

Vitamin D and the Immune System

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ABSTRACT. Evidence of the role of vitamin D in the regulation of T and B cells, macrophages, dendritic cells, and keratinocytes continues to accumulate and provides a link between vitamin D and many autoimmune diseases, including Crohn's disease, juvenile diabetes mellitus, multiple sclerosis, asthma, and rheumatoid arthritis. Considering the influence of vitamin D on the immune system, it may have potential as a treatment for immune-mediated diseases, even if additional research is required to better quantify dosage. But the biggest obstacle to its clinical use is its potent hypercalcemic effect. The calcium status of the host may influence the effect of vitamin D on immunity. (First Release Jan 15 2010; J Rheumatol 2010;37:491–5; doi:10.3899/jrheum.090797)

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B LYMPHOCYTES

T LYMPHOCYTES

Vitamin D is a seco-steroid, that is, a steroid characterized by the breaking of 1 of the bonds in the steroid rings. Vitamin D acts on target cells in a steroid hormone-like manner, by binding to a specific nuclear receptor called the vitamin D receptor (VDR), which functions as a heterodimer, generally with the retinoid X receptor. This ligand-receptor complex interacts with a specific vitamin D responsive element, i.e., a DNA sequence involved in initiating transcription of messenger RNA coding for calcium-transporting proteins and growth factors responsible for calcium-phosphate metabolism and other cellular actions¹.

Because of differences in its side chain, vitamin D is distinguished by 2 biological forms, called ergocalciferol (vitamin D₂), present in some vegetable and fungal sources, and cholecalciferol (vitamin D₃), produced in the skin after ultraviolet B radiation exposure. Each form, to be biologically active, must be converted to 25 hydroxyvitamin D₃ (25OHD₃) by hydroxylation in position 25 by the enzyme 25-hydroxylase, mostly but not exclusively found in the liver^{2,3}. Further, to be completely active, 25OHD₃ is additionally hydroxylated in position 1 by the enzyme 1-alpha-hydroxylase, mainly present in the proximal renal tubule. The enzyme 1-alpha-hydroxylase has also been described in immune cells, bone, epithelia, and parathyroid glands⁴. In the proximal renal tubule, parathyroid hormone (PTH) is capable of stimulating 1-alpha-hydroxylase, while fibroblast growth factor-23 is involved in 1-alpha-hydroxy-

lase inhibition^{5,6}. In macrophages and keratinocytes the main role in 1-alpha-hydroxylase regulation is played by Toll-like receptor (TLR) activation and by the availability of vitamin D^{7,8}. Moreover, in keratinocytes, 1,25(OH)₂D₃ production is increased by tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ)^{9,10}. Both the proximal renal tubule cells and keratinocytes may express 25-hydroxyvitamin D₃-24-hydroxylase, a mitochondrial P450 enzyme that catabolizes both 1,25(OH)₂D₃ and 25OHD^{11,12}. A nonfunctional alternatively spliced form of 25-hydroxyvitamin D₃-24-hydroxylase has been seen in the cytoplasm of macrophages that may be responsible for impeding substrate access to the mitochondrial 25-hydroxyvitamin D₃-24-hydroxylase¹³.

Moreover, even if the activity of 1-alpha-hydroxylase is mainly regulated by calcium-phosphorus blood levels, vitamin C may also be involved in the transformation of vitamin D into the active metabolite by influencing 1-alpha-hydroxylase¹⁴.

Role of Vitamin D in Immune System Regulation

The primary role of vitamin D is the regulation of bone mineral homeostasis; however, its importance in the regulation of the immune system has emerged in the last 30 years. In 1983 it was demonstrated that macrophages were involved in vitamin D production and that VDR were isolated in activated human inflammatory cells^{15,16}. In 1984, Rigby, *et al*¹⁷ found that vitamin D may inhibit T cell proliferation. Evidence grew of the role of vitamin D in the regulation of the immune system.

It has been shown that the enzyme 1-alpha-hydroxylase is present not only in renal tissues but also in activated macrophages and dendritic cells^{18,19}, even if PTH inhibition has been seen only in renal tissues. In murine macrophages, 1-alpha-hydroxylase may be regulated by other mediators, such as IFN-γ²⁰.

