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PERSPECTIVES

Vitamin D and the Immune System: Getting It Right

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Abstract

1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the active form of vitamin D, is an important player in calcium and bone metabolism, but $1,25(OH)_2D_3$ also has a physiological role beyond its well-known role in skeletal homeostasis. Receptors for $1,25(OH)_2D_3$ are present in various immune cells, including monocytes, macrophages and dendritic cells, as well as T and B lymphocytes, thus suggesting a role for $1,25(OH)_2D_3$ in both innate and adaptive immune responses. Besides being targets, immune cells express vitamin D-activating enzymes, allowing local conversion of inactive vitamin D into $1,25(OH)_2D_3$ within the immune system. Data from epidemiological studies are clear: vitamin D deficiency, especially in early life, increases the risk of autoimmune diseases later on and is associated overall with an increased risk of infections. Moreover, higher levels of $25(OH)D_3$ are associated with relative protection against infections and autoimmune diseases. These association data are corroborated by experiments in preclinical animal models, where data exist that even supplementing with high doses of vitamin D or analogues of $1,25(OH)_2D_3$ can interfere with the course of immune diseases, especially autoimmune diseases like colitis, multiple sclerosis and type 1 diabetes. In humans, however, intervention trials demonstrating strong protection are lacking. This *Perspective* discusses the complex immune-regulatory effects of $1,25(OH)_2D_3$ on immune cells as well as its role in infectious and autoimmune diseases, using tuberculosis and type 1 diabetes as examples. *IBMS BoneKEy*. 2011 April;8(4):178-186.

Keywords: Vitamin D; Immune modulation; Autoimmune disease; Infectious disease

Vitamin D Is an Immunomodulator *In Vitro*

The awareness of a role for vitamin D in the regulation of immune responses was triggered by the discovery of the vitamin D receptor (VDR) in almost all immune cells, including activated $CD4^+$ and $CD8^+$ T cells, B cells, neutrophils, and antigen-presenting cells (APCs), such as macrophages and dendritic cells (DCs) (1;2). Monocytes and macrophages are crucial members of the innate immune compartment and exhibit a great ability to sense pathogen-associated molecular patterns (PAMPs) of various infectious agents by means of pattern-recognition receptors, such as Toll-like receptors (TLRs), and thus provide a first-line defense against dangerous microbial invaders. In this context, $1,25(OH)_2D_3$ has been recognized as an important mediator of innate immune responses, enhancing the antimicrobial properties of immune cells such as monocytes and macrophages. Thus, vitamin D increases autophagy and facilitates destruction of *Mycobacterium tuberculosis* (3).

In the adaptive immune system, the part of the immune system involved in autoimmunity and graft rejection, APCs, such as DCs, present antigens to other immune cells, in particular T lymphocytes, that will develop into effector cells (further propagating the immune reaction and eventually destroying the target organ) or into regulator cells (that will taper the immune reaction and bring everything back to a steady state). Exposure of dendritic cells *in vitro* to $1,25(OH)_2D_3$ dramatically alters the phenotype and behavior of these cells, with inhibition of DC maturation as evidenced by decreased levels of DC markers (CD1a), MHC class II, costimulatory molecules (CD40, CD80, and CD86), and other maturation-induced surface markers

(*e.g.*, CD83) (4-7). Furthermore, $1,25(OH)_2D_3$ also modulates DC-derived cytokine and chemokine expression, by inhibiting the production of IL-12 and IL-23 (known as major cytokines driving Th1 and Th17 differentiation, respectively), and enhancing the release of IL-10 (a cytokine exerting broad-spectrum anti-inflammatory activities) and the chemokine MIP-3α (also known as CCL22, a chemokine involved in the recruitment of CCR4-expressing regulatory T cells (Tregs)) (4-7).

