There is a clear and well-documented relationship between serum levels of 25-hydroxyvitamin D_3 (25[OH]D_3) and fracture risk (3). According to the available information, the optimal levels of circulating 25(OH)D_3 should be >50 nM (20 ng/ml) to reduce bone-fracture risk (3). Lifeguards and other individuals who work outdoors have circulating levels of 25(OH)D_3 of >100 nM (>40 ng/ml) (4). Toxicity after sunlight exposure has never been reported. The vitamin D deficiency diseases occur when 25(OH)D_3 levels are <12.5 nM (5 ng/ml). More recently, the benefits of vitamin D have been shown against other chronic diseases besides osteoposrosis (2, 5). The circulating level of 25(OH)D_3 that is optimal for all organ systems is unknown; however, it is safe to assume that the level must be at least that amount required to reduce bone-fracture risk (>50 nM).

The question of how much vitamin D intake is needed to maintain circulating levels above 50 nM is a controversial one. Many vitamin D experts agree that the current recommendations are far too low (2, 3, 5–7). Because there is no definition for "optimal" health, and because the best studied biological outcome for vitamin D status is bone-mineral density or fracture risk, the information available is based on achieving healthy bones. Vitamin D supplementation below 800 IU/day has uniformly shown to have no effect on the incidence of bone fractures, perhaps because this dose will not raise serum 25(OH)D_3 levels above 50 nM (3). At a recent meeting held at the National Institutes of Health (Vitamin D and Health in the 21st Century: Bone and Beyond, Bethesda, MD, October 9–10, 2003), the vitamin D experts suggested that new guidelines for vitamin D are necessary. Furthermore, four of five of the experts agreed on a target of 70 nM for circulating 25(OH)D_3 levels and intakes of 1000 IU/day for adults over the age of 18. This level is 2.5- to 5-times higher (depending on age) than the current U.S.-recommended intake values for vitamin D. The current upper level of vitamin D intake was set at 2000 IU/day for all age groups, but more recent reviews of the data concluded that this amount was based on insufficient scientific evidence (5, 6). Vieth et al. (8) have shown that intakes of vitamin D at 4000 IU/day for up to 5 months are safe and do not result in hypercalciuria, which occurs when circulating levels of 25(OH)D_3 are above 250 nM (6). Estimated daily intakes of vitamin D supplements in excess of 10,000 IU are required to achieve 25(OH)D_3 levels above 250 nM (6). More data are needed to determine the upper and safe level of vitamin D. High doses of vitamin D (>1000 IU/day) might induce vitamin D toxicity over long periods of time. However, the data demonstrate a need for vitamin D intakes in the range of 800–1000 IU/day and for serum 25(OH)D_3 target levels of 50–70 nM.

Vitamin D and Autoimmunity

The early suggestions that vitamin D status might affect the prevalence of autoimmune diseases were largely based on anecdotal evidence (9). For example, MS and IBD are diseases prevalent in Canada, the northern parts of the United States, and Europe. The underlying hypothesis would be that the availability of the vitamin D ligand in the environment through either sunlight or food is an environmental factor that affects autoimmune disease prevalence. The Northern hemisphere receives less sunlight, especially during the winter. The severity of MS has been shown to fluctuate seasonally, with exacerbations occurring more in the spring than in other months (10–13). Circulating levels of vitamin D also fluctuate seasonally, with low levels of 25(OH)D_3 in the winter months and high levels during the summer months. It seems reasonable that a lag time may exist between the dip in 25(OH)D_3 levels and the increased MS exacerbations. In a German population, vitamin D status was shown to strongly correlate with MS lesion frequency given a 2-month lag time (14). Recently, a large population study determined that vitamin D intake was inversely correlated with MS incidence (15). Munger et al. (15) looked at the vitamin D intake in more than 187,000 women from two separate cohorts (one cohort was followed for 20 years, the other for 10 years). Overall, the risk of MS was 40% lower in women in the upper quintile of vitamin D intake (15). The contributions of sunlight were not accounted for, and all the women in the upper quintile were taking vitamin D supplements; vitamin D from food had no effect on the incidence of MS (15). A second study showed that vitamin D intake (sunlight not accounted for) was inversely associated with the risk of developing RA in a population of 29,000 women (16). Vitamin D supplementation (2000 IU/day) during infancy (10,366 children) also significantly reduced the development of IDDM (rate ratio of 0.22) when evaluated 30 years later (17). Regarding IBD, no prospective study has been conducted that examined vitamin D intake and the risk of developing IBD. However, vitamin D deficiency is common in patients with Crohn's disease even when the disease is in remission (18, 19). New evidence in large prospective studies supports the hypothesis that high vitamin D intakes, regardless of sunlight exposure, are associated with the reduced risk of developing IDDM, RA, and MS.

