

Vitamin D, infections and immune-mediated diseases

Vitamin D has been an exciting field of research in recent years, with more than 1400 publications published on the subject in 2008. The lay press has published articles, the internet is full of information and pharmacies have prominent displays of vitamin D supplements. Historically, vitamin D has been thought only to have an effect on calcium metabolism and bone. Vitamin D deficiency was thought only to cause rickets or osteomalacia. Recent work has found that vitamin D affects many other cells and tissues. Basic science, epidemiological, case-control and cohort studies have been conducted that show an effect of vitamin D on infections and autoimmune diseases such as multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus and Type 1 diabetes. Further research is needed in each of these areas to elucidate the mechanism vitamin D uses to affect the immune system and to develop prevention and/or treatment guidelines.

KEYWORDS: 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D, autoimmune, immunity, infection, vitamin D

Definition & prevalence of vitamin D deficiency

The definition of vitamin D deficiency has been controversial, but most experts would agree that a 25-hydroxyvitamin D (25(OH)D) level of less than 20 ng/ml is deficient, and that more than 30 ng/ml would be a better level based on calcium homeostasis end points such as parathyroid hormone suppression [1-3] and maximum calcium absorption [4].

Depending on which definition is used, the prevalence is high among several different populations. At one time, we thought the risk was only in the elderly and in those living in nursing homes, but we have seen that the elderly living in the community, as well as children and younger adults, are also at risk of deficiency. For example, approximately 50% of young people of all races living at a northern latitude were considered to be vitamin D deficient [5,6]. In another study, 32% of young indoor workers were vitamin D deficient, despite drinking milk and taking multivitamins [7]. There also appears to be a risk even in sunny areas if people are indoors or skin is extensively covered. Studies in Saudi Arabia, Australia, Turkey, India and southern Florida have found that 30-50% of children and adults are vitamin D deficient [8,9].

This high prevalence of vitamin D deficiency is especially worrisome when we consider new research that links low vitamin D status with increased risks of several illnesses, including cancer, autoimmune disorders and cardiovascular disease [10].

History of vitamin D discovery

The history of vitamin D discovery began in the early 19th century with the discovery that sun exposure, cod liver oil and, subsequently, artificial ultraviolet light cured rickets [11]. The chemical structure of vitamin D was determined in 1936 and it was shown that cod liver oil contained the same chemical. In 1971, it was established that vitamin D undergoes hydroxylation in the liver and subsequently the kidney. In 1975, the vitamin D receptor was discovered and shortly after, it was located in most tissues and cells throughout the body [12]. This leads us to the present (and future) discovery of what vitamin D is doing in all these different tissues.

Vitamin D pathophysiology

Vitamin D is formed in the epidermis, and to a small extent in the dermis, when UV-B light (290-315 nm wavelength) strikes 7-dehydrocholesterol generating previtamin D₃. The warmth of the skin converts the previtamin D₃ to native vitamin D₃. Native vitamin D₃ is transferred to the circulation, where it is carried by vitamin-D-binding protein [13].

Native vitamin D can also be obtained in oral form as D₂ (from irradiated yeast) or D₃ (from irradiated 7-dehydrocholesterol derived from lanolin). Dietary forms of native vitamin D are absorbed in the proximal small bowel and transported through the lymphatic system bound to chylomicrons [13].

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Native vitamin D (of either form) is carried by vitamin-D-binding protein to the liver, where it is hydroxylated to form 25(OH)D. This is the major circulating form that is measured to assess vitamin D status. 25(OH)D is further hydroxylated either by the kidney to produce 1,25-dihydroxyvitamin D (1,25(OH)₂D) for systemic use or by individual cells for internal cell regulation (see below for more detail on intracellular production and use of 1,25[OH]₂D). Although 1,25(OH)₂D is classically considered the active form of vitamin D, its systemic level is highly regulated by calcium homeostasis signals, so it does not indicate overall vitamin D status. In fact, because of this tight regulation, serum 1,25(OH)₂D levels can be normal or elevated in the face of vitamin D deficiency. For details on vitamin D nomenclature, see **Box 1**.

Like other steroid hormones, 1,25(OH)₂D acts on a nuclear receptor. It is taken up by the cell and transported to the nucleus where it binds to a vitamin D receptor (VDR). This complexes with a retinoic acid X receptor, which then binds to vitamin D response elements in the genome and modifies over 200 genes. This interaction regulates gene transcription of a variety of factors. Many of these factors are involved, not surprisingly, with calcium homeostasis and bone remodeling. The vitamin D response elements also modify genes responsible for cell regulation, including proliferation, differentiation and apoptosis. The VDR is present in most tissues and organs in the body and it has been known for 30 years that 1,25(OH)₂D localizes to many tissues and organs. The immune cells, T and B lymphocytes, neutrophils, macrophages and dendritic cells all contain a VDR. When this was first discovered, 1,25(OH)₂D looked promising as a treatment targeting the VDR, but this direct approach was limited by severe hypercalcemia.

Box 1. Glossary of vitamin D nomenclature.

- Native vitamin D: vitamin D before it is hydroxylated by the liver. It can be obtained through skin production or vitamin D supplements.
- 25-hydroxyvitamin D (25[OH]D): vitamin D after it is hydroxylated by the liver. This is the lab measurement that indicates vitamin D status.
- 1,25-dihydroxyvitamin D (1,25[OH]₂D): vitamin D after it is hydroxylated by both the kidney and the liver. This can be obtained in oral or intravenous forms for use in renal patients and for clinical trials.
- Vitamin D analogs: several different forms of 1,25[OH]₂D that have been modified or synthesized to avoid the hypercalcemic effects of 1,25[OH]₂D.

