REVIEW

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The Active Metabolite of Vitamin D_3 as a Potential Immunomodulator

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Abstract

In the past, vitamin D was known for its classical, skeletal action as a regulator of calcium and bone homoeostasis. Currently, vitamin D was found to have a role in numerous physiological processes in the human body; thus, vitamin D has pleiotropic activity. The studies carried out in the past two decades showed the role of vitamin D in the regulation of immune system functions. Basically, these effects may be mediated not only *via* endocrine mechanism of circulating calcitriol but also *via* paracrine one (based on cell–cell communication that leads to production of signal inducing the changes in nearby/adjacent cells and modulating their differentiation or behaviour) and intracrine mechanism (the action of vitamin D inside a cell) of 1,25-dihydroxycholecalciferol (1,25 $(OH)_2D_3$) synthetized from its precursor 25-hydroxyvitamin D_3 ($25(OH)D_3$). Both vitamin D receptor (VDR) and 25-hydroxyvitamin D_3 1-α-hydroxylase (CYP27B1) are expressed in several types of immune cells (i.e. antigen presenting cells, T and B cells), and thus, they are able to synthetize the bioactive form of vitamin D that modulates both the innate and adaptive immune system. This review discusses the role of vitamin D as regulator of immune system, and our understanding of how vitamin D regulates both adaptive and innate immunity as well as inflammatory cascade on the cellular level.

Introduction

Vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) are two major physiological forms of vitamin D. In humans, the main source of vitamin D is the endogenous photosynthesis from the 7-dehydrocholesterol – pro-vitamin D_3 in the epidermis (only cholecalciferol) rather than absorption from the diet (ergocalciferol and cholecalciferol). The ergocalciferol from pro-vitamin D_2 is synthetized only in yeast, fungi and plants under action of sunlight [1]. Vitamin D_3 is more effective than vitamin D_2 in maintaining 25-hydroxyvitamin D_3 ($25(OH)D_3$) serum level – vitamin D status marker and circulating form of vitamin D in the human body [2, 3]. Additionally, vitamin D_3 binds better to vitamin D receptor (VDR) than vitamin D_2 [4].

After photosynthesis in the skin, vitamin D_3 25-hydroxylase metabolizes vitamin D_3 to $25(OH)D_3$ in the liver. The circulating form of vitamin D_3 is then transported to the proximal tubules of the kidney and is hydroxylated by 25-hydroxyvitamin D_3 1-α-hydroxylase (CYP27B1) to 1,25-dihydroxycholecalciferol (1,25 $(OH)_2D_3$), calcitriol [5]. The biologically active form of vitamin D_3 -1,25$(OH)_2D_3$ is capable of binding and activating VDR. This receptor belongs to superfamily of nuclear receptors and acts as ligand-activated transcription factor [6]. Complex of 1,25$(OH)_2D_3$-VDR forms heterodimer with the retinoid receptor (RXR) and induces the expression of vitamin D responsive genes *via* binding the heterodimer to vitamin D responsive elements (VDRE) in the promoter region of these genes [7].

Nowadays, apart from classical action of vitamin D, numerous non-classical, non-skeletal activities have been established. Vitamin D hormonal, classical actions are related to stimulation of osteoclasts' differentiation, enhancement of the absorption of calcium and phosphorus from the intestines as well as reabsorption of calcium from the bones and promotion of the bone matrix mineralization [8]. One of the non-classical action of vitamin D activities is its influence on the immune system [9]. Initially, it was observed that monocytes/macrophages derived from patients with granulomatous disease are capable of synthetizing 1,25$(OH)_2D_3$ from its precursor – $25(OH)D_3$ [10]. Currently, the immune responsiveness determinant role of 1,25$(OH)_2D_3$ is better known. Calcitriol may modulate the immune system [9, 11–13] *via* endocrine, paracrine and intracrine mechanisms. The endocrine mechanism refers to the substances directly secreted into

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the bloodstream and may influence the activity of different organs. The paracrine mechanism is associated with the effects in nearby/adjacent cells while intracrine mechanism is related to hormone activities inside a cell [14, 15].