In addition to the data that show vitamin D status as an environmental factor affecting autoimmune disease prevalence, patients with autoimmune diseases have also been shown to express genetic polymorphisms for vitamin D regulatory genes. Polymorphisms in the vitamin D receptor (VDR) have been correlated with increased susceptibility of MS (20, 21), IBD (22, 23), RA (24, 25), and IDDM (26–31). Polymorphisms in other vitamin D regulatory genes have been examined. Polymorphisms in the vitamin D binding protein and the 1α-25(OH)D$_3$ hydroxylase gene were determined in one Canadian and one Japanese cohort but were not found to be associated with MS (32, 33). The genetic differences in the VDR might be one of the many genes that predispose individuals to develop autoimmunity. Unfortunately, no functional phenotype is associated with the different VDR polymorphisms. In addition, the vitamin D regulatory system is extremely complex, and other genes and complicated interactions could occur between genes that may affect autoimmune disease susceptibility.

Few trials have used vitamin D interventions in patients with autoimmune diseases. A small double-blind placebo-controlled intervention using daily 1000 IU vitamin D and 800 mg calcium supplementation showed that vitamin D supplements increased circulating levels of the anti-inflammatory cytokine transforming growth factor (TGF) $\beta1$ in patients with MS (34). Transforming growth factor–$\beta1$ levels have been shown to be low in patients with MS, and treatments that increase TGF$\beta1$ should decrease autoimmune T-cell function (35). A second study in patients with MS used calcium, magnesium, and vitamin D (cod liver oil: 5000 IU/day) supplements for 1 year (36). The number of MS-related exacerbations decreased 2.4- to 2.7-fold after supplementation. However, the study did not include control subjects. A third study showed that supplementation with fish oil (containing vitamin D) and several other vitamins significantly reduced clinical severity of MS in 11 of 16 patients (37). The study was uncontrolled, and supplementation was accompanied with recommendations of overall lifestyle changes that could have affected observed results (recommendations regarding smoking, reduced saturated fat intake, and so on). Supplementation with the synthetic 1,25-dihydroxy vitamin D$_3$ (1,25[OH]$_2$D$_3$) precursor alphacalcidiol (1α-hydroxyvitamin D$_3$) decreased the severity of RA symptoms in a small case-controlled study (38). Placebo-controlled studies have shown that calcium and vitamin D supplementation were effective for preventing bone loss in patients with autoimmune diseases, including Crohn's disease (19, 39). Unfortunately, the investigators did not look at the effects of vitamin D on the symptoms of Crohn's disease. Two studies have shown that fish oil, which is a rich source of vitamin D, decreased the severity of IBD (40, 41). More vitamin D intervention studies are needed in patients with autoimmune diseases. In patients with active autoimmune disease, the hormonally active form or analogs of the vitamin (1,25[OH]$_2$D$_3$) should be tested. The limited number of studies cited do show a small benefit associated with taking vitamin D supplements.

