Mechanisms of Vitamin D—Controlling Infections and Autoimmunity

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Vitamin D is critical in protecting humans from hyper-inflammation, invasive pathogens, and autoimmunity risks and in maintaining good health. In contrast, low 25(OH)D status increases susceptibility to infections and developing autoimmunity. Data strongly suggested that maintaining serum 25(OH)D concentrations of more than 50 ng/mL is associated with significant risk reduction from viral and bacterial infections, sepsis, and autoimmunity. This is because, above this level, immune cells get sufficient diffusion of vitamin D and 25(OH)D from the circulation to generate intracellular calcitriol for their biological and physiological actions. Vitamin D deficiency treatment costs less than 0.01% of the cost of investigating worsening comorbidities associated with hypovitaminosis D. Despite cost-benefits, the prevalence of vitamin D deficiency remains high worldwide. This was clear among those who died from COVID-19 in 2020/21—most had severe vitamin D deficiency. Herein, the critical mechanisms of how immune cells maintain their robust activities are summarized.

25(OH)D epidemiology vitamin D Autoimmunity

1. Introduction

Calcitriol functions as a hormone following secretion into the bloodstream from the kidneys. This alters the behavior of the cells involved in calcium–phosphate–bone metabolism, intestinal mucosal cells, and bone and parathyroid cells. Generally, the circulatory concentrations of vitamin D and 25(OH)D (in ng/mL) are approximately 900-fold higher than calcitriol (in pg/mL) [1]. Consequently, the diffusion of hormonal calcitriol from the blood to peripheral target cells is too little to influence their biological or physiological activity [2]. Thus, unsurprisingly, circulatory calcitriol has no clinical and evidentiary impact outside the musculoskeletal, parathyroid, and fat cells [2]. Consequently, peripheral target cells' physiological activities depend on the synthesis of calcitriol intracellularly [3][4][5]. This requires sufficient diffusing of vitamin D and/or 25(OH)D into immune cells [2]. In response to membrane-based signaling from TLR-4, immune cells increase intracellular synthesis of calcitriol and VDR, generating genomic functions [6].

Calcitriol down-regulates inflammation and oxidative stresses by suppressing inflammatory cytokines and enhancing anti-inflammatory cytokines' synthesis via the abovementioned overlapping mechanisms [7]. The immunomodulatory effects of vitamin D include activation of immune cells such as T and B cells, macrophage and dendritic cells, and enhanced production of several antimicrobial peptides and neutralizing antibodies [8][9][10].

2. The Importance of Intracellular Generation of Calcitriol for Immune Cell Signaling

Approximately 75% of the innate [11] and over 50% of the adaptive [12] immune systems are driven by intracellularly generated calcitriol [3]. The average circulatory concentration of calcitriol is approximately 0.045 ng/mL. However, its diffusible free form is less than half in the blood [13]. Even though unbound (free-form) calcitriol is fully diffusible into target cells, it occurs in a low pico-molar range: much less than the minimum concentration needed to stimulate immune cells. These minute concentrations are far below the threshold required to initiate intracellular signaling or genomic activity [2]. Whether such has any biological function is not yet known. Considering this, there is no rationale for giving calcitriol to acutely ill patients, especially with serious infections, as it will not enter immune cells sufficiently for intracellular signaling (autocrine/intracrine functions) or to alter cellular functions.

As per present data, calcitriol's hormonal form would not impact intracellular biological signal transduction or genomic functions in immune cells. This is yet another reason to avoid using pharmacological doses of synthetic calcitriol in infections or to overcome autoimmune conditions. It can only add adverse effects, making the situation worse. The correct approach is to provide appropriate higher amounts of the precursor—vitamin D (including upfront loading doses between 100,000 and 400,000 IU as a stat dose, if indicated) [14]. The better option is the oral administration of calcifediol, especially in emergencies [3][4].

Intracellular calcitriol is critical for both modulating genomic [15] and non-genomic activities such as signal transduction [16][17]. The non-genomic functions include the tightening of the gap-junctions [18] and autocrine (intracrine) and paracrine signaling [9][10][4]. Initiating intracrine signaling following the detection of foreign proteins, microbes, etc., by a series of cell surface receptors. The most important detection and signaling mechanism is the membrane-bound (sensing/detecting) TLR-4 [19], which is also involved in the production of antimicrobial peptides [20]. Intermittent signals derived from TLR-4 lead to transient, over-drive production of "calcitriol and VDR" in mitochondria/microsomes [21] (see below for details).