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Vitamin D plays a role in T and B cell regulation. Indeed, VDR overexpression in active CD4+ T cells and 1,25(OH)₂D₃ inhibition of T cell proliferation have been demonstrated^{21,22}. Mahon, *et al*²², using microarray technology, identified over 102 genes that were targets of 1,25(OH)₂D₃ in CD4+ T cells. Of the 102 genes, 57 genes were downregulated and 45 were upregulated by 1,25(OH)₂D₃. Some of the identified targets were regulators of nuclear factor-kB; others were genes for interleukin 2 (IL-2) receptor β and for IgE binding factor. In addition, 1,25(OH)₂D₃ is responsible for preventing supplementary recruitment of T cell and antigen presentation by downregulation of CD4+ T cell production of IL-2 and IFN-γ, respectively^{23,24}. Moreover, 1,25(OH)₂D₃ increases the production of the T helper 2 cell-associated cytokines IL-4 and IL-5²². To investigate the relationship between 1,25(OH)₂D₃ and T CD4+ cells, Froicu, *et al*²⁵ examined CD4+ T cells from VDR knockout (KO) mice. In those mice, CD4+ T cells produced more IFN-γ and less IL-2, IL-4, and IL-5 than did CD4+ T cells from wild-type (WT) mice. Moreover, VDR KO mice were characterized by an amplified antigen-specific IFN-γ response and an increased mixed lymphocyte reaction²⁵. These data suggest that WT mice, which can respond to vitamin D, produce less IFN-γ, which is a T helper 1 cell-associated cytokine, and more IL-4 and IL-5, which are T helper 2 cell-associated cytokines. Further, 1,25(OH)₂D₃ has an inhibitory effect on T helper 1, by suppressing IL-12 synthesis, and on T helper 17, by suppressing IL-6 and IL-23 production²⁶. Using an *in vivo* model of T helper 1-mediated colitis in mice, Daniel, *et al*²⁶ demonstrated that 1,25(OH)₂D₃ may upregulate IL-4 and downregulate IL-6 and IL-17, as well as IL-12p70 and IL23p19, two dendritic cell mediators responsible for the induction of a proinflammatory differentiation of naive T cells toward T helper 1 and T helper 17, respectively.

Recently, 1,25(OH)₂D₃ inhibition of T helper 17 has been demonstrated in mice at several levels, including a direct reduction of dendritic cell ability to activate T helper 17, a reduced ability to support T helper 17 polarization of naive CD4+ T cells, and an inhibition of T helper 17-related IL-17 production²⁷. As demonstrated in a murine experimental autoimmune uveitis model, 1,25(OH)₂D₃ oral administration prevented and partially reversed disease and suppressed immunological response, shown by reduction of both ROR-γ-t (retinoic acid receptor-related orphan receptor gamma t) and IL-17 in CD4+ T cells, 2 indicators of T helper 17 cell function. By contrast, *in vitro*, 1,25(OH)₂D₃ inhibited IL-17 expression in naive CD4+ T cells purified from these mice, without suppressing T helper 17 cell function, as reflected by unaltered ROR-γ-t, STAT3, and FoxP3 expression. Moreover, 1,25(OH)₂D₃ inhibited bone marrow-derived dendritic cell ability to influence T helper 17 polarization of naive CD4+ T cells²⁷.

Regarding B cell regulation, 1,25(OH)₂D₃ is involved in

the suppression of immunoglobulin production and B cell proliferation and differentiation²⁸. Because patients with systemic lupus erythematosus (SLE), particularly those with antinuclear autoantibodies and amplified disease activity, had reduced 1,25(OH)₂D₃ levels, suggesting that vitamin D might be involved in the regulation of autoantibody expression, Chen, *et al*²⁸ studied the effects of 1,25(OH)₂D₃ on B cells from patients with SLE. That study showed that 1,25(OH)₂D₃ has a direct effect on B cells, including an inhibitory effect on proliferation, on generation of class-switched memory B cells, on plasma cell differentiation, and on immunoglobulin production²⁸.