Similarly to DCs, the antigen-presenting and T cell stimulatory capacities of monocytes/macrophages are reduced upon exposure to $1,25(OH)_2D_3$ (8). Interestingly, our group observed significant differences in the protein profiles of DCs being exposed to a VDR agonist, showing major alterations in three specific protein groups, including proteins involved in protein biosynthesis/proteolysis, metabolism and cytoskeleton structure (9). Such alterations in cytoskeleton proteins may contribute to the altered trafficking capacities of $1,25(OH)_2D_3$-modulated DCs towards inflammatory and lymph node-homing chemokines, but may also affect the formation of DC-T cell contacts. Considering their position at the interface of innate and adaptive immunity, with antigen-presentation and T cell activation as their main functions, modulation of DCs by $1,25(OH)_2D_3$ indeed has a major impact on the outcome of T cell responses. $1,25(OH)_2D_3$-modulated DCs have a reduced capacity to trigger T cell proliferation (4;10). Moreover, $1,25(OH)_2D_3$-mediated modulation of DC-derived cytokines alters the Th balance, by limiting inflammatory Th1 and Th17 responses, while skewing the T cell response towards a Th2 phenotype (4-10). Importantly, the reduced expression of costimulatory molecules and the ability of DCs to produce IL-10 are recognized as tolerogenic features, enabling $1,25(OH)_2D_3$-modulated DCs to favor the development of Tregs with suppressive capacity. Indeed, the ability of VDR agonists to enhance Treg induction *in vitro* has been observed by different groups (4-11).

In contrast to monocytes and DCs, resting T lymphocytes do not express the VDR. However, these are dramatically up-regulated upon immune stimulation (12;13). Exposing T lymphocytes to $1,25(OH)_2D_3$ directly alters the cytokine profiles of T cells, by inhibiting the production of inflammatory Th1 cytokines such as IL-2, IFN-γ, and TNF-α and the Th17-derived cytokines IL-17 and IL-21 (14). Thus far, the direct effects of $1,25(OH)_2D_3$ on the emergence of Th2 cytokines are less clear: some studies show that $1,25(OH)_2D_3$ favors the emergence of Th2 cells by upregulating the expression of the Th2-specific transcription factors GATA-3 and c-maf and concomitant cytokines, including IL-4, whereas others contradicted these findings (15-18). Also at the level of Treg induction by $1,25(OH)_2D_3$, the involvement of tolerogenic DCs does not seem to be a prerequisite, as it has been shown that $1,25(OH)_2D_3$, either alone or in combination with dexamethasone, could induce IL-10-producing Tregs in an APC-free *in vitro* system (17;19). In this respect, we have found that a vitamin D analog triggered the emergence of a $CD4^+CD25^{high}CD127^{low}$ Treg phenotype and selectively induced IL-10 expression within the $CD4^+$ T cell subset (20).

Exposing B lymphocytes to $1,25(OH)_2D_3$ inhibits their proliferation, plasma cell differentiation and immunoglobulin secretion (IgG and IgM), and memory B lymphocyte generation and induces B cell apoptosis (21) (Fig. 1).

Epidemiology Points Towards a Role for Vitamin D in the Immune System

Multiple groups have reported a correlation between vitamin D deficiency and susceptibility to respiratory infections, especially in the context of infection by *Mycobacterium tuberculosis* and gram-negative bacteria (reviewed in (22)). For example, a higher susceptibility to tuberculosis is seen in subjects with relatively low serum vitamin D levels, including elderly, uremic patients, and dark-skinned people (23).

In addition to its effects on innate immune responses (as described above), a growing amount of data strongly supports the proposed role of vitamin D as a regulator of adaptive immune responses. Different