Vitamin D may also modulate host defence against foreign pathogens and antigens. This potential activity is based on four findings:

(1) The immune cells are capable of producing CYP27B1 and converting $25(OH)D_3$ into $1,25(OH)_2D_3$.
(2) VDR is expressed in majority of immune cells, especially after stimulation.
(3) Impaired status of vitamin D is a common problem and may contribute to various pathogen infections.
(4) A correct supplementation of vitamin D may prevent some infectious diseases [16–19].

Several clinical studies confirmed the role of vitamin D in the modulation of innate immune response [20]. Additionally, the recent studies showed that vitamin D may be a regulator of adaptive immune response in autoimmune and inflammatory diseases [21, 22].

The review provides a general summary of the role of vitamin D as an immunomodulator – a regulator of the immune system. It focuses on the effects of vitamin D on the immune system on the cellular level and mechanisms that link the action of vitamin D with the regulation of both innate and adaptive immunity as well as inflammatory cascade.

How does vitamin D regulate immunity?

The action of $1,25(OH)_2D_3$ is based on its binding to VDR. Then, VDR dimerizes with RXR and binds to VDRE in target genes [23–25]. Moreover, VDR-RXR heterodimers may replace the nuclear factors in activated T cells leading to inhibition of cytokine-related gene transcription [26].

Vitamin D is thought to have an influence on the human immunity. VDR is expressed in the majority of immune cells including B cells, $CD4^+$ as well as $CD8^+$ T cells, APCs (i.e. DCs, macrophages), neutrophils [27, 28]. Moreover, some of the immune cells (i.e. DCs, macrophages, T and B cells) may produce CYP27B1 – the vitamin D-activating enzyme, so they are capable of converting $25(OH)D_3$ into $1,25(OH)_2D_3$ *via* activity of CYP27B1 and providing physiologically significant local level of $1,25(OH)_2D_3$ [9, 29–31]. However, the level of $1,25(OH)_2D_3$ production in the immune cells is dependent on the level of CYP27B1 expression in these cells and various vitamin D enzymatic machinery pathway such as CYP24A1 – the enzyme responsible for inactivation of calcitriol. For instance, *in vitro*, it has been observed that stimulated macrophages are able to increase the amount of $1,25(OH)_2D_3$ production compared with DCs that expressed truncated transcript of CYP27B1; thus, DCs expressed lower level of CYP27B1 protein and increased CYP24A1 expression [32]. Vitamin D influence both innate and adaptive immune system. In addition, it is not associated only with the $1,25(OH)_2D_3$ classical, endocrine mechanism of action but also with the intracrine and paracrine mechanisms [17, 33].

Vitamin D and modulation of immunity

Vitamin D and cathelicidin production, innate immunity

Macrophages and monocytes play a crucial role in cell-mediated immunity, that is in the process of defence against pathogens such as *Mycobacterium tuberculosis*. The role of these innate immune cells is phagocytosis of pathogens and elimination or assimilation of waste minerals. The active metabolite of vitamin D may enhance the antibacterial effects *via* enhancement of their phagocytic and chemotaxis abilities [34, 35].