Immune cells do not have active (energy-dependent) cell-membrane transportation mechanisms, such as megalin–cubulin; only diffusible low concentrations of calcitriol get into immune cells from circulation. Consequently, in addition to a smaller quantity via endocytosis, only the diffusible calcitriol can enter immune cells from the circulation. The estimated intracellular calcitriol concentration in active status exceeds 1 ng/mL—an estimated minimum intracellular concentration needed for initiating immune cell functions. However, the average circulating concentration of hormonal calcitriol is approximately 0.045 ng/mL [12]. The estimated diffusible calcitriol into peripheral cells is about 0.023 ng/mL, less than the 40-fold minimum concentration required to initiate intracellular signaling [22]. Therefore, at equilibrium conditions in immune cells, the free hormonal form of calcitriol diffusing into immune cells (~0.02 ng/mL) is too little to activate their functions, such as intracellular signaling or binding with VDR, leading to gene transcription [12].

This is another reason not to administer pharmacological doses of calcitriol (e.g., one or more micrograms/day), as these would not enter immune cells. Unsurprisingly, several clinical studies on infectious diseases have reported a

lack of beneficial effects, including failed RCTs in SARS-CoV-2 [23][24]. The exceptions for using calcitriol include calcitriol/VDR-resistant syndromes, hypoparathyroidism, and chronic renal failure, where exogenous calcitriol is lifesaving [25].

3. Intracellular Calcitriol Signaling

When external threats such as circulating antigens are detected by immune cell membrane receptors—key innate immunity pattern recognition receptors such as membrane-bound TLR-4 [26], they send signals to microsomal-apparatus to increase the expression of 1α-hydroxylase and VDR [12], to increase cytoplasmic concentrations of both [7][22]. As a result, immune cells synthesize higher (nmol range) concentrations of non-hormonal calcitriol and VDR *in situ* [2][12]. This crucial step results in generating peaks of calcitriol in the cytosol, driving autocrine and paracrine signaling [1][9][10][22] and calcitriol/VDR complexes (not from the circulatory hormonal calcitriol) when needed [4][27][28]. As described above, note that calcitriol does not enter meaningful amounts into peripheral target cells.

This provides a physiologically balanced intracellular autocrine/intracrine and paracrine signaling, essential for robust immune cell functions [12]. This critical early warning TLR-detection system evolved to identify and overcome threats from infection (or foreign antigens) and autoimmune responses. It is noteworthy that, since this is also a threshold mechanism, further increasing serum 25(OH)D concentrations (i.e., beyond 60 ng/mL) would not produce additional beneficial immune cell functions from an infection's point of view.

When no external signaling exists, calcitriol and VDR synthesizing revert to a baseline steady state, curtailing intracellular signaling. This is an efficient evolutionary mechanism to stimulate immune cells as needed, intermittently, as needed—when an external threat—detecting unfamiliar (foreign) proteins or antigens in the circulation or local tissues. This sporadic phenomenon ensures the formation of sufficient calcitriol-VDR complexes to modulate gene transcriptions and calcitriol for intra-cellular autocrine regulations when needed and enough intracellular concentration of calcitriol for internal signaling, as described in the next section.

4. The Importance of Autocrine and Paracrine Signaling for Immune Cell Functions

TLR-4-mediated calcitriol synthesized within the immune cells also enhances the expression of anti-microbial peptides and antibodies [29][30]. The exact mechanism of stimulation of these pathways is unclear, but it is known to involve transcription factors C/EBPβ and the inhibition of NR4A2, an orphan receptor [31]. The regulation of the CYP27B1 gene (1α-hydroxylase enzyme) by a transcription factor promoter, NR4A2, is inhibited by C/EBP-beta. Furthermore, over-expression of C/EBP-beta decreases NR4A2 and CYP27B1 mRNA levels [31].

In contrast, FGF-23 counteracts the activity of the 1α-hydroxylase enzyme through FGF receptors in the presence of the co-receptor (an aging-related factor), Klotho [32]. The ablation of Klotho leads to over-expression of FGF23,

which is consistent with Klotho deficiency [32]. This signaling also activates the mitogen-activated protein kinase (MAPK), but its role in CYP27B1 expression remains unclear [33].

When the circulating D and 25(OH)D are low and at insufficient concentrations to enter immune cells, it hinders the generation of intracellular calcitriol. One key example of calcitriol intracrine signaling is switching T helper cell 1 (Th1) to T helper cell 2 (Th2) and Th17 to Treg cells, which transforms pro-inflammatory status to anti-inflammatory status [9][10]. This maintains the inflammatory statutes of Th1 and Th17 cells; severe viral infections such as SARS-CoV-2 in vulnerable people could initiate cytokine storms and the development of ARDS [34][35].