Vitamin D also plays a role in macrophage regulation. Indeed, 1,25(OH)₂D₃ is responsible for monocytes developing into “resident” tissue macrophages, and influences their cytokine expression. It stimulates macrophages to produce prostaglandin E₂, which is involved in the inflammatory process and inhibits the expression of granulocyte-macrophage colony-stimulating factor. Moreover, 1,25(OH)₂D₃ reduces MHC II antigen expression on the cell membrane surface and induces macrophages and epithelial cells to produce cathelicidin, a peptide involved in antimicrobial action²⁹⁻³¹. Cathelicidin is responsible for activating the innate immune response by binding to its transmembrane receptor and is correlated to higher levels of the enzyme 1-α-hydroxylase in macrophages and keratinocytes^{7,8}. The enzyme 1-α-hydroxylase further increases the production of cathelicidin through the production of 1,25(OH)₂D₃.

Further, 1,25(OH)₂D₃ is responsible for inhibiting the differentiation of monocytes into dendritic cells and inhibits the differentiation, maturation, activation, and survival of dendritic cells, leading to reduced dendritic cell stimulatory activity on T cells, as demonstrated in studies on human and murine monocyte-derived dendritic cell cultures exposed to 1,25(OH)₂D₃³²⁻³⁶.

Recently, 1,25(OH)₂D₃ has been regarded as important for the interaction of monocytes with T cells, because 1,25(OH)₂D₃ reduced both the CD40L-related production of proinflammatory cytokines, including IL-1 and TNF-α, and the expression of surface costimulatory molecules, such as CD80 and CD86. Under the influence of 1,25(OH)₂D₃ and CD40L, monocytes reduce T cell proliferation and IFN-γ production, and amplify IL-10 synthesis³⁷. Moreover, 1,25(OH)₂D₃-related reduction of CD40, CD80, and CD86 expression in antigen-presenting cells, such as dendritic cells, is responsible for reduced T cell activation³⁸.

As shown in a recent study²⁶, 1,25(OH)₂D₃ can also promote dendritic cells to induce CD4+/CD25+ regulatory T cells (T reg). The study used mice with a reporter for augmented FoxP3 expression and IL-10 production, which are decisive for T reg development. Moreover, Gregori, *et al*³⁹ found in transplanted mice that 1,25(OH)₂D₃ induces dendritic cells with a tolerogenic phenotype and an augmented

percentage of CD4+/CD25+ T reg in the spleen and in the transplant-draining lymph nodes. These regulatory T cells are responsible for an increased tolerance and for a transfer of transplantation tolerance.

Vitamin D and Autoimmunity

Many autoimmune diseases are linked to vitamin D deficiency, including Crohn's disease, juvenile diabetes mellitus, multiple sclerosis (MS), asthma, and rheumatoid arthritis (RA)^{29,40-44}. It has been suggested that 1,25(OH)₂D₃ is responsible for suppression of autoimmune diseases by decreasing IL-2 and IFN-γ production, and increasing IL-4 expression, as has been demonstrated in mice^{24,45,46}. Nevertheless, the role of 1,25(OH)₂D₃ in IL-4 expression is controversial. On one hand, 1,25(OH)₂D₃ is responsible for inhibition of IL-4 production from murine T helper 1 and T helper 2 cells⁴⁷. On the other hand, Cantorna, *et al*⁴⁸ and Boonstra, *et al*²¹ have demonstrated that 1,25(OH)₂D₃ is involved in increasing IL-4 production through murine T helper 2 cell stimulation.

Administration of an analog of 1,25(OH)₂D₃ to diabetic mice reduces IL-12 and IFN-γ expression and prevents dendritic cell maturation and T helper 1 cell infiltration of the pancreatic islets and inhibits diabetes progression⁴⁹. CD4+/CD25+ T reg are probably involved in the protection of mice from diabetes because under 1,25(OH)₂D₃ treatment, the number of CD4+/CD25+ T reg in the pancreatic lymph nodes is increased⁴⁹.

Numerous studies have demonstrated that serum levels of 25(OH)D₃ are significantly lower in many cases of autoimmune diseases, including SLE, RA, and insulin-dependent diabetes mellitus, than in the healthy popula-

tion^{40,50-53}. Moreover, in RA, lower 25(OH)D₃ serum levels were directly correlated to higher disease activity⁵². A recent study has shown lower 25(OH)D₃ serum levels in patients with undifferentiated connective tissue disease than in controls. The study suggested that in some of these patients, vitamin D insufficiency plays a role in the successive development of a well established autoimmune disease, such as RA, SLE, mixed connective tissue disease, Sjögren's syndrome, systemic vasculitis, and antiphospholipid syndrome⁵⁴.

