CURRENT STATUS IN VITAMIN D AND REGULATORY T CELLS - IMMUNOLOGICAL IMPLICATIONS

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CURRENT STATUS IN VITAMIN D AND REGULATORY T CELLS (IMMUNOLOGICAL IMPLICATIONS) (Abstract): There has been a continuous effort to understand possible non-Ca metabolism roles of vitamin D, including its role in the immune system and, in particular, in T cell-mediated immunity. Vitamin D receptor is found in significant concentrations in the T lymphocyte and macrophage populations, when we refer to immune system, and pretty much in any human tissue and cells. Until the eighties, no one had imagined that vitamin D might play a role in the functioning of the immune system. Today we accepted that the normal immune system harbors a regulatory T cell (Treg) population specialized for immune suppression. Currently, the most commonly known regulatory T-cell lineage is called CD4+CD25highFoxP3+ regulatory T cells. Several autoimmune disorders have been linked to a deficiency in vitamin D$_3$. In some autoimmune diseases, including multiple sclerosis (MS), a compromised Treg function is believed to be critically involved in the disease process. Vitamin D insufficiency has ramifications not only for bone health, but also in other non-skeletal areas of vitamin D function, such as immune cells, muscle cells and, perhaps, adipocytes. As a final conclusion, further researches in the field of vitamin D, Tregs, immunity (inflammatory processes, rejection, autoimmune diseases, etc.), either in vitro on cell cultures or in vivo using lab animals or volunteers are still necessary. Keywords: VITAMIN D, REGULATORY T CELLS (TREG), AUTOIMMUNE DISEASES, VITAMIN D RECEPTOR (VDR), FORK-HEAD BOX P3 (FOXP3), ADIPOGENESIS, ADIPOCYTE

In recent years there has been an effort to understand possible non-Ca metabolism roles of vitamin D, including its role in the immune system and, in particular, on T cell-mediated immunity. Vitamin D receptor is found in significant concentrations in the T lymphocyte and macrophage populations, when we refer to immune system, and pretty much in any human tissue and cells. However, its highest concentration is in the immature immune cells of the thymus and the mature CD8 T lymphocytes. Until 1980, no one had imagined that vitamin D might play a role in the functioning of the immune system. The function of vitamin D was largely considered to be in the area of calcium, phosphorus, and bone metabolism. It prevents rickets in children,
osteomalacia in adults, and hypocalcemic tetany. Continued pursuit of the metabolism of vitamin D resulted in the understanding that vitamin D must first be hydroxylated in the liver to form 25-hydroxyvitamin D$_3$ (25(OH)D$_3$), the major circulating form of the vitamin (1). This form of vitamin D was subsequently found to be metabolically inactive and must be further converted to a final active form, 1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$) (2, 3). The attempt to understand how the active form of vitamin D carries out its functions led to the discovery of vitamin D receptor (VDR) in 1974 and 1975 (4, 5). It became clear that this 1,25(OH)$_2$D$_3$ (radio-labelled) is localized almost entirely in the nucleus in a specific fashion in target tissues. This localization was also found in other tissues not previously considered targets (6, 7). For example, keratinocytes of skin, islet cells of the pancreas, lymphocytes, and promyelocytes, adipocytes, etc. showed specific nuclear localization of 1,25(OH)$_2$D$_3$ and the presence of VDR. The discovery of VDR in these tissues resulted in the idea that vitamin D hormone had functions beyond calcium and phosphorus metabolism, which prompted investigations into the noncalcemic actions of vitamin D (8). Noting VDR in promyelocytes, Abe et al. and Tanaka et al. (9, 10), demonstrated that vitamin D hormone can suppress proliferation of promyelocytes and cause their differentiation into the monocyte. Similar effects of vitamin D hormone on several cancerous cell lines ensued (11). A role of vitamin D hormone in cellular differentiation thus became known.