Mechanism of vitamin D in the immune system

The disparate effects of vitamin D on everything from diabetes to seasonal affective disorder to protection from infectious diseases and cancer seem on the surface to be unrelated, but a recent series of experiments demonstrate the mechanism vitamin D uses in the immune system, and also provide hints of the role that vitamin D plays in other cells. The key to the wide-reaching effects of vitamin D is the intracellular 1- α -hydroxylase that is located in cells throughout the body. The exact role that vitamin D plays in every cell has not yet been elucidated, but investigators are working on possible mechanisms. There are two main outcomes of vitamin D on immunity: increased immunity against antigens and modulation of the autoimmune response. These actions of vitamin D are accomplished through a variety of mechanisms. Below is a basic explanation of the mechanisms as far as we understand them today.

■ Increased immunity against antigens

The mechanism for increased immunity against antigens has been elegantly explained by Liu and colleagues [14] (see **FIGURE 1**). It was known for several years that many cells contain a 1- α -hydroxylase enzyme, but the purpose was unclear until a series of experiments in human macrophages showed that 25(OH)D is converted intracellularly to 1,25(OH)₂D in response to the interaction of a Toll-like receptor with a bacterial antigen. This interaction activates the expression of genes for 1- α -hydroxylase and cathelicidin. This leads to an elevated production of cathelicidin, a bactericidal peptide, but only in the presence of 25(OH)D or 1,25(OH)₂D. Cathelicidin is effective not only against bacteria, but also viruses [15] such as herpes simplex [16] and influenza [17]. Vitamin D could be used in either of two forms: 1,25(OH)₂D could be used by the cell directly, or 25(OH)D could be converted to 1,25(OH)₂D intracellularly and then used. In this work, they also showed a dose-response effect of 25(OH)D in human serum. Serum containing higher levels of 25(OH)D (mean: 78 nmol/l) versus serum with lower 25(OH)D levels (mean: 22 nmol/l) increased cathelicidin gene expression by twofold. This explains the use of circulating 25(OH)D to produce 1,25(OH)₂D intracellularly, so the person is not at risk of hypercalcemia from high systemic levels of 1,25(OH)₂D. When this intracellular mechanism is activated, the enzyme responsible for catabolism of 1,25(OH)₂D is also activated, keeping the 1,25(OH)₂D production and

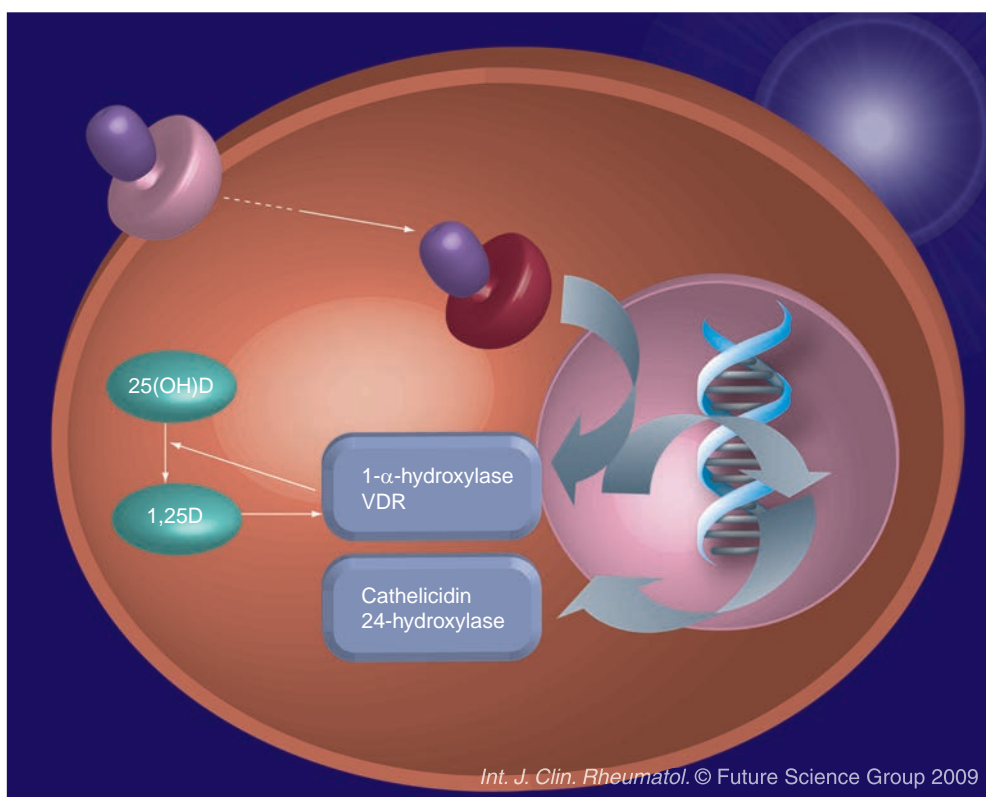


Figure 1. Vitamin D increases immunity. An antigen interacts with a Toll-like receptor. The complex is taken into the cell where the genes for 1- α -hydroxylase and the vitamin D receptor (VDR) are activated. If sufficient 25(OH)D is present, it is converted to 1,25-dihydroxyvitamin D (1,25[OH]₂D) by the 1- α -hydroxylase enzyme. This active form of vitamin D interacts with the VDR and promotes the expression of cathelicidin (a bactericidal peptide) and 24-hydroxylase (the catabolic enzyme that inactivates 1,25[OH]₂D, thus completing the reaction). Adapted with permission from Robert P Heaney.

catabolism completely self contained. This mechanism not only explains the role of vitamin D in macrophages specifically, but also explains the role that vitamin D is likely to play in other types of cells. It is probable that other cells are activated by a stimulus (e.g., antigen), and in the presence of adequate 25(OH)D, express genes specific to that cell's function (e.g., T cells and cytokines).