Early findings suggested that vitamin D may stimulate innate immunity and may be effective in the treatment of tuberculosis with cod liver oil [36]. Initially, it was thought that vitamin D stimulates the differentiation of monocytes to mature macrophages with phagocytic abilities [37–40]. Monocytes/macrophages and DCs are target cells of innate immunity that express VDR and CYP27B1 and are able to utilize $25(OH)D_3$ for intracrine activity leading to promotion of antibacterial response to pathogens [14] and sense the pathogen associated molecular pattern (PAMP) *via* toll like receptors (TLRs). The activation of TLRs leads to upregulation of VDR and CYP27B1 (i.e. the exposition of monocytes to *M. tuberculosis* activates TLR2 leading to upregulation of CYP27B1 and VDR). In turn, it contributes to induction of the cathelicidins (hCAPs) [12, 41] – the complex of $1,25(OH)_2D_3$+VDR+RXR induces transcription of genes encoding antimicrobial peptides, that is hCAP18 that is cut from LL-37, and defensin $\beta 2$ (DEFB) [14, 42, 43]. An antibacterial peptides – hCAPs are capable of killing of pathogens or binding to endotoxin *via* formation of ion channels and increasing of membrane permeability [44, 45]. Human DEFB is modestly upregulated by $1,25(OH)_2D_3$ which may be chemoattractant for monocytes and neutrophils and may trigger antiviral activities [46, 47]. Interestingly, the enhancement of $1,25(OH)_2D_3$-mediated DEFB stimulation was also shown as a consequence of the nuclear factor kappa B (NF-κB) induction following cytokine-treated (i.e. IL-1β) monocytes [48]. Despite the fact that the neutrophils granules store majority of hCAPs released at infection's sites, hCAP18 is expressed in some immune cells (i.e. NK cells, monocytes, B cells) [49]. Interaction of $1,25(OH)_2D_3$ with the promoter of hCAPs may lead to the enhancement of antibacterial potential [16] *via* promotion of microbial killing in phagocytic vacuoles [42], and thus, the

stimulation of hCAPs production enhances killing of *M. tuberculosis* [12]. Chen *et al.* showed that TLR signalling may be regulated by 1,25(OH)$_2$D$_3$ in macrophages by the mechanism stimulating SOCS1 *via* downregulated miR-155. It is a newly discovered mechanism of negative feedback regulation and control of innate immunity by vitamin D [50]. Additionally, not only TLR signalling but also cytokines including IL-4, IFN-ϒ may have influence on the CYP27B1 expression [51]. The presence of IFN-ϒ stimulates macrophage CYP27B1 [51, 52]. In contrast, Th2-produced IL-4 stimulates the catabolism of 25(OH)D$_3$ to its inactive metabolite – 24,25(OH)$_2$D$_3$ [51], and thus, the cell-mediated and the innate immune responses may be linked *via* the mechanism based on the vitamin D metabolism; however, the precise role of vitamin D in this process is not fully understood [53]. Moreover, the involvement of p38 MAP kinase, JAK-STAT as well as NF-κB pathway in stimulation of CYP27B1 expression in the presence IFN-ϒ or lipopolisaccharide (LPS) – TLR4 ligand, was shown in cultured monocytic cell lines [54].

Decidual cells, epithelial cells, trophoblasts respond to systemic 1,25(OH)$_2$D$_3$ and intracrine hydroxylation of 25 (OH)D$_3$ to stimulate antibacterial responses [55]. Interestingly, the calcitriol may also inhibit the expression of TLRs leading to decreased responsiveness to molecular cascades induced by pathogens. This is a negative feedback mechanism that self-inhibits excessive activation of TLRs and inflammation in further stages of infections [56].

Besides the hCAP activities against *M. tuberculosis*, these peptides also have activities against other bacteria and viruses. During the viral infections, the lung epithelial cells are capable of converting inactivate vitamin D to its active form leading to increased hCAP production [57].

However, a sufficient level of 25(OH)D$_3$ supporting intracrine 1,25(OH)$_2$D$_3$ production is necessary to enhance macrophages functions and hCAP activation [55]. The enhancement of monocyte-driven response of innate immunity to infection may be related to variations of vitamin D status. It was shown that the level of LL-37 production following activation of TLR 2/1 is dependent on vitamin D status variation [12]. *In vivo*, the supplementation with vitamin D in vitamin D- insufficient individuals was found to improve monocyte TLR-mediated induction of hCAPs [58] and protection against infection.

Vitamin D also promotes bacteria killing by monocytes. 1,25(OH)$_2$D$_3$ treatment of monocytes elevates the level of autophagy [59]. The autophagy and process of autophagosome formation are crucial for pathogen isolation in the cells and combating pathogens by antibacterial proteins [60].