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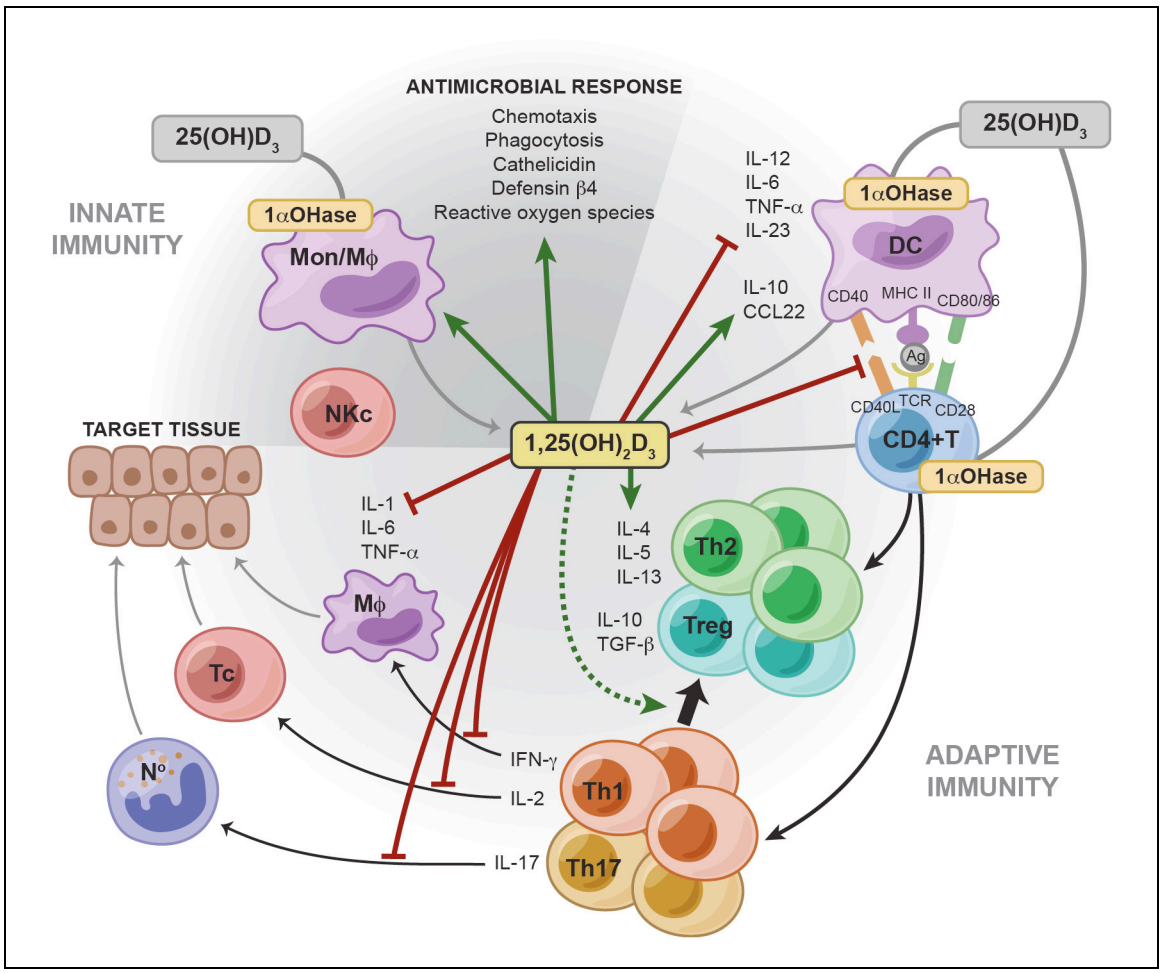

Fig. 1. The immunomodulatory effects of $1,25(OH)_2D_3$. $1,25(OH)_2D_3$ targets different players of the innate and adaptive immune compartment. $1,25(OH)_2D_3$ stimulates innate immune responses by enhancing the chemotactic and phagocytotic responses of macrophages as well as the production of antimicrobial proteins such as cathelicidin. On the other hand, $1,25(OH)_2D_3$ also modulates adaptive immunity. At the level of the APC (like the DC), $1,25(OH)_2D_3$ inhibits the surface expression of MHC-II-complexed antigen and of co-stimulatory molecules, in addition to production of the cytokines IL-12 and IL-23, thereby indirectly shifting the polarization of T cells from a Th1 and Th17 phenotype towards a Th2 phenotype. In addition, $1,25(OH)_2D_3$ directly affects T cell responses, by inhibiting the production of Th1 cytokines (IL-2 and IFN-γ) and Th17 cytokines (IL-17 and IL-21), and by stimulating Th2 cytokine production (IL-4). Moreover, $1,25(OH)_2D_3$ favors Treg cell development via modulation of DCs and by directly targeting T cells. Finally, $1,25(OH)_2D_3$ blocks plasma cell differentiation, IgG and IgM production and B cell proliferation.

epidemiological studies report an inverse correlation between vitamin D status and the incidence of autoimmune diseases, such as type 1 diabetes (T1D), systemic lupus erythematosus (SLE), multiple sclerosis (MS), inflammatory bowel disease (IBD), and rheumatoid arthritis (RA) (24-28). For example, a considerable percentage of the population living in more northern areas of the Northern hemisphere (and thus receiving less UV radiation) is vitamin D-deficient and this deficiency positively correlated with higher incidences of autoimmune diseases. Also, similar to the serum levels of $25(OH)D_3$, the onset and exacerbation of different autoimmune diseases have been documented to vary with seasonality. Furthermore, patients suffering from different autoimmune diseases such as MS, SLE, RA, and T1D display lower serum $25(OH)D_3$ levels in comparison to healthy individuals. In the context of T1D, a Finnish birth cohort study revealed a three-fold increased disease incidence in individuals that were vitamin D-deficient during early life (29).