■ Modulation of the immune response

With the above intracellular 1- α -hydroxylase in mind, the effect of vitamin D on autoimmune disease occurs through several mechanisms, each specific to the cell it is targeting. Vitamin D acts both directly and indirectly on several immune cells, including B and T lymphocytes, dendritic cells and macrophages (see FIGURE 2).

Lymphocytes

The T lymphocytes (T cells) are central players in autoimmune disease. They respond to antigens on or inside cells, such as tumor cells or viruses.

When stimulated by these antigens, the T cells produce the inflammatory cytokines IFN- γ , IL-2 and TNF- α . There are two types of helper T cells, Th1 and Th2. Th2 cells are important for antibody-mediated immunity, while Th1 cells can react against 'self' proteins causing autoimmune diseases such as Type 1 diabetes (T1D), inflammatory bowel disease (IBD), multiple sclerosis (MS) and rheumatoid arthritis (RA). 1,25(OH)₂D directly inhibits the proliferation of Th1 cells and decreases their cytokine production [18]. In murine models, if vitamin D is deficient or the VDR is absent, Th1 actions are more prominent [19,20]. *In vitro*, treatment with 1,25(OH)₂D inhibits the production of Th1 cells and promotes Th2 cell development [18,21]. The overall effect of T cells is to increase self-tolerance [22].

B lymphocytes (B cells) are also affected by vitamin D. Cell cultures show some inhibitory effects of 1,25(OH)₂D and 25(OH)D on B-cell responses including proliferation, plasma cell differentiation and secretion of immunoglobulins, especially IgG and IgM [23].

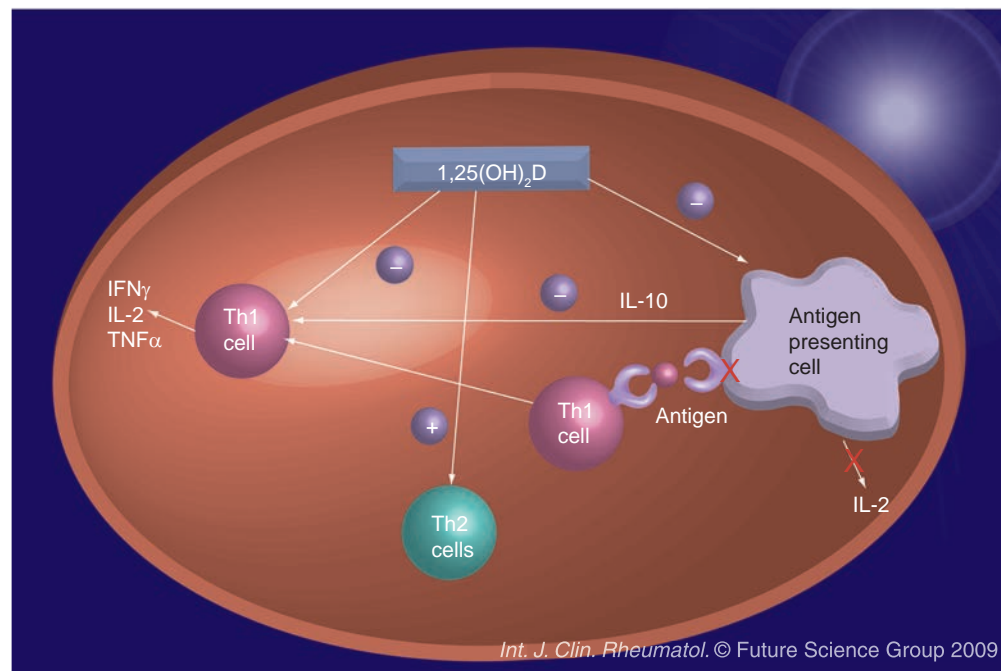


Figure 2. The effect of vitamin D on immune cells. 1,25-dihydroxyvitamin D ($1,25[\text{OH}]_2\text{D}$) directly inhibits the Th1 lymphocyte from producing inflammatory cytokines ($\text{IFN-}\gamma$, IL-2 and $\text{TNF-}\alpha$). $1,25(\text{OH})_2\text{D}$ also indirectly inhibits the Th1 lymphocyte by inhibiting the antigen-presenting cell from producing IL-2 and inducing it to produce IL-10, which inhibits Th1 lymphocytes. $1,25(\text{OH})_2\text{D}$ promotes Th2 cell development.

Antigen-presenting cells

Dendritic cells and macrophages are key to regulating immune activation and response to self proteins. Both contain an intracellular $1\text{-}\alpha$ -hydroxylase and are capable of producing $1,25(\text{OH})_2\text{D}$. The latter inhibits the differentiation of monocytes into dendritic cells [24], and also inhibits the antigen-presenting capacity of the cells [25]. Dendritic cells are uniquely capable of signaling to lymphocytes to specialize and migrate to specific tissues [26].

The effects of vitamin D on macrophages have some similarities to those on dendritic cells. $1,25(\text{OH})_2\text{D}$ promotes monocyte differentiation to macrophages and prevents them from producing inflammatory cytokines [27]. By inhibiting antigen-presenting cells from producing cytokines (specifically IL-2) that activate Th1 cells or stimulating their production of cytokines (IL-10) that inhibit Th1, $1,25(\text{OH})_2\text{D}$ modulates the immune system to become more self-tolerant [28–30].

Research to date

Much has been done in this field in determining the effects of vitamin D on calcium and bone, but this review will focus exclusively on work in the immune system (see TABLE 1).

■ Infectious disease

The connection between infectious diseases and vitamin D is well over 100 years old. It was well known that patients with rickets were more prone to infections and disease mortality was, in large part, due to lung infections such as pneumonia.