5. Mechanisms of Decreasing Inflammation with Vitamin D Adequacy

Vitamin D has anti-inflammatory, anti-oxidant, and anti-mitotic actions. In addition, it stabilizes endothelial cells and improves smooth muscle cell functions. This is highlighted by the reported statistically significant inverse relationship between serum 25(OH)D concentrations less than 21 ng/mL and higher serum C-reactive protein (CRP) levels (an inflammatory marker) [36]. Further evidence for vitamin D is an essential anti-inflammatory effect of vitamin D in humans. Such chronic inflammation increases the risks for multiple diseases, including myocardial infarction, strokes, and deterioration of renal and pulmonary functions. In contrast, vitamin D adequacy suppresses this generalized inflammatory effect, thus reducing the risks for cardiovascular diseases [37][38].

Vitamin D signaling decreases inflammatory responses, including reduction of the expression of pro-inflammatory cytokine and mediators (e.g., cyclooxygenases; 5-lipoxygenase), as demonstrated by genome- and transcriptome-wide studies. It also modulates transcription factors, such as the nuclear factor kappa light-chain (NF-κB) of activated B cells that regulate inflammatory gene expression and reduce mitogen-activated protein kinases' activation [39][40]. Calcitriol also downregulates cytokine production and the biosynthesis of pro-inflammatory cytokines in the prostaglandin pathway and through NF-κB [40]. These actions explain a strong association between low serum 25(OH)D concentrations and the many inflammatory diseases mentioned. Despite these findings, no vitamin D or analog has been used in adequately powered RCTs to test efficacy in controlling inflammatory conditions [40][41][42].

Increased local generation of calcitriol has been reported in those with diabetic foot ulcers. This is considered a physiological response to chronic inflammation and an attempt to enhance immunity in local tissues to combat infections [43][44]. However, such ongoing inflammation (in this case, vitamin D deficiency-induced) will have generalized negative effects and increase risks for myocardial infarction and stroke as described above. Moreover, an increased intake of micronutrients during high stress is known to reduce inflammatory processes and plasma lipids with favorable clinical outcomes [45]. This may also have clinical relevance for not only those with diabetic foot ulcers but also other chronic infections and conditions. These data support vitamin D's important immunomodulatory and anti-inflammatory roles [44]. Examples of these are discussed below.

6. Anti-Microbial Activities of Vitamin D

Mycobacteria and/or activation of macrophages leads to enhanced intracellular 1α-hydroxylase activity in macrophages (e.g., in granulomatous tissues), leading to the generation of $1,25(OH)_2D_3$. These increases in intracellular calcitriol accompany the increasing expression of the VDR in macrophages., as a defense mechanism in those with sufficient vitamin D status to overcome infections and autoimmune reactions. Since this activity is not subjected to feedback control, if not identified intervened in a smaller number of patients, it may increase serum concentrations of $1,25(OH)_2D_3$, leading to (granuloma-related) hypercalcemia.

The seasonal peaks of influenza/flu have been attributed to a higher prevalence of vitamin D deficiency during winter [46]. Thus, persons with hypovitaminosis D are more susceptible to viral infections, especially respiratory viruses [47]. During winter, with the colder and dryer weather, viruses live longer, thus increasing their virulence. This is supported by meta-analyses and several RCTs, such as in postmenopausal women with a baseline mean 25(OH)D concentration of 48 nmol/L [48] and the other was in a group of schoolchildren in Japan with low serum vitamin D [49]. In the latter, daily supplementation with 1000 IU of vitamin D_3 significantly reduced the risks of type-A influenza by two-thirds but did not affect type-B influenza. These RCTs are conducted before the current knowledge of the requirement of higher serum 25(OH)D concentrations, such as over 50 ng/mL, to have robust immune systems to overcome infections [3][4].

In addition, a meta-analysis of 11 RCTs on vitamin D supplementation concluded that once-daily dosing with vitamin D supplements had a significantly better response rate than did intermittent dosing regimens, such as monthly dosing (odds ratio = 0.51 vs. 0.86; $p = 0.01$) [50]. Those who experience pneumonia also had an increased prevalence of vitamin D deficiency [51]. Low serum 25(OH)D concentration is associated with low cellular immune functions and an increased risk for hyponatremia, as reported with H7N9 pneumonia [52]. However, the relationship between these two entities is unknown, and not all RCTs support this concept [53][54].

7. Vitamin D Enhances the Expression of Bactericidal Proteins

All immune cells, including T cells and macrophages, have a high concentration of VDRs [55][56]. Vitamin D—receptor interactions increase the expression of potent bactericidal and viricidal protein cathelicidin, which combats mycobacterium organisms, such as tuberculosis and lepra, and other intracellular bacteria [57]. In addition to cathelicidin, VDR activation increases the synthesis and secretion of multiple other bactericidal peptides, including defensins [58][59]. Thus, calcitriol adequacy, while reducing the expression of inflammatory cytokines, also increases the secretion of bactericidal peptides *in vivo* [37][60], complementing the immune system to combat invading microbes.