Vitamin D, VDR, and Experimental Autoimmunity

The disease symptoms in murine models of MS (experimental autoimmune encephalomyelitis [EAE]), RA, and IDDM are all suppressed by 1,25(OH)$_2$D$_3$ treatment in vivo (9). Conversely, vitamin D deficiency has been shown to accelerate the development of experimental MS and to increase the incidence of experimental IDDM (42, 43). More recently, experimental IBD has been shown to be accelerated by vitamin D deficiency and suppressed by 1,25(OH)$_2$D$_3$ treatment (44). Experiments with VDR knock out (KO) mice have further suggested the importance of signal transduction through the VDR in autoimmunity. In two different models of experimental IBD, the absence of the VDR has resulted in an accelerated and fulminating form of colitis (45). The increased prevalence and severity of experimental IBD was not a result of changes in calcium homeostasis because the VDR KO mice had normal serum calcium values (45). Interestingly, VDR deficiency reduced the incidence of EAE (46) and reduced the incidence of streptozotocin-induced experimental IDDM (47). The reduction of EAE and IDDM in VDR KO mice is the opposite of the effect of vitamin D ligand deficiency. However, the incidence of streptozotocin-induced IDDM was normal in VDR KO mice that were fed lactose or high-fat diets that resulted in normalized serum calcium (47). Perhaps the effect of VDR deficiency depends on the tissue that the autoimmune response targets, whereas vitamin D ligand deficiency may have broader effects on the immune system. The gastrointestinal tract is an organ that is known to be a vitamin D target. Important vitamin D targets may be in the gastrointestinal tract and the immune system that explain the increased severity of IBD in both the VDR-deficient and vitamin D ligand–deficient mice. Future work will have to sort out the role of the VDR and the vitamin D ligand in experimental autoimmunity.

Vitamin D and the Immune System: New Insight into Mechanism

Because CD4+ T cells control experimental autoimmunity, the data suggest that vitamin D regulates the differentiation and activity of CD4+ T cells either directly or indirectly to suppress autoimmune disease pathology (9). 1,25-dihydroxy vitamin D$_3$ has been shown to reduce the production or expression of IL-2 and interferon-γ (IFN-γ) in CD4+ T cells in vitro (9). These cytokines are characteristic of Th1 responses and are associated with the progression of RA, MS, IDDM, and IBD. The production of the Th2-associated cytokine IL4 has been shown to be upregulated by 1,25(OH)$_2$D$_3$ treatment in vivo (48). 1,25-dihydroxy vitamin D$_3$ addition to purified CD4 + T cells inhibited Th1 cell development and cytokine production and resulted in

Table 1. 1,25-Dihydroxy Vitamin D_3 (1,25[OH]$_2$D$_3$) and Immunoregulation[a]

| Cell	Effect of 1,25(OH)$_2$D$_3$	Reference
Th1	decreased number	48, 49
	suppress function	48, 49
Th2	increase number/function	48, 49
	suppress function	50
T reg	increase function	48, 52, 55
	increase number	54
DC	inhibit maturation	56–60
	inhibit Th1 induction	56–60
	increase Th2 induction	58, 60

[a] Th1 and Th2, T helper cells; T reg, regulatory T cell; DC, dendritic cell.

Th2 cell expansion and increased IL-4 production (49). The role of 1,25(OH)$_2$D$_3$ in the regulation of IL-4 production is controversial because Staeva-Vieira and Freedman (50) have reported 1,25(OH)$_2$D$_3$ inhibition of both Th1 and Th2 cell cytokine production, including the inhibition of IL-4. 1,25dihydroxy vitamin D_3 directly regulates Th cell cytokine secretion. Th1 cytokines are inhibited by 1,25(OH)$_2$D$_3$ treatment, whereas the role of 1,25(OH)$_2$D$_3$ in regulating Th2 cytokines is still unclear.