With regard to T1D, distinct studies have found that supplementation with regular vitamin D in early life is associated with a lower risk of disease onset. In 1999, the results of a large-scale study sponsored by the European Community were published: the Concerted Action on the Epidemiology and Prevention of Diabetes showed a 33% reduction of T1D in children who received vitamin D supplementation early in life (30). In accordance with these results, Hyppönen *et al.* also found that the risk of T1D development was significantly reduced when high doses of vitamin D supplementation (up to 2,000 IU/d) were given during infancy (29). Furthermore, a meta-analysis of data from 4 case-control studies and one cohort study support the beneficial effects of vitamin D in T1D prevention, since infants receiving vitamin D supplementation showed a 29% reduction in disease onset (31). Overall, these studies suggest that vitamin D-mediated diabetes protection may be dose-dependent, with individuals receiving higher amounts of vitamin D having a lower risk of developing T1D. On the other hand, some studies did not find a correlation between T1D prevention and vitamin D supplementation. In Norway, intake of cod-liver oil by children < 1 year did not result in significant effects on T1D prevention, though there was a tendency for a negative association between cod-liver oil intake and diabetes development (32). More recently a study in Sweden with 1- to 2.5-year-old children who received vitamin D supplementation could not find a correlation between supplementation and development of diabetes-related auto-antibodies (33). However, despite the fact that some studies failed to show an association between the reduction of T1D risk and vitamin D supplementation during infancy, none of them found any association with an increased risk.

Although promising results were obtained in a few clinical trials, there is still a lack of non-biased large cohort studies that can sustain the proposed benefits of vitamin D supplementation for optimal immune function. Small sample sizes, a short follow-up duration and a lack of control groups constitute major limitations of the reported studies. In addition, different doses of vitamin D have been employed, and the initial vitamin D status of the individuals included was not always known, making it unclear whether the administered vitamin D supplements restored existing deficiencies or augmented circulating vitamin D levels in already sufficient individuals.

Vitamin D and Its Derivatives Can Be Exploited as Immunomodulators in Preclinical Models of Disease

The proposed relation between circulating vitamin D levels and immune function is further confirmed by various experimental studies investigating the consequences of impaired vitamin D signaling on immune function. Disease progression following infection with *Mycobacterium tuberculosis* was severely aggravated when mice were rendered vitamin D-deficient (34). The increased susceptibility due to the lack of vitamin D resulted in impaired macrophage functions, with defective chemotaxis, phagocytosis, respiratory burst capacity, and proinflammatory cytokine production, all being essential for their antimicrobial activity (35;36).

Also in animal models of various autoimmune diseases, vitamin D deficiency profoundly affects disease incidence and severity (reviewed in (24)). For example, in non-obese diabetic (NOD) mice (a mouse model spontaneously developing T1D with a pathogenesis similar to human disease), vitamin D deficiency during early life resulted in more aggressive disease manifestation and a higher incidence (36). Administration of 1,000 IU of regular vitamin D_3 (intraperitoneally) in early life to vitamin D-sufficient NOD mice did not prevent diabetes development, though pancreatic insulin content was higher in treated mice compared to controls (37). It is possible that higher doses, a different route of administration or a longer time-frame of supplementation with regular vitamin D may be required to observe prevention.

Many studies yielded beneficial results using active vitamin D to prevent and/or intervene in different autoimmune disease models, including T1D, SLE, experimental autoimmune encephalomyelitis (EAE),

collagen-induced arthritis, IBD, prostatitis and Heymann nephritis (38-44). Unfortunately, clinical application of pharmacological doses of $1,25(OH)_2D_3$ is obstructed by toxicity issues, since the supraphysiological doses needed to modulate immune responses elicit concomitant calcemic side effects. To overcome this limitation, structural analogs of $1,25(OH)_2D_3$ are being designed that have reduced calcemic effects with similar immunoregulatory activity.