Lately, hCAPs and DEFB have been found to act as vitamin D-regulated antimicrobial peptides but also as hepcidin – protein that is a key modulator of iron distribution in tissue *via* inhibiting export of cellular iron mediated by ferroportin. It was shown that hepcidin expression in cultured monocytes and hepatocytes may be downregulated by both 25(OH)D$_3$ and 1,25(OH)$_2$D$_3$ [61]. It may be a tool against intracellular pathogens. Downregulation of hepcidin expression reduces concentration of iron in the cell by increasing iron export. This mechanism may limit the proliferation of pathogens in the cell as the growth and survival of bacteria depend on iron. However, the regulation of extracellular iron may have an opposite effect in controlling systemic infections [62, 63].

The number of innate granulocytic cells including neutrophils is high in severe infection. Neutrophils may be a source of hCAPs [64]. However, the CYP27B1 expression has not been shown in the neutrophils, and thus, these cells are not able to convert 25(OH)D$_3$ into 1,25(OH)$_2$D$_3$ but may be regulated *via* endocrine effect of calcitriol synthetized by kidneys [65].

Vitamin D and DCs, antigen presentation, innate immunity

Despite proper management of intracellular bacteria by innate immunity for infection management, the adequate activity of the acquired or adaptive immunity is required. The innate immunity and adaptive immunity are linked by action of APCs – monocytes/macrophages and DCs derived from one haematopoietic lineage. APCs have a crucial role in the process of the adaptive immune response initiation as they are responsible for the presentation of antigens to T cells. Moreover, APCs are capable of modulating B and T cells by tolerogenic or immunogenic signals, that is cytokines and co-stimulatory molecules expression; these cells are responsible for promotion of T cell and B cell responses. APCs are also targets for immunomodulatory action of vitamin D [66–68].

VDR and CYP27B1 are expressed both in monocytes and DCs, so active intracrine fashion of vitamin D action is exhibited in these cells [69–71].

However, antigen presentation is fulfilled more effectively by DCs than macrophages [72]. In DCs, vitamin D may inhibit process of maturation and contribute to modulation of CD4$^+$ T cell function *via* intracrine mechanism. The response of T helper (Th) cells may be also modulated by action of 1,25(OH)$_2$D$_3$ on VDR expressed CD4$^+$ T cells in paracrine mechanism. Additionally, the CD4$^+$ T cells may be targets for the systemic, biologically active vitamin D [33].

Several studies demonstrated that bioactive form of vitamin D and its analogs may modify morphology and function of DCs through suppression of DCs maturation and promotion of tolerogenic phenotype [29, 34, 73–75]. Interestingly, these effects were more visible in mDCs than pDCs despite similar level of VDR expression in both types of DCs. *In vitro*, 1,25(OH)$_2$D$_3$ may suppress activation of naïve T cells *via* regulation of mDCs function. However, tolerogenic pDCs may respond to 1,25(OH)$_2$D$_3$ *via* local, intracrine mechanism as the pattern of VDR expression is

similar in both mDCs and pDCs [73]. Alternatively, 1,25 $(OH)_2D_3$ produced by pDCs may not regulate DC maturation but may act on T cells expressed VDR *via* paracrine fashion [63].

Both native and active vitamin D, cholecalciferol and calcitriol induce tolerogenic properties in DCs because CYP27B1 is expressed in these cells, and thus, the local high concentration of $1,25(OH)_2D_3$ is necessary for immunomodulatory effect [34]. The importance of immunomodulatory role of $1,25(OH)_2D_3$ as immunomodulator of DCs function was confirmed in the studies carried out on the CYP27B1 and VDR knockout mice that showed increasing number of mature DCs, abnormal chemotaxis of DCs and abnormal DCs trafficking [76–78].

The reduced antigen presentation and decreased secretion of Il-12 as well as increased generation of tolerogenic IL-10 in immature DCs is a result of decreased expression of co-stimulatory molecules (i.e. CD-40, 80, 86) and MHC class II in these cells [29, 34].