It is now widely accepted that the normal immune system harbors a regulatory T cell (Treg) population specialized for immune suppression (12). Tregs exert functions in the induction and maintenance of immune tolerance (13-16). The family of Tregs is represented by a heterogeneous cell population that includes adaptive and naturally occurring Tregs (13-16). According to the current paradigm, adaptive Tregs develop from naïve T cells in the periphery and can produce IL-10 and transforming growth factor β (TGF β), whereas naturally occurring Tregs originate in the thymus as CD4$^+$CD25$^+$ cells and perform their suppressive functions through cell-to-cell contacts and membrane-bound TGF β and cytotoxic T-lymphocyte antigen-4 (CTLA-4) (13-16). The transcription factor FoxP3 is necessary for the development of this subpopulation of CD25$^+$ Tregs, being their characteristic marker (17).

**Vitamin D and Tregs interactions and possible pathways**

Broadly speaking, Tregs have the capability to suppress the activity of the immune system and to regulate self-tolerance. However, it is not possible to unambiguously define Tregs as a homogeneous group, since T cells of different phenotypes have been shown to exhibit immune regulating potential (18). Currently, the most commonly known regulatory T-cell lineage is called CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells. The fork-head box p3 (FoxP3) transcription factor is the cell lineage–determining master transcription factor of this T-cell subtype (18). Generation of Tregs is shown to depend on the cytokine transforming growth factor (TGF)-β (19). A new population of Tregs has been described lately that is under the regulation of interleukin IL-35 (TR$_{35}$) (18). Although the first report showed them to be FoxP3$^+$, a more recent study suggests that TR$_{35}$ cells
are independent of FoxP3 (20). Because not much information exists regarding the influences of dietary products on generation or function of this population, within this report, Tregs are defined as CD4+CD25+FoxP3+ T cells unless otherwise stated (18).

Vitamin D3 can be generated in the skin through processes using ultraviolet B (UVB)-mediated photolysis of a cholesterol derivative and a spontaneous isomerization step. Vitamin D3 needs to be enzymatically active to its hormonal form! Because endogenous production of Vitamin D depends on UVB exposure, the concentration is highly influenced by environmental factors such as seasonal sunshine length and latitude, behavioral factors such as time and surface area of exposed skin, skin pigmentation and age.

Vitamin D3 is also taken up through diet. These factors lead to great variations in its circulating form and calcidiol, spurring an ongoing discussion about “natural,” “normal” and “desired” levels of Vitamin D and its derivatives. A daily intake of 100 μg (4,000 IU) is suggested as a safe dose, elevating the 25(OH)D3 levels to desired concentrations in most humans (21). This number is higher than the recommended nutrient intake of between 5 and 15 μg/day (200–600 IU) by WHO (World Health Organization).

This section deals with the role of Vitamin D and its derivatives on the immune system, focusing on their effects on FoxP3+ Treg generation and function in different diseases. UVB light exposure as well as local calcipotriol (a synthetic derivative of calcitriol) treatment can lead to vitamin D receptor (VDR)-dependent induction of FoxP3+ Tregs in the skin (18). Vitamin D3 supplementation is reported to reduce the CNS autoimmune disease EAE (experimental autoimmune encephalomyelitis) in females but not in males or ovariectomized female mice. The disease protection correlated with high local 1,25(OH)2D3 (active form) levels in the inflamed spinal cord, but not in serum. It was suggested a slower degradation of 1,25(OH)2D3 process in the CNS, mediated through a suppression of the enzyme CYP24A1, a 24-hydroxylase with 1,25(OH)2D3–inactivating properties (18, 22, 23). The protective effect in females could be restored in ovariectomized females through estrogen supplementation (24).

An additional pathway for the treatment effect of 1,25(OH)2D3 has been shown by induction of high numbers of FoxP3+ Tregs in combination with IL-2 (25). In support of this, dietary administration of 1α-(OH)-vitamin D3, an active vitamin D3 analog, led to reduced diabetes in non-obese diabetic mice and increased FoxP3+ T cells (26). Previously, it was shown that a 1,25(OH)2D3 analog also had a therapeutic effect in a spontaneous autoimmune non-obese diabetic model, resulting in an increased protective CD25+ Treg population (28).