TB

Long before anti-TB drugs were developed, TB patients were treated by exposure to the sun. Interestingly, in 1946, Dr Raab was using native vitamin D as a bactericidal agent by injecting it directly into the pleural cavity of TB patients who were too ill to undergo lung resection, the standard treatment for pleural TB at that time [31]. He reported that the pleural fluid was negative for TB bacilli after 3 weeks of vitamin D treatment, and all but one patient clinically improved as well. This work was forgotten when effective TB antibiotics were developed. However, in the basic science field, $1,25(\text{OH})_2\text{D}$ was known to inhibit the development of TB in macrophage cultures [32].

Recent studies have found associations between high rates of TB infection and vitamin D deficiency in immigrants in London [33] and in immigrants in Australia [34]. While association studies suggest, but do not prove, causality,

Table 1. Evidence of vitamin D effects on disease.

Disease	Mice models	Epidemiology	Observational	RCTs
TB			√	√
Respiratory infections		√	√	√
Inflammatory bowel	√	√	√	
Multiple sclerosis	√	√	√	√
Rheumatoid arthritis	√	--	±	
Lupus erythematosus	√		±	
Type 1 diabetes	√	√	√	±

√: Evidence present; ±: Evidence mixed; --: Evidence negative.
RCT: Randomized controlled trial.

a recent randomized clinical trial successfully used 10,000 IU of native vitamin D daily in addition to standard TB multidrug treatment to improve clearance of mycobacterium from sputum [35]. The placebo group had 76% sputum conversion, while the vitamin-D-treated group had 100% conversion. Martineau and colleagues also conducted a randomized, controlled trial using a single dose of 100,000 IU native vitamin D to enhance antimycobacterial immunity in tuberculin skin-test-positive patients [36].

Respiratory infections

Epidemiologic data have shown an association between season and epidemic influenza. This has been hypothesized to be related to the seasonal differences in vitamin D status [37,38]. Low serum levels of 25(OH)D were also associated with increased respiratory infections in an Indian study [12], and there was a 13-times greater rate of pneumonia in Ethiopian children [39]. A Finnish study found an association between low 25(OH)D levels and a variety of acute respiratory infections in army recruits [40]. Further evidence of a possible cause and effect relationship was provided by a 3-year, double-blind, placebo-controlled trial of native vitamin D supplementation in African-American women. Reported cases of influenza were lower in the vitamin-D-treated group than the placebo group. This effect was especially pronounced in the winter [41].

Helicobacter pylori

A group of elderly female nursing home residents with osteoporosis who had been treated for over 20 years with α -calcidiol (1α (OH) D_3), a vitamin D analog, had lower rates of *Helicobacter pylori* infections than patients who were not treated [42].

When we consider the evidence that vitamin D is needed to initiate a bactericidal response against TB, these findings are highly suggestive of the protective effect of vitamin D against other infections. Further basic research into the effect of vitamin D on different bacteria and viruses is needed. Randomized, controlled clinical trials with infectious disease as a predefined outcome are also needed to determine the efficacy of vitamin D and its dose-response relationship to infectious disease prevention.

■ Autoimmune diseases

Epidemiological studies have linked autoimmune diseases such as MS, IBD, RA, systemic lupus erythematosus (SLE) and T1D to latitude [43–45]. I will review here further work that has been carried out in mouse models on these diseases, observational studies and a few randomized, controlled trials. All of these autoimmune diseases are mediated by specific T lymphocytes, Th1 cells. These cells are inhibited both directly and indirectly by $1,25(OH)_2D$ in cell cultures.

The cause of autoimmune diseases is multifactorial. With the high prevalence of vitamin D deficiency in the general population, it is unlikely that this is the only factor predisposing someone to autoimmune disease. There appears to be a genetic component involved as well as environmental factors, such as viruses, that predispose a person to develop an autoimmune disease. Vitamin D deficiency appears to be one modifiable factor in autoimmunity development.

Inflammatory bowel disease

IBD is an immune (Th1)-mediated disease with inflammation focused on the GI tract. The prevalence of IBD exhibits a north-south latitude

gradient similar to that of MS [46]. Seasonal exacerbation of IBD has been described with higher incidence in the winter [47].

Vitamin D deficiency is quite common in patients with IBD, possibly caused by a poor dietary intake, poor absorption, lack of sun exposure and/or steroid use, which affects vitamin D metabolism. Several studies have looked at the prevalence of vitamin D deficiency in patients with IBD. The definition of vitamin D deficiency varies throughout the various studies; some use 25(OH)D levels of less than 15 ng/dl as being indicative of vitamin D deficiency, while others use a higher level, 20–32 ng/ml. One study showed 30% of the subjects with IBD having 25(OH)D levels below 16 ng/ml [48]. In the Manitoba IBD Cohort study, the investigators found that 80% of recently diagnosed IBD patients had 25(OH)D levels of less than 30 ng/ml [49]. Another study in children and young adults aged 5–22 years, found 25(OH)D levels of less than 15 ng/ml in 16% of the patients, and looking at their data further, it appears that approximately 80% of the patients had vitamin D levels below 30 ng/ml [50]. Another study of vitamin D status in children found a high prevalence of vitamin D deficiency of approximately 38% using a level of 15 ng/ml, and approximately 80% using a level of 32 mg/ml. This study also found an association between low vitamin D status and winter, having dark skin, a low body mass index, high sedimentation rate, new diagnosis or upper GI tract disease involvement. They found the most predictive factor in low vitamin D status to be low albumin levels [51]. Vitamin D bound to vitamin-D-binding protein could potentially be lost through the gut lumen as the loss of albumin and immunoglobulins is well documented in IBD [52]. The association of vitamin D deficiency with IBD is quite strong, but it does not point to a cause and effect relationship. While it is quite likely that the IBD would cause vitamin D deficiency, whether the vitamin D deficiency plays a role in the onset of IBD remains to be seen. The experimental studies in mice point to the likely role that vitamin D plays in IBD.