Opposite to monocytes/macrophages, the primary intracrine function of vitamin D in DCs is the regulation of cell maturation and the cells ability of antigen presentation to T cells [70]. Paradoxically, the process of DCs differentiation to mature APCs is related to simultaneously increased expression of CYP27B1 and decreased VDR expression [70]. DCs may utilize paracrine mechanism of vitamin D action because immature VDR-expressing DCs respond to $1,25(OH)_2D_3$ produced by mature DCs with decreased VDR expression. This mechanism allows maturation of some DCs and promotion of T cell activation. It may be considered as part of adaptive immune responses although it prevents an excessive response leading to inflammatory complications. A similar fashion of differential regulation of VDR and CYP27B1 was observed for differentiation of monocytes towards macrophages [41].

Adaptive immunity and vitamin D

The nuclear VDR and CYP27B1 expression has already been described in B and T cells [28]. The expression of VDR by resting B and T cells is very low while in activation and proliferation, the expression of VDR is significantly upregulated by these cells. It permits modulation of approximately 500 genes which influence proliferation and differentiation of adaptive immunity cells [11, 79, 80]. The tissue-specific production of 1,25 $(OH)_2D_3$ is essential for the immune response of B and T cells.

T cells

DCs maturation is inhibited *via* intracrine mechanism of $25(OH)D_3$ activation which allows induction and modulation of initial $CD4^+$ T cell response [70]; calcitriol is an inhibitor of T cell proliferation [81]. Additionally, the calcitriol signalling suppresses the transcription of genes that encode Th-1 cytokines (i.e. INF-γ) [80, 82] and Th17 cytokines (i.e. IL-17) [13] to achieve the polarization of the $CD4^+$ T cells response to regulatory T cells (Treg) [9, 83] or regulatory Th2 [84] phenotypes. Both phenotypes are vital for vitamin D ability to inhibit Th1-mediated autoimmunity response [81, 83]. Jeffrey *et al.* also demonstrated that $1,25(OH)_2D_3$ suppresses generation of proinflammatory cytokines including IL-17, IL-21, INF-γ as well as stimulates the development of Treg with expression of cytotoxic T-lymphocyte antigen 4 and FOXP3 [85].

Moreover, Th17 cells play an important role in the defence against certain pathogens including *Helicobacter pylori*, *M. tuberculosis*, *Candida albicans*, *Klebsiella pneumoniae* and *Staphyloccocus* which are related to inflammation and damage of tissue [86]. The particular role vitamin D plays in the regulation of Th17 cells is not completely clarified [9]. The paracrine mechanism is responsible for the response of $CD4^+$ T cells to $25(OH)D_3$ -$1,25(OH)_2D_3$ produced *via* DCs acting on the VDR expressed in $CD4^+$ T cells. It seems that the bioactive metabolite of vitamin D balances self-tolerance *via* suppression of excessive response of adaptive immune system and enhancement of response of protective innate immune system [13]. However, the correlation between the vitamin D status variations on the T cell functions is still not fully understood. The following effects of $1,25(OH)_2D_3$ on the T cells may be distinguished:

(1) the endocrine, direct effects of systemic 1,25 $(OH)_2D_3$ on T cells
(2) the indirect effects through intracrine production of $1,25(OH)_2D_3$ by CYP27B1-expressed DCs
(3) the paracrine, direct effects through $1,25(OH)_2D_3$-produced by DCs or monocytes (CYP27B1 expressed cells)
(4) direct, intracrine effects *via* abilities of T cells to conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$

The exposure to vitamin D causes alteration of proinflammatory immune status to more tolerogenic pattern with different effects on T cell subtypes (i.e. inhibiting differentiation, proliferation of Th cells and modulating cytokine production by these cells) [87]. The exposure of T cells on calcitriol or its analogs leads to suppression of proinflammatory: Th1 (i.e. INF-γ, IL-2), Th-9 (IL-9) as well as Th-22 (IL-22) cytokine production [71, 88–92] and promotion of anti-inflammatory Th2 (i.e. IL-3, -4, -5 -10) cytokine secretion [93]. It has been proposed that vitamin D may be used as an adjunct to anti-inflammatory therapy in Th2-mediated disease – asthma. The studies carried out in the murine models showed that pretreatment with 1,25 $(OH)_2D_3$ strengthens the immunotherapy effects of inhibiting allergic airway inflammation [94–96]. Moreover, dexamethasone with different concentrations of $1,25(OH)_2D_3$