Although individual studies indicate positive results, when data from multiple microbiological studies are pooled, the results may not always show a positive direction. This is mostly due to diluting the data by poorly designed studies and the heterogeneity of clinical studies used in meta-analyses and selection biases. Inclusion of studies that differ

concerning population, taking cut-off limit varying from 20 to 40 ng/mL to compare groups, ethnicity, age range, types of infections, severity, study design, and duration, mode of observation or randomizations, and marked variation of baseline serum 25(OH)D concentrations (consisting some subjects with vitamin d deficiency and others with sufficiency), the failure to measure the serum 25(OH)D concentrations achieved, and using the dose provided rather than the serum 25(OH)D levels for statistical correlations have muddled the situation and publish inaccurate conclusions.

Vitamin D reduces risks for and the spread of chronic infections [61][62][63], particularly mycobacterium tuberculosis, by regulating innate and adaptive immunity. Sufficient amounts of intracellular calcitriol in immune cells augment innate responses (monocytes/macrophages with anti-microbial activity) and suppress adaptive immunity (T- and B-lymphocyte activities) [64]. In addition, calcitriol modulates B lymphocytes, immunoglobulin production, and B-cell homeostasis [64].

As described above, because of the variability of studies and poor study designs, vitamin D dosing, and recruitment, the pooled RCT data from a vitamin D, many meta-analyses cannot be relied upon [65]. Therefore, to generate meaningful conclusions, future RCTs should be focused on "subjects with documented vitamin D deficiency measured at recruitment to confirm low serum 25(OH)D concentration and subjects in the treatment arm provided with adequate vitamin D supplements to achieve a predefined target serum 25(OH)D concentration (but prohibited from taking over the counter nutrients in both arms)," and standardized measurable hard outcomes.

8. Multiple Sclerosis and Autoimmune Encephalomyelitis

Without supplements, serum 25(OH)D concentrations can be a reliable surrogate marker of UVB exposure. However, there are additional, non-vitamin D-related beneficial effects of UVB exposure, such as reductions in the severity of depression and the risk of MS [66][67][68][69], of which mechanisms are ill-understood. In addition, exposure to UVB potentiates the suppression of experimentally induced autoimmune encephalomyelitis in animal models [70][71]. In people with MS, low 25(OH)D concentrations are an independent, positive predictor of disease progression [72]. Furthermore, a better response has been reported with interferon beta (IFN beta) in those with higher serum 25(OH)D concentrations [73][74].

In persons with MS, serum adipocytokine concentrations are positively correlated with inflammatory mediators and negatively correlated with Foxp3 expression [75]. In that study, positive correlations were also reported between leptin and resistin concentrations with TNF-alpha and interleukin 1β (IL-1β), with the highest levels of TNF-alpha, IL-1β, CRP, resistin, and leptin reported in persons with progressive MS [75]; some of these are positively modulated by calcitriol.

Overall data suggest a clinically meaningful suppression of autoimmune disorders when serum 25(OH)D concentrations are maintained at greater than 40 ng/mL [32][76][77], preferably over 50 ng/mL (range 50 to 80 ng/mL). Supporting this, a longitudinal, prospective observational study by the author demonstrated that in those

with chronic MS (n = 64), keeping serum 25(OH)D concentrations above 40 ng/mL over an average 2-year period resulted in an 80% reduction in recurrences (i.e., reactivation rate) [32].

9. Conclusion

During the past decade, many advances have been made in understanding the physiology and biology of vitamin D, and its receptor ecology has emerged. Evidence supports strong physiological associations of vitamin D with disease risk reduction and improved physical and mental functions. Together, these data have facilitated our understanding of new pathways for intervention to prevent and treat human diseases. Recent epidemiological, cross-sectional, and longitudinal studies support the idea that having physiological serum concentrations of 25(OH)D, levels greater than 40 ng/mL, significantly reduces the incidence of extra-musculoskeletal disorders. The latter includes diabetes [78][79][80], MS [81], rheumatoid arthritis [82], osteoporosis [83][84], autoimmune diseases [85], and certain types of cancer [86][87][88][89], as well as reducing all-cause mortality [90]. The proper functioning of the vitamin D endocrine, paracrine, and autocrine systems is essential for many physiological activities and maintaining good health.

The dosages of vitamin D prescribed for non-obese deficient persons of average weight of 70 kg should be between 4,000 and 7,000 IU/day, 20,000 IU once or twice a week, or 50,000 IU once or once in 10 days [2]. Such doses would allow approximately 97.5% of people to maintain their serum 25(OH)D concentrations above 40 ng/mL [76][91][92]. However, intermittent doses at intervals longer than once a month are unphysiological and thus ineffective [93][94]. Studies have shown that daily vitamin D supplements are more beneficial than supplementation administered less frequently [95][96][97][98][99].

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