In addition to Th1 and Th2 cells, CD4+ T cells can also develop into regulatory or suppressive T cells (51). The regulatory CD4+ T cells' main function appears to be the maintenance of self-tolerance. Expression of the regulatory T-cell (T reg) receptor CD25 heterodimeric partner (IL-2 receptor β) was increased by 1,25(OH)$_2$D$_3$ treatment of CD4+ T cells (52). The function of CD25+ T cells is thought to involve IL-2 signaling, which requires both the α and β chain of the IL-2 receptor (53). Inhibition of IBD symptoms by 1,25(OH)$_2$D$_3$ was ineffective in the IL-2 KO mouse, and IL-2 KO mice do not make CD4+ CD25+ T reg (18, 19). Furthermore, in vivo, 1,25(OH)$_2$D$_3$ treatment of experimental autoimmune diabetes induced a population of CD4+ CD25+ T reg that correlated with the protection of the mice from diabetes (54). Barrat et al. (55) have shown that a combination of 1,25(OH)$_2$D$_3$ and dexamethasone induced IL-10-producing T reg in human and mouse CD4+ T cells. In addition, the T reg–associated cytokine TGFβ1 was increased by 1,25(OH)$_2$D$_3$ treatment (48). Some of the identified targets of 1,25(OH)$_2$D$_3$ in CD4+ T cells have been shown to include genes that indicate an increase in either the number or the function of the T-reg compartment.

Dendritic cells (DCs) are professional antigen-presenting cells that play an important role in the initiation and propagation of T-cell–mediated immune responses. In vitro, 1,25(OH)$_2$D$_3$ has been shown to inhibit the differentiation of monocytes into DCs and the T-cell stimulatory activity of DCs (56–60). In vivo experiments also suggested that 1,25(OH)$_2$D$_3$ has immunosuppressive effects on DCs (56–60). 1,25(OH)$_2$D$_3$ has been shown to inhibit IL-12 production (58) and promote IL-10 production (58, 60) in
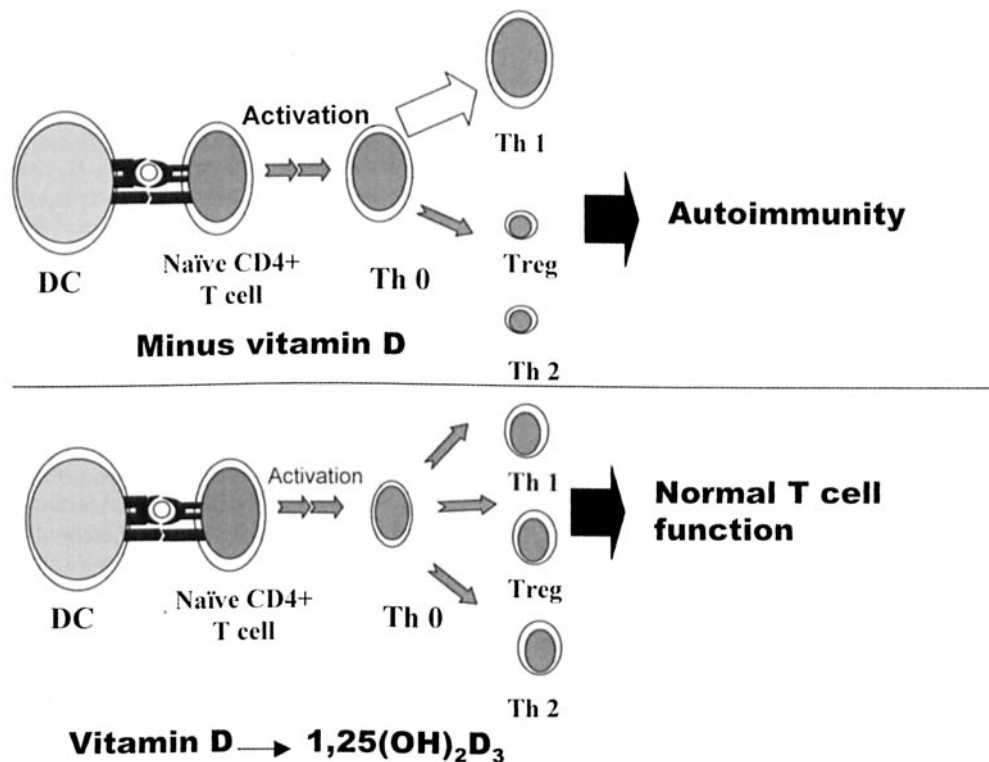
Figure 1. A model for the effects of vitamin D and 1,25-dihydroxy vitamin D_3 (1,25[OH]$_2$D$_3$) and T-cell development and function. The hypothesis is that vitamin D and 1,25(OH)$_2$D$_3$ regulate T helper cell (Th1) development by inhibiting Th1 and inducing other CD4+ T cell populations, including regulatory T cells and Th2 cells. In the absence of adequate vitamin D, the immune system favors the development of self-reactive T cells and autoimmunity.