Additional evidence of the role of vitamin D in autoimmunity is the demonstration of genetic polymorphisms for the VDR gene in patients with autoimmune conditions such as inflammatory bowel diseases, RA, MS, and juvenile diabetes mellitus⁵⁵⁻⁵⁸.

In vivo studies using murine models, however, suggest that not only vitamin D but also an adequate calcium intake plays a role in regulation of the autoimmune response in the gastrointestinal tract and the central nervous system. A reduced inclination toward inflammatory bowel diseases such as ulcerative colitis and Crohn's disease has been associated with calcium intake coupled to vitamin D treatment⁵⁹. Moreover, dietary calcium doses are directly linked to vitamin D effectiveness in preventing experimental T helper cell-dependent autoimmune diseases such as experimental autoimmune encephalomyelitis. The observation that vitamin D may reduce the incidence of this autoimmune disease only if associated with adequate calcium intake underlines the importance of serum calcium in regulation of autoimmune response. Even hypercalcemic doses of vitamin D fail in preventing experimental autoimmune encephalomyelitis if not associated with adequate calcium intake. However,

Table 1. The influence of 1,25 (OH)₂D₃ in the regulation of mediators produced by immune cells.

Action of 1,25 (OH) ₂ D ₃ on Immune Mediators	Producing Cells	1,25 (OH) ₂ D ₃ Effects	References
↓ IL-1	Dendritic cells, macrophages	↓ Flogosis	37
↓ IL-2	T helper 1 cells	↓ T cells	23
		↓ Antigen presentation	
↑ IL-4	T helper 2 cells	↑ T helper 2 cell	22, 25
↑ IL-5	T helper 2 cells	↑ B cell, ↑ eosinophil	22, 25
↓ IL-6	T cells, macrophages	↓ T helper 17	26
↑ IL-10	Dendritic cells	↑ T reg activation	26, 37
↓ IL-12	Dendritic cells, macrophages	↓ T helper 1	26
↓ IL-17	T helper 17 cells	↓ Immunological response	27
↓ IL-23	Macrophages	↓ T helper 17	26
↓ Immunoglobulins	B cells	↓ Immunological response	28
↓ TNF-α	Macrophages	↓ Flogosis	37
↓ IFN-γ	T helper 1 cells	↓ T cells	24, 25
		↓ Antigen presentation	
↓ CD40	Antigen-presenting cells	↓ T cell activation	38
↓ CD80	Antigen-presenting cells	↓ T cells activation	38
↓ CD86	Antigen-presenting cells	↓ T cell activation	38
↑ FoxP3	Dendritic cells	↓ T reg activation	26

T reg: CD4+/CD25+ regulatory T cells; TNF: tumor necrosis factor; IFN: interferon.

vitamin D doses that do not cause hypercalcemia may reduce the incidence of experimental autoimmune encephalomyelitis, probably because the function of vitamin D may be both calcium-dependent and calcium-independent⁶⁰.

Although the real mechanism responsible for the role of calcium in autoimmune response is unknown, it may involve intracellular mechanisms, considering that calcium is an important intracellular messenger. However, the relationship of dietary calcium and intracellular calcium is still unidentified.

Conclusions

We have reported a link between vitamin D and the immune system (Table 1). It is suspected that vitamin D exerts immunoregulatory activities because it is produced by numerous cells of the immune system and VDR are expressed by many of these cells, and because vitamin D has a role in the inhibition of T helper 1, T helper 17, and dendritic cell differentiation, and in the induction of T reg.

Considering the influence of vitamin D on the immune system, it is suggested that vitamin D can be used in the treatment of immune-mediated diseases. The incidence and severity of autoimmune diseases, including RA, juvenile diabetes mellitus, inflammatory bowel disease, and MS may be reduced by increasing vitamin D intake²⁹.

Another question is how much vitamin D is needed to affect autoimmune response. Studies showed that a high pharmacologic dosage of vitamin D, regardless of sunlight exposure, is required to play a role in the regulation of autoimmune response, even if additional research is required to better quantify how much vitamin D intake is needed^{29,61}. The most significant obstacle blocking its clinical use is its potent hypercalcemic effect, even though the calcium status of the host may influence the effect of vitamin D on immunity^{59,60}.

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