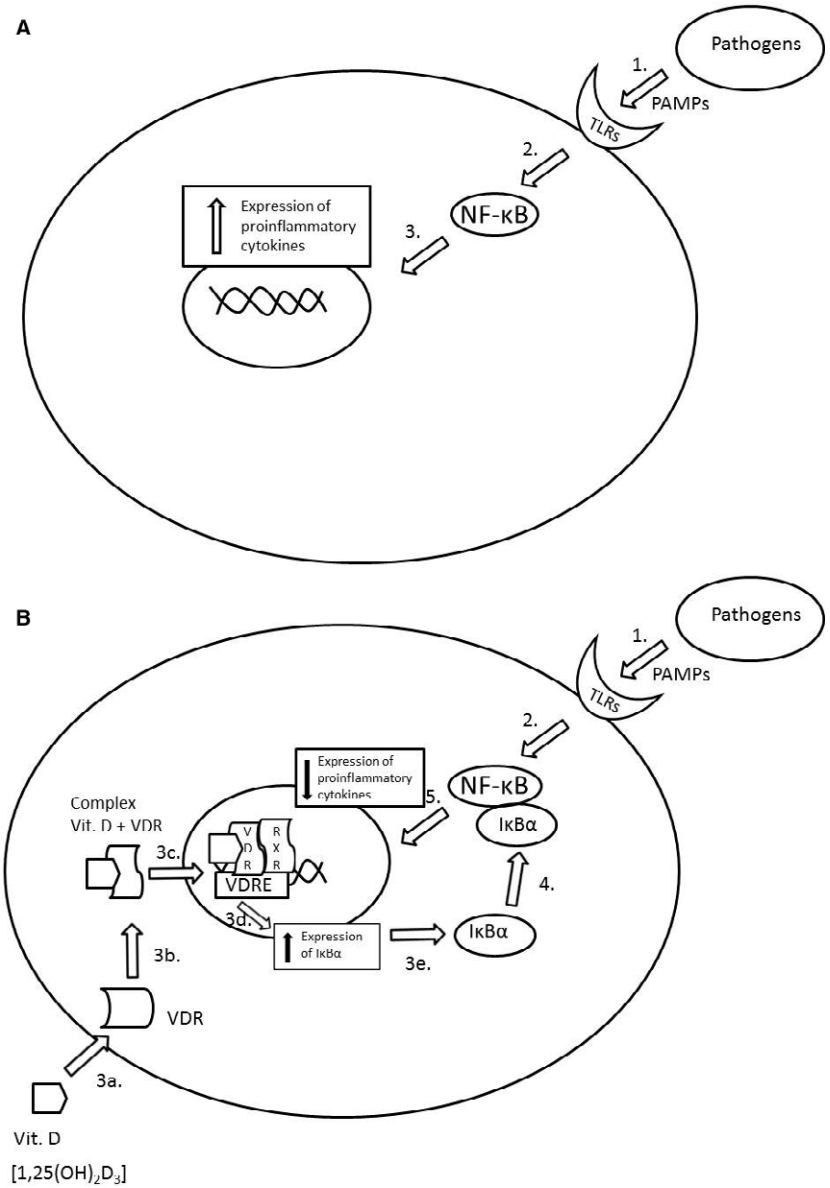

Figure 1 The NF-κB-related inflammatory cascade: (A) NF-κB pathway upregulates expression of proinflammatory cytokines: 1 – PAMPs activate TLRs on various types of immune cells; 2 – various PAMPs-activated TLRs signalling pathway induces NF-κB pathway; 3 - NF-κB pathway upregulates expression of proinflammatory cytokines. (B) Vitamin D downregulates proinflammatory cytokine expression: 1 – PAMPs-mediated activation of TLRs on various types of immune cells; 2 – various PAMPs-activated TLRs signalling pathway induces NF-κB pathway; 3 – Vit. D [1,25(OH)$_2$D$_3$] upregulates IκBα: 3a. Vit. D acts on the VDR; 3b. – Vit. D binds to VDR; 3c. – Vit. D + VDR complex binds to VDRE in DNA; 3d. – increased expression of IκBα; 3e. – increased IκBα level; 4 – IκBα attaches to NF-κB leading to decreased expression of NF-κB pathway; 5 – decrease in NF-κB pathway leading to decreased expression of proinflammatory cytokines.