A combination of 1,25(OH)2D3 and the anti-inflammatory corticosteroid dexamethasone synergistically induced primarily IL-10–producing FoxP3+ Tregs (28, 29). Anyway, in an experimental IBD (inflammatory bowel disease) model, 1,25(OH)2D3 and dexamethasone had a FoxP3 expression–promoting effect in the gut (30). Not only was the amount of FoxP3 expression increased by 1,25(OH)2D3 treatment, but the potency of FoxP3+ Tregs was accentuated, even in a Th2-driven in vivo asthma model (31).

The 1,25(OH)2D3 precursor calcidiol
[25(OH)D₃] is mainly 1α-hydroxylated in the kidney to its active form, but also it has been shown to be processed in immune cells (18). CD3⁺ and CD28⁺ activated T cells as well as dendritic cells (DCs) are able to produce 1,25(OH)₂D₃ from its precursor, but T cells could not directly produce precursor from Vitamin D under these conditions (18). To support this, it is shown that 1α-hydroxylase is highly up-regulated in activated human T cells (32). Furthermore, 1,25(OH)₂D₃ up regulates VDR expression, which has been shown to correlate with the protective effects of 1,25(OH)₂D₃ in EAE (18). Nevertheless, it is reported that 1,25(OH)₂D₃ induces FoxP3⁺ Treg conversion. Was also showed that multiple sclerosis (MS) patients have lower levels of calcidiol and 1,25(OH)₂D₃ during active disease, suggesting that Vitamin D₃ metabolites might play a role in controlling Treg formation and the inflammatory process. This role is further underlined by findings about positive correlations of serum calcidiol and Treg function in MS patients (32, 33). Upper studies also received support in a retrospective analysis of serum samples preceding disease onset and from epidemiological studies showing an inverted correlation of sun exposure and MS prevalence (34). Although CNS-specific conversion of encephalitogenic T cells into FoxP3⁺–induced Tregs is described (18), its correlation to local vitamin D levels is still obscure. The instability of pancreas-retrieved Tregs has been suggested (35), but it is not reported if this is correlated to levels of vitamin D derivatives in pancreas of diabetic mice. Studying the endogenous levels of vitamin D derivatives in other target tissues of immune attacks, such as in gut, joint and lung, and their role in induction of tissue-induced Tregs could be valuable.

**Fig. 1.** Overview of dietary components and conditions (represented by dark grey boxes) influencing regulatory T cells (Tregs). The Treg-modulating effects of dietary components could be direct by mediating expansion and/or suppressive function of Tregs or indirect via modulation of DCs and their subsequent actions on Tregs. Additionally, the indirect influence of dietary components could be mediated by regulation of naïve T cells to convert to Tregs or by blocking the function of inflammatory effector T cells (Teffs). The positive anti-inflammatory effects of Tregs are mediated by expression of FoxP3 and production of IL-10 (indicated by black boxes). ROS = reactive oxygen species. IL-10 hi = high expression of IL-10. (18 modified)
**Vitamin D + Treg cells in autoimmune diseases**

Several autoimmune disorders have been linked to a deficiency in vitamin D$_3$ (17, 36).

In some autoimmune diseases, including multiple sclerosis (MS), a compromised Treg function is believed to be critically involved in the disease process. In vitro, the biologically active metabolite of vitamin D has been shown to promote Treg development. A poor vitamin D status has been linked with MS incidence and MS disease activity (33). Moreover, high circulating levels of vitamin D have been associated with lower risk of multiple sclerosis (32).