In mouse models of IBD, it has been demonstrated that absent vitamin D receptors or IL-10 deficiency add to the symptoms of colitis [53]. Vitamin D deficiency accelerates the development of IBD in experimental models [54]. Also in a mouse model, 1,25(OH)₂D prevents and ameliorates the symptoms of IBD [55]. Vitamin D analogs have been used in lieu of 1,25(OH)₂D to

avoid the risk of hypercalcemia. The vitamin D analog, 22-ene-25-oxa vitamin D, was used in an IBD mouse model to reduce colitis severity. It did this without causing hypercalcemia and it was noted that the inflammatory cytokines TNF- α and IFN- γ were reduced as well [56]. The vitamin D analog 1 α (OH)D₃ has also been used to prevent colitis in mice [57].

Vitamin D uses at least two mechanisms to reduce inflammation in IBD; reducing inflammatory cytokines and maintaining the intestinal mucosal barrier. As in other autoimmune diseases, reduction of inflammatory cytokines is an important part of reducing inflammation. Treatment of mice with 1,25(OH)₂D decreases TNF- α production, but this effect was greatest if high dietary calcium was also used in the treatment [58]. Treating cells from Crohn's disease and ulcerative colitis patients with a vitamin D analog decreased the production of TNF- α [59]. Treating T cells from Crohn's patients with a combination of 1,25(OH)₂D and dexamethasone downregulated IFN- γ and increased production of IL-10. Interestingly, the cytokines had the same response when 1,25(OH)₂D was used by itself in the absence of dexamethasone [60]. There also appears to be a direct effect of 1,25(OH)₂D on the intestinal barrier. It has been recently demonstrated in mouse models that 1,25(OH)₂D and the vitamin D receptor are needed to maintain the intestinal mucosa barrier. It does this through enhancing intracellular junctions and healing mucosal injuries. 1,25(OH)₂D also increases tight junctions in cell cultures [61].

While the murine models and basic science is strong for suggesting the role of vitamin D in IBD, the levels of 25(OH)D needed for prevention or treatment have not been determined. There also needs to be more clinical intervention trials in humans.

Rheumatoid arthritis

RA is an immune (Th1)-mediated disease causing inflammation and subsequent destruction of joints. The data supporting vitamin D prevention or treatment of RA is not as strong as for the other diseases, but it does show the same T-mediated autoimmunity and so, theoretically, should be influenced by vitamin D status. Unlike some of the other autoimmune diseases, RA has not been shown to be related to latitude [62]. Mouse models have been developed that imitate RA. These include Lyme-induced arthritis and collagen-induced arthritis, and 1,25(OH)₂D has

been shown to prevent initiation and progression of inflammatory arthritis in these mouse models [63].

In human studies the association between vitamin D status and RA has been mixed. Vitamin D deficiency has been noted in patients with RA [64]. A recent large observational study reported higher disease activity in spring and lower in autumn, which may be a result of waning vitamin D stores during winter. Unfortunately, 25(OH)D levels were not assessed as part of this study [65]. In Europe, serum measurements of 25(OH)D have been inversely related to the severity of RA [66]. Another study did not find a relationship between 25(OH)D levels and disease activity, measured as C-reactive protein or erythrocyte sedimentation rate [67,68]. This was carried out in patients on active treatment, which could mask the potential role of vitamin D. A study performed with newly diagnosed patients before treatment did find an inverse correlation between 25(OH)D levels and disease activity [20].

The Women's Iowa Health Study, a large observational study, found that vitamin D intake from food and supplements was inversely correlated with RA incidence [69]. Interestingly enough, this study also found an inverse relationship between milk products and RA. However, since many milk products are fortified with vitamin D, this study could not separate out the effects. As an aside, many of the mouse models that studied human autoimmune disease also noted that the effect of vitamin D on disease was blunted, unless the animal was on a high calcium diet, indicating that it is likely other nutritional factors play a role. Another large prospective observational study, The Nurses Health Study I and II, did not find a relationship between native vitamin D intake and RA. They also did not have serum 25(OH)D measurements, but they did take into account variables such as skin color, latitude and UV exposure to make an estimate of vitamin D production in the skin [70]. A retrospective study in the Netherlands obtained 25(OH)D levels from blood donors who later developed RA. In this study they did not see an association between vitamin D deficiency, defined as less than 8 ng/ml, and the development of RA [71]. However, this study used such a low cut-off to indicate vitamin D deficiency that they may not have seen an effect if a higher level was needed for prevention. Only one clinical treatment trial used a vitamin D analog, 1α (OH) D_3 , in an open-label trial of RA patients with some success [72,73]. A

controlled, randomized trial of native vitamin D for RA prevention or treatment has not been published. Much more work needs to be done in determining the optimal level of 25(OH)D needed to prevent or treat RA.

Multiple sclerosis

MS is an immune (Th1) mediated, inflammatory, demyelinating disease of the CNS. Much of the literature linking vitamin D and MS has been epidemiological and observational in nature. Basic science work has been done in mice with experimental allergic encephalitis (EAE; a useful animal model of MS). In this model, vitamin D deficiency exacerbates EAE and treatment with vitamin D suppresses expression of EAE [74].

It has been observed that MS prevalence is related to latitude, with higher incidence occurring at a greater distance from the equator. It has also been noted that there is a seasonal fluctuation in appearance of new brain lesions on MRI, with more active lesions being seen in the spring compared with the fall [44]. The seasonal fluctuation in MS exacerbations has also been associated with lower 25(OH)D levels [75]. In large observational studies (Nurses Health Study I and II), women with the highest levels of vitamin D intake, largely from multivitamin supplements, were 40% less likely to develop MS [76]. Measurements of 25(OH)D in a subset of these subjects with the highest intake had a mean 25(OH)D level of approximately 30 ng/ml. One case-control analysis in the Netherlands found that, in women, for every 4 mg/dl increase in serum 25(OH)D levels, the odds ratio for developing MS decreased by 19% [77]. This study also found an inverse relationship between disability score and 25(OH)D levels. A retrospective study of USA military personnel using stored serum samples found increased MS risk in those with lower 25(OH)D levels. They calculated an odds ratio of 0.59 for a 20 ng/ml increase in 25(OH)D levels [78].