maturing DCs. IL-12 production increases Th1 development, whereas IL-10 promotes Th2 cell development. CD4+ T cells cultured with 1,25(OH)₂D₃-treated DCs have been shown to secrete less IFN-γ than do CD4+ T cells cultured with control DCs (58). Indirectly 1,25(OH)₂D₃ regulates the autoimmune CD4+ T cell response by regulating DC function.

Many cells of the immune system contain VDRs. 1,25(OH)₂D₃ regulates the presentation of antigens by DCs and macrophage (Table 1). Dendritic-cell production of IL-12 is suppressed and IL-10 production is increased by 1,25(OH)₂D₃ treatment (Table 1). Regulatory T-cell numbers and function are increased in the presence of 1,25(OH)₂D₃ (Table 1). 1,25(OH)₂D₃ directly and indirectly suppresses the development and function of Th1 (Table 1). The evidence suggests that the net result for the immune system to develop in an environment where vitamin D is limiting is an increase in the emergence of self-reactive T cells (Fig. 1). All the reported actions (Table 1) of 1,25(OH)₂D₃ on the immune system would result in fewer and less active Th1 cells (Fig. 1).

Summary

A growing body of evidence supports the hypothesis that vitamin D is an environmental factor important in the etiology of T-cell–mediated autoimmune diseases. Mechanistically, the 1,25(OH)₂D₃ targets in the immune system include CD4+ T cells, DCs, and T reg (Table 1 and Fig. 1). The net result of 1,25(OH)₂D₃ treatment in vivo is a reduction in the autoimmune Th1 response and an amelioration of symptoms of experimental IBD, RA, IDDM, and MS. Vitamin D receptor deficiency and vitamin D ligand deficiency both result in more severe experimental IBD. Unexpectedly, IDDM and MS were less severe in VDR deficiency and more severe in vitamin D ligand deficiency. There may be both immune-mediated and target-organ–mediated effects of vitamin D in autoimmune diseases.

Clinical data confirming the connection between vitamin D and autoimmunity are lacking. New data in large prospective studies suggest that vitamin D supplementation reduces the incidence of RA, IDDM, and MS. Patients with MS, IDDM, RA, or IBD express different VDR polymorphisms, which are associated with their disease, and have been shown to have low circulating levels of 25(OH)D₃ and to be at an increased risk for bone fractures. In areas with a high prevalence of autoimmunity, adequate vitamin D from the environment is difficult to acquire. Vitamin D supplementation is a safe and cost-effective way to increase circulating vitamin D levels. More clinical trials using either vitamin D or 1,25(OH)₂D₃ analogs are needed. The risk of the 1,25(OH)₂D₃ analog treatments is hypercalcemia. However, new 1,25(OH)₂D₃ analogs may effectively regulate the immune system without increasing serum calcium. Increasing circulating vitamin D is known to decrease the risk of bone fracture. An added benefit associated with increasing vitamin D intakes may be to decrease the incidence and severity of IDDM, MS, RA, and IBD. Improving vitamin D status in patients and relatives of patients with autoimmune disease should be a priority.

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