Focusing primarily on T1D models, administration of $1,25(OH)_2D_3$ or its analogs has been documented to inhibit insulitis and disease or delay the onset of T1D in NOD mice (45-49). Some possible mechanisms explaining the observed disease reduction have been proposed, suggesting that $1,25(OH)_2D_3$ and analogs act both at a central and peripheral level. $1,25(OH)_2D_3$ was found to affect thymic differentiation of DCs and T cells. In addition, $1,25(OH)_2D_3$ or analog treatment is proposed to increase the number of Tregs, which are likely to suppress effector T cells and to halt β-cell destruction. Indeed, work by Gregori *et al.* showed that administration of a $1,25(OH)_2D_3$ analog resulted in decreased Th1 cell infiltration in the pancreas and increased $CD4^+CD25^+$ Tregs in the pancreatic lymph nodes of treated mice (47). Furthermore, $1,25(OH)_2D_3$-treated mice displayed a Th1-Th2 shift in the pancreas and pancreas-draining lymph nodes. Importantly, since T1D only becomes clinically overt after destruction of the majority of β-cells, the ability of $1,25(OH)_2D_3$ and analogs to intervene at a later stage and to revert ongoing autoimmunity has been investigated as well. Here, structural analogs of vitamin D could successfully block progression of insulitis in pre-diabetic NOD mice, along with preventing recurrence of autoimmune diabetes in NOD mice after syngeneic islet transplantation, when combined with other immunomodulating agents (reviewed in (50)).

Perspective

Where do we go from here? Receptors for $1,25(OH)_2D_3$ are present in almost all immune cells, and vitamin D deficiency is associated with poorer immune outcomes in association studies and in studies in animal models of immune disease. Finally, in preclinical models, administering vitamin D, and in particular its active form, $1,25(OH)_2D_3$, or less calcemic analogues thereof, in pharmacological doses can prevent autoimmune diseases. Translating this present knowledge to the human situation should follow two paths. First, the data are already strong enough to state that vitamin D deficiency should be avoided in all populations. For this purpose, nationwide guidelines specifying how to avoid vitamin D deficiency need to be implemented, focusing on food supplementation and guidelines for extra supplements in vulnerable populations such as neonates. Second, we need to answer the question whether the immunomodulatory potential of the vitamin D system can be exploited in a pharmacological way to prevent or treat immune diseases in populations at risk or already affected. To address this issue, very different approaches need to be taken. Here the data are not as strong, since solid large-scale intervention trials in humans that support the findings in preclinical models are lacking.

Several trials are underway, but major problems in organizing such trials exist. When using vitamin D itself, there is the issue that this product is off-patent and thus, any trial is an investigator-driven initiative, with no support from the pharmaceutical industry in the current climate where clinical trials are characterized by administrative nightmares. Second, even when support is found, organizing these trials is not easy, in particular when studying autoimmune disease. In animal models, prevention is more efficient than intervention and high doses of $1,25(OH)_2D_3$ are needed to see clear effects. For most autoimmune diseases this would mean treating subjects at genetic risk, but without any symptoms. Moreover, currently the predictive value of genetic screening is imperfect, which means treating not only symptom-free individuals, but also treating people with only a statistical chance of getting the disease. Still, it would be interesting to pursue this path, considering the interesting profile of the system: tolerance-inducing potential rather

than immunosuppression – the holy grail of immunotherapy.

How can we proceed? A first approach is to target those genetically at risk, but to administer the product at doses we deem high enough to see some effect, but safe enough to warrant use in still symptom-free people. For instance, this is now being considered in a large study in neonates for T1D. A second approach is to manipulate the immune system outside the patient. Indeed, immune cells can be isolated from the patient, manipulated *ex vivo* (*e.g.*, by exposing them to high levels of $1,25(OH)_2D_3$ or one of its even more potent analogues), and then reintroduced into the patient. For example, this approach is being studied in NAIMIT, a collaborative effort within Framework 7 of the European Union in several laboratories in Europe (www.naimit.eu).

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