MS patients are at high risk of vitamin D deficiency. One study found that at the time of diagnosis, more than 64% of MS patients had 25(OH)D levels below 20 mg/dl [79]. All of these observations have led to the suggestion that native vitamin D or $1,25(OH)_2D$ supplementation may reduce the development of MS [80]. Unfortunately, clinical trial evidence is limited. A year long open-label trial of $1,25(OH)_2D$ was done in relapsing-remitting patients with MS. This study did not have a control group so it

is difficult to assess efficacy, although they had fewer relapses during the treatment part of the trial compared with the following year. Two of the subjects did have symptomatic hypercalcemia and two subjects required dose adjustments for asymptomatic hypercalcemia [81]. Vitamin D analogs have been tried experimentally because of the risk of hypercalcemia with active vitamin D ($1,25(\text{OH})_2\text{D}$). $1\alpha(\text{OH})\text{D}_3$, a vitamin D analog, was administered to five MS patients for 6 months [82]. Another vitamin D analog, 19-nor-1,25-dihydroxyvitamin D_2 , was used in 11 patients with relapsing–remitting MS for 6 months. In this study, there were no changes in clinical symptoms or MRI lesions [83].

Now that we know that cells make $1,25(\text{OH})_2\text{D}$ internally, it makes sense to try native vitamin D supplements. A 6-month, double-blind, randomized, controlled trial of 1000 IU native vitamin D daily versus a placebo in MS patients found a significantly increased level of the anti-inflammatory cytokine, TGF- β -1, in the native vitamin-D-treated group [84]. TGF- β -1 is a cytokine that has been found to be inversely correlated with symptoms and level of disability in patients with MS [85]. Another small treatment trial using magnesium, calcium and cod liver oil containing 5000 IU native vitamin D was conducted in subjects with MS. In this study, the numbers of MS exacerbations were reduced (nine) compared with the patients' expected exacerbation rate (25) [86]. A tolerability trial of vitamin D in MS patients using escalating doses of up to 280,000 IU weekly over 28 weeks in MS patients did not increase serum or urine calcium concentrations. These huge doses did not effect clinical disease progression or activity, but did decrease the number of lesions seen on MRI [87].

Overall, the association studies and the basic science data are quite strong in suggesting an effect of vitamin D on MS. The effect on MS exacerbations is likely to be through reducing inflammation, but $25(\text{OH})\text{D}$ may also have a direct effect on muscle. Other studies have shown that native vitamin D supplementation increases muscle strength [88]. The efficacy of treating or preventing MS with vitamin D is largely unknown. Studies to determine optimal $25(\text{OH})\text{D}$ levels to prevent MS or to decrease MS exacerbations are needed. There is a great need for controlled clinical trials addressing these outcomes.

Type 1 diabetes mellitus

T1D is an autoimmune disorder characterized by destruction of the pancreatic islet cells. As

with other autoimmune diseases, T1D has been shown to have a latitude gradient, with higher rates found in areas with lower UV-B irradiance. It has been noted that T1D is almost nonexistent in areas with high UV-B irradiance [89,90]. There is also a seasonal variation in incidence of T1D diagnosis [91]. A study in Newfoundland, Canada, reported peak T1D incidence in the winter months when UV-B exposure and the production of vitamin D is negligible [92].

It is known that both newly diagnosed diabetic patients and those with established disease have lower $25(\text{OH})\text{D}$ and $1,25(\text{OH})_2\text{D}$ levels than age- and sex-matched controls [93–95]. In Sweden, investigators found low $25(\text{OH})\text{D}$ levels in patients who were newly diagnosed with T1D. In this population, they saw greater rates of both T1D and vitamin D deficiency in young men compared with women. They postulated that the increased incidence of the T1D could be partially related to vitamin D deficiency [87].

In several Nordic countries, vitamin D supplementation of infants was recommended. There have been several case–control studies that have examined the effects of cod liver oil or native vitamin D supplementation during pregnancy or infancy on later development of T1D. The results of these studies have not always been congruent depending on the design of the study, which variables were included and the timing of supplementation. Hyppönen conducted a birth cohort study in Finland where high levels of vitamin D supplementation (2000 IU daily) were recommended in infants. She found that children who were compliant with this recommendation had a decrease in frequency of T1D (relative risk was 0.12, 95% confidence interval was 0.03–0.51) compared with those without supplementation [96]. The source of native vitamin D was from cod liver oil as well as other native vitamin D supplements. In Sweden, native vitamin D supplementation of 400 IU is recommended in children, and a study was carried out by looking at compliance with native vitamin D supplementation and the incidence of T1D. The investigators noted nearly 100% compliance, so they were unable to compare the effect of vitamin D supplementation. They did find that taking supplements containing vitamin D during pregnancy was associated with lower rates of developing autoantibodies (a risk factor for T1D) in the infants at 1 year [97]. The Diabetes Autoimmunity Study in the Young (DAISY) reported that the presence of autoantibodies in children inversely correlated

with maternal native vitamin D intake from food, but not supplements, during pregnancy [98]. Another case–control study looking at cod liver oil supplementation during pregnancy or early childhood found a decreased rate of T1D in children who used cod liver oil during their first year of life, but did not see an effect of supplementing native vitamin D during pregnancy [99]. This study did not ask about the amounts of vitamin D taken, but typical prenatal- and multi-vitamins available contained only 200 IU. Another retrospective case–control study in Italy also found an inverse relationship between native vitamin D supplementation in infancy and the development of T1D [100]. A recent meta-analysis of several of these case–control and cohort studies found that overall the risk of T1D was significantly reduced in infants who were supplemented with native vitamin D (an odds ratio of 0.71) compared with controls. Most of the cases were supplemented with approximately 400 IU of native vitamin D from either cod liver oil or supplements. They also noted that patients with rickets diagnosed early in life had the greatest risk of developing T1D (with an odds ratio of 3.0) [101]. Unfortunately, these studies did not use 25(OH)D levels to give an objective measurement of the levels needed to provide protection from T1D.