Vitamin D is an important regulator of the immune system in general and multiple sclerosis in particular. Experimentally, invariant natural killer T (iNKT) cells have been shown to be important suppressors of autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE; an animal model of MS), as shown in literature. On the other hand, in experimental allergic asthma iNKT cells are required for disease induction and are therefore pathogenic. The active form of vitamin D (calcitriol) suppresses EAE. The development of EAE symptoms is accelerated in vitamin D deficiency. Interestingly experimental asthma is less severe in vitamin D deficiency although there is no effect of calcitriol on disease severity. The data suggest that an important target of vitamin D in EAE and asthma are the iNKT cells. Vitamin D and/or vitamin D receptor deficiency results in the impaired development of iNKT cells. Vitamin D is critical very early during development of the immune system. Low levels of vitamin D in utero resulted in significantly reduced numbers of iNKT cells that failed to recover when calcitriol was used to supplement neonatal or adult mice. The data suggest that one of the consequences of early vitamin D deficiency is a reduction in the numbers of iNKT cells that develop. The iNKT cells are required for the beneficial effects of calcitriol in EAE. The important role of vitamin D on iNKT cells could impact the development of human immune-mediated diseases including MS and asthma (37).

![Fig. 2. Vitamin D and vitamin D receptor (VDR) targets in invariant natural killer T (iNKT) cell development (38, 39 modified).](image)

iNKT cells develop in the thymus following several different phenotypic changes. The earliest iNKT cell precursor, DPdim expresses the invariant T-cell receptor (tetramer + ) and CD24$. The early iNKT cells down-regulate CD24 and diverge from the
other CD4/CD8 DP cells that go on to become conventional T-cells. Expression of two transcription factors (Fyn and NF-kB) is important in the movement of iNKT cells from stage (S) 0 to S1. Vitamin D and VDR deficiency affect the number of iNKT cells that rapidly expand and enter the S1 stage in maturation. There is no effect of vitamin D deficiency on the further maturation of iNKT cells. VDR knockout (KO) iNKT cells have an additional block in maturation at the S2 stage and fail to fully develop into mature iNKT cells. T-bet and NF-kB expression is associated with the transition of iNKT cells from S2 to S3. VDR KO iNKT cells express significantly less T-bet than their fully mature S3 wild-type counterparts (see Fig. 2) (37, 39).

Vitamin D3 is known to induce Treg cells by rendering antigen-presenting cells with immunologic tolerance (tolerogenic), its direct effect on human naturally occurring Treg cells is unclear. Some groups investigated if and how 1,25(OH)\(_2\)D\(_3\) can directly affect the proliferation and function of human naturally occurring Treg cells in vitro. So they demonstrated that these Treg cells express vitamin D receptors that were up-regulated following anti-CD3/CD28-bead stimulation. 1,25(OH)\(_2\)D\(_3\) inhibited proliferation of Treg cells even when exogenous interleukin-2 was provided. Treg cells were more susceptible to the inhibitory effect of 1,25(OH)\(_2\)D\(_3\) than conventional T cells 1,25(OH)\(_2\)D\(_3\) neither affected the anergic state nor the suppressive function of Treg cells but induced a subtle increase in interleukin-10-secreting cells. The cell-division-inhibiting effect of 1,25(OH)\(_2\)D\(_3\) on Treg cells was also demonstrated in vivo by supplementing vitamin D-deficient HIV-1-infected patients with 2000 IU cholecalciferol (vitamin D\(_3\)), conform with literature. Increased serum 1,25(OH)\(_2\)D\(_3\) levels were associated with a drop in the number and percentage of Treg cells, which may be attributed to a decrease in the proliferating FoxP3\(^+\) Treg cell population (40).

Treg cells are characterized by a CD25\(^{high}\) CD4\(^+\) phenotype and signature transcription factor, fork-head box protein 3 (FoxP3). They are a part of the immune system and are crucial in the regulation of immune homeostasis (40). It has been demonstrated that the regulatory function of CD4\(^+\)CD25\(^+\) Treg cells is hindered in autoimmunity, allergy and infectious diseases, indicating that these cells play a huge role in immune-mediated pathology. In last years, evidence has conducted researchers to the potential therapeutic application of Treg cells either in potentate their regulatory activity in inflammatory diseases such as autoimmunity, allograft rejection, graft versus allergic diseases and/or host disease also in blocking their suppressive activity related to tumor immunity or vaccine development (40).