In mice models of T1D (nonobese diabetic mice or NOD model), treatment with 1,25(OH)₂D from the time of weaning reduces the incidence of insulinitis and development of T1D [102,103]. As in other autoimmune diseases, vitamin D analogs have been used to directly stimulate the vitamin D receptor, and these have also been protective [104]. While the mice models have given us some idea of the role vitamin D plays in the incidence of T1D, the Bio-Breeding rat models of diabetes did not have the same response to 25(OH)D, 1,25(OH)₂D or vitamin D analogs, implying a species-specific effect [105].

There have been very few randomized trials looking at the effects of vitamin D metabolites or vitamin D analogs on the incidence or control of diabetes in humans. A randomized, open-label trial investigating 0.25 µg of 1,25(OH)₂D taken every other day versus niacin in newly diagnosed T1D patients had no major effect on the diabetes, but did decrease insulin requirements slightly. This is likely because the timing and dose of supplementation is so important. It appears that supplementation during pregnancy or infancy has a greater effect than supplementing at the

time of diagnosis, after most of the insulin-producing β-cells have been destroyed. On the other hand, it is encouraging that the patients exhibited some decrease in insulin requirements, and this has been interpreted as protection of residual β-cell function. Unfortunately, in this study there were no measurements of 25(OH)D or 1,25(OH)₂D to indicate the level needed to improve diabetic control [106]. Another pilot study of late-onset autoimmune diabetes used 1,25(OH)₂D to improve residual β-cell function as measured by C-peptide secretion [107]. There are many unanswered questions. Before vitamin D supplementation can be used to prevent T1D, the timing and 25(OH)D levels needed must be worked out. As in the other autoimmune diseases, there is a need for prospective clinical research in this area.

Systemic lupus erythematosus

Systemic lupus erythematosus is a chronic inflammatory disease of unknown cause which can affect the skin, joints, kidneys, lungs, nervous system, serous membranes and/or other organs of the body. Lupus mouse models treated with vitamin D analogs had improved longevity and decreased proteinuria, renal arteritis and absent alopecia and scab formation [108,109]. Other studies showed no effects on SLE manifestations [110].

Vitamin D deficiency has been appreciated in patients with SLE [111–115]. The reasons for this could be steroid use, photosensitivity leading to decreased sun exposure, or poor dietary intake related to disease activity. Another reason could be antivitamin D antibodies that have been reported in SLE patients [108]. A cross-sectional study of 112 SLE patients noted very low 25(OH)D levels (mean: 11.5 ng/ml). This level was seen in both the newly diagnosed patients as well as those with established disease, and it was not related to renal disease or to steroid use [109]. A cross-sectional study found that 65% of patients with SLE had vitamin D deficiency and 25(OH)D levels inversely correlated with disease severity [116]. Other studies have also shown the relationship between serum levels of 25(OH)D and scores of severity [117,118]. African–Americans, who tend to have a higher prevalence of vitamin D deficiency due to the melanin content of their skin, have a higher incidence and severity of SLE [119].

On the other hand, a large prospective observational study, the Nurses Health Study I and II, found no correlation with SLE incidence and vitamin D intake from food or supplements [70].

The lack of finding in this large study may be explained by the fact that vitamin D intake from food or supplements contributes a very small amount to vitamin D status compared with sun exposure. Another cross-sectional study found vitamin D deficiency in 75% of patients with no relation to disease severity [120]. Interestingly, they did find that subjects with very low 25(OH)D levels, those with less than 10 ng/ml, had a high score on their fatigue scale. It has been noted in clinical patients that treating SLE patients with 800 IU native vitamin D₃ reduced reported fatigue episodes [121]. The association of vitamin D with SLE is clear, but the evidence for the treatment or prevention of SLE with vitamin D is unknown at this point. Further clinical research needs to be done.

Other

Periodontal disease is a chronic inflammatory disease characterized by loss of periodontal attachment. It has elements of both autoimmune and infectious disease. The host's production of proinflammatory cytokines to bacteria in dental plaque leads to the damage. The Third National Health and Examination Study (NHANES III) found that 25(OH)D concentrations were inversely correlated with periodontal attachment loss in older adults [122].

Clinical implications

The definition of vitamin D sufficiency has been established with calcium system end points, including parathyroid hormone response [2], calcium absorption [4] and bone mineral density measurements [123]. These end points are optimal when 25(OH)D is at approximately 30–34 ng/ml. Unfortunately, we do not have the data to establish the level of 25(OH)D that is sufficient for optimal function of the immune system. It may well be that more vitamin D is required to protect against infections, autoimmune diseases or cancer. This was suggested in work by Lappe and colleagues, which described a decrease in cancer incidence in a randomized, controlled trial of vitamin D and calcium when 25(OH)D levels increased from 28.8 ± 8.0 ng/ml to 38.5 ± 8.6 ng/ml [124]. Recommendations to keep 25(OH)D levels above 30 ng/ml are based on current consensus from data collected on calcium homeostasis end points [2,4,123]. Others have suggested that even higher levels (e.g., 36–40 ng/ml) may be needed to affect outcomes such as bone mineral density, lower-extremity function, dental health, and risk of falls, fractures and colorectal cancer [123,125].

Native vitamin D can be obtained through sun exposure or oral supplements, and in some countries intramuscular injections of native vitamin D are also available. Oral supplements of native vitamin D are the simplest way for a person to get an adequate amount. Nutritional supplements of vitamin D come in two forms: vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol). Several studies have shown that intermittent doses of vitamin D₃ are between three- and nine-times more potent at maintaining 25(OH)D levels than D₂ [126–128]. This has been recently challenged by Holick's work in which he showed that daily vitamin D₂ or vitamin D₃ doses were equally effective at maintaining 25(OH)D levels [129]. This is consistent with previous studies that show initial 25(OH)D levels to be equal after dosing with native vitamin D₂ or D₃, suggesting that absorption and 25-hydroxylation are the same [4]. The differences between the two forms of vitamin D may become apparent only during further steps in metabolism or in action on the VDR. The differences between the findings of these studies are possibly due to the daily versus intermittent dosing regimen. Further studies need to clarify whether or not there is a true difference between the forms of vitamin D. We currently use vitamin D₃ because the majority of the clinical trials with bone mineral density and fracture outcomes were conducted using a native vitamin D₃ supplement rather than D₂. The associations between higher 25(OH)D levels and lower incidence of infectious disease, autoimmune disease and cancer were observed in people whose vitamin D was mainly derived from sun exposure. There needs to be further basic science research exploring the action of vitamin D on the VDR to confirm if the forms are truly interchangeable.

The question always arises as to how much to give. Rather than rely on a 'one-size-fits-all' recommendation, which does not account for differences in skin pigmentation, sun exposure, age or weight, the simplest method is to measure the patient's 25(OH)D level. In calculating supplement dose, an estimate is that 100–150 IU daily will raise 25(OH)D levels by approximately 1 ng/ml [130]. In practice, this translates to between 1000 and 2000 IU daily for typical patients with baseline 25(OH)D levels greater than 10 ng/ml. Occasionally, patients with malabsorption or gastrointestinal surgery may require substantially more vitamin D.

Vitamin D has experienced an explosion of popularity recently and hence, supplements are much easier to find in the USA. Vitamin D₂ is

available in some multivitamin preparations and is also available as a prescription preparation of 50,000 IU. In addition, vitamin D₃ is available in multivitamin supplements as well as over-the-counter preparations of 200–50,000 IU. Availability is significantly different in other countries. In the UK, vitamin D₂ is the form most readily available, either combined with calcium or in over-the-counter preparations. Vitamin D₃ is typically prepared in calcium supplements as 400 IU increments, and is also available as over-the-counter supplements [131]. With vitamin D available to the general population, caution must be given, as toxicity can be a risk. From studies of case reports of toxicity and controlled dosing studies, it appears that the daily tolerable upper limit is approximately 10,000 IU [132]. This is a much greater amount than is needed by a typical patient. As we define what is an optimal 25(OH)D level for different disease states, we will need more dosing studies to clarify safety.

Conclusion

The evidence for the role of vitamin D in prevention and treatment of autoimmune diseases is just beginning to accumulate. The studies from basic science, mice models and epidemiologic studies point to a protective role of vitamin D in the immune system. Vitamin D deficiency is highly prevalent in the general healthy population, and even more prevalent in those with chronic disease. It is undisputed that vitamin D sufficiency is essential for optimal bone health. As moderate amounts of supplemental vitamin D are safe, it makes sense to treat and avoid vitamin D deficiency while the evidence for the role of vitamin D in the immune system accumulates.

Future perspective

In the future, we will have a thorough understanding of the multiple mechanisms that vitamin D uses to affect cells throughout the body. There will be many more randomized,

Executive summary

- The prevalence of vitamin D deficiency is high among the general population.

The history of vitamin D discovery

- The vitamin D receptor is found in many different tissues and cells throughout the body.
- The 1- α -hydroxylase is also present in many different tissues and cells and can convert 25-hydroxyvitamin D (25[OH]D) to the active vitamin D metabolite, 1,25 dihydroxyvitamin D (1,25[OH]₂D).

Vitamin D physiology

- Vitamin D is formed in the skin, hydroxylated by the liver and then the kidney.
- 25(OH)D can also be hydroxylated by intracellular 1- α -hydroxylase for each individual cell's internal use.
- Through the vitamin D receptor, 1,25(OH)₂D acts on vitamin D response elements on the genome to regulate gene transcription.
- These genes are responsible for calcium homeostasis, bone remodeling and cell regulation, including proliferation, differentiation and apoptosis.

Mechanism of vitamin D in the immune system

- There are two distinct end points of the effect of vitamin D on the immune system:
 - Increased immunity against antigens
 - Modulation of the immune response against the body (self)

Current clinical research

- Vitamin D has protective effects against infectious diseases such as TB.
- Vitamin D deficiency is associated with immune-mediated diseases:
 - Multiple sclerosis
 - Inflammatory bowel disease
 - Rheumatoid arthritis
 - Systemic lupus erythematosus
 - Type 1 diabetes

Clinical implications

- 25(OH)D is the form of vitamin D clinically measured to assess vitamin D status.
- Calcium system end points support a 25(OH)D level of approximately 30 ng/ml.
- Oral native vitamin D supplementation is the simplest way to get vitamin D.
- 100–150 IU of native vitamin D daily will raise 25(OH)D levels by approximately 1 ng/ml.

Conclusion

- Vitamin D deficiency should be prevented and treated, while the evidence for its role in the immune system accumulates.

controlled studies that will address the efficacy of native vitamin D. We will know the optimal levels of 25(OH)D needed to impact specific endpoints such as infectious disease, autoimmune disease and cancer. There will be further recommendations on vitamin D intake for the population as a whole to reap the benefits of optimal vitamin D status.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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▪ of interest

▪▪ of considerable interest

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