**VITAMIN D AND ADIPOGENESIS**

Despite the ability to synthesize vitamin D in the skin and the availability of vitamin D in some foods, vitamin D deficiency is increasingly recognized as a widespread global nutritional problem, as shown in literature (41). Vitamin D insufficiency has ramifications not only for bone health, but also in other non-skeletal areas of vitamin D function, such as immune cells, (42) muscle cells and, perhaps, adipocytes (43, 44).

Studies conducted in Japan almost 20 years ago indicated that treatment of 3T3-L1 pre-adipocytes in culture with 1,25(OH)\(_2\)D\(_3\), the bioactive form of vitamin D, affected differentiation and adipocyte metabolism. (47). These early studies indi-
icated that nM concentrations of 1,25-(OH)\(_2\)-vitamin D could inhibit adipogenesis and reduce the accumulation of triacylglycerol by 50% compared to fully differentiated control cells. In addition, treatment of pre-adipocytes with other vitamin D metabolites, such as 24,25-(OH)\(_2\)-vitamin D, could also inhibit pre-adipocyte differentiation, but at higher concentrations than 1,25(OH)\(_2\)D\(_3\) that paralleled their reduced affinity for the vitamin D receptor. These early studies also noted that specific 1,25(OH)\(_2\)D\(_3\) binding was evident in pre-adipocyte 3T3-L1 cells, but there was no evidence of specific binding for 1,25(OH)\(_2\)D\(_3\) in mature adipocytes (42). This finding is consistent with the idea that any influence of vitamin D on adipogenesis would likely be exerted early in the pre-adipocyte to adipocyte transition when more VDR was available. Theoretically, the sensitivity of the pre-adipocyte to 1,25(OH)\(_2\)D\(_3\) would be increased by a greater amount of vitamin D receptor or other limiting VDR-associated proteins. More than a decade ago, it was shown that one of the early effects of 1,25(OH)\(_2\)D\(_3\) treatment of 3T3-L1 pre-adipocytes was an increase in vitamin D receptor mRNA expression in pre-adipocytes, which occurs within 4h of treatment in cell culture (42). Little was appreciated, however, about any direct importance of the unliganded VDR in adipogenesis, or the molecular details whereby 1,25(OH)\(_2\)D\(_3\) treatment inhibited differentiation of pre-adipocytes.

**DISCUSSION**

Summarizing all written up, we could say that definitely exist profound metabolic links via multiple metabolic and signaling pathways between vitamin D (provided as food intake or endogenous produced), its active hormonal form 1,25(OH)\(_2\)D\(_3\) , VDR from various cells (bone, muscle, hepatocytes, epithelial cells, neurons, white cells, adipocytes), various other signaling proteins (cytoplasmic and nuclear) or enzymes, transcriptional and translational factors in nuclei, and various genes (vitamin D direct related or others).

So, various groups studied different aspects of vitamin D intake amount, in those various levels in its pathway (metabolically, signaling, etc.) and its effect over immunity (hence its relation with Tregs), beyond vitamin D effects on calcium metabolism. Various groups oriented their research over vitamin D and adipogenesis and inflammation related to adipose tissue. Numerous groups studied interrelations between vitamin D and Tregs in many autoimmune diseases, infections, allergies, organ rejection and so on.

Starting from all this work done or still in progress, we could summarize it is still necessary to study on in vitro and in vivo (on lab animals and voluntary patients) the non-calcemic effects in metabolism by vitamin D (and her derivatives) in an healthy organism or in many diseases, to either ameliorate there, the effects of that disease (or medication related disease) or as co-treatment even.

**CONCLUSIONS**

The work in the field of vitamin D, Tregs, immunity (inflammatory processes, rejection, autoimmune diseases, etc.) is still necessary either in vitro on cell cultures or in vivo, using lab animals or volunteers. Also, studies on the relation between vitamin D, Tregs and adipocytes are still needed, this avenue requiring a huge amount of work, to which our research group is hoping to contribute in the future.
REFERENCES

Current status in vitamin D and regulatory T cells - immunological implications