acts synergistically and increase Th2 cytokines (i.e. Il-5, -13) and decrease Th-1 cytokines (i.e. IFN-ϒ) in comparison with dexamethasone alone [97]. In response to dexamethasone, Tregs of steroid resistant asthma patients produce less anti-inflammatory cytokine IL-10. *In vitro* studies on CD4$^+$ T cells in patients with steroid resistant asthma demonstrated that the addition of 1,25(OH)$_2$D$_3$ may strengthen the response to glucocorticoids by restoration of the impaired IL-10 response induced by steroids [98].

However, the direct effect of bioactive metabolite of vitamin D on the differentiation and function of naïve T cells is still not fully clarified [9, 17]. Although the expression of VDR in the naïve T cells is very small or even non-existent, the recent data showed that 1,25(OH)$_2$D$_3$ may directly modulate the TCR signalling [99, 100]. Complex pathway leads through the alternative mitogen-activated protein kinase p38 to inducing VDR and phospholipase C gamma 1 (PLA-γ1). Both VDR and

PLA-γ1 are essential to TCR signalling and activation of T cells [17].

B cells

VDR is expressed in human B cells and may be upregulated by activation of B cells. The results of recent *in vitro* studies showed that B cells are capable of intracrine response to bioactive metabolite of vitamin D. Initially, the antiproliferative effects of $1,25(OH)_2D_3$ (i.e. stimulation of apoptosis, suppression of proliferation and differentiation, decreased production of immunoglobulin) in B cells were thought to be indirectly driven by Th cells [79]. Latest studies showed more direct effects of $1,25(OH)_2D_3$ on homoeostasis of B cells, that is apoptosis promotion of immunoglobulin producing B cells, suppression of generation of plasma and memory cells [11, 29, 34].

The bioactive form of vitamin D regulates expression of VDR in B cells so calcitriol may have different influence on both resting and activated B cells and individuals with different $1,25(OH)_2D_3$ level in serum [11]. Additionally, the appropriate threshold of VDR engagement is required for inhibitory effect of the bioactive metabolite of vitamin D mediated by VDR regulation to appear. The expression of CYP27B1 was also found in resting B cells. It may not be further induced by $1,25(OH)_2D_3$ but by stimulation of these cells. Interestingly, the significant upregulation of CYP24A1 was observed in human B cells after the incubation with $1,25(OH)_2D_3$. It seems that vitamin D influences B cells not only *via* expression of VDR but also degradation of active molecules. However, following the activation of human B cells, no alteration of CYP24B1 and VDR expression was found. Thus, these cells are capable of directly responding to the bioactive metabolite of vitamin D. The upregulation of VDR may be reflected by increase of activated B cells susceptibility to many $1,25(OH)_2D_3$ effects [11]. High concentration of vitamin D precursor-$25(OH)D_3$ and bioactive form of vitamin D had similar effects on the purified B cells [9]. Indeed, $1,25(OH)_2D_3$ suppresses ongoing B cells proliferation and modulates B cell response. However, its effects on plasma and memory cell differentiation are the result of inhibition of ongoing B cell differentiation [10]. The process is necessary before differentiation stages [101]. These effects are essential in expanded B cell activation (i.e. systemic lupus erythematosus) [102]. Other $1,25(OH)_2D_3$-modulated targets of B cells are CCR10 [103] and IL-10 [104]. It suggests that response of B cells to vitamin D is extended by regulation of mucosal immunity [13, 71] and allergic immune response [105].

Vitamin D and the inflammatory response

Vitamin D modulates NF-κB pathway leading to regulation of the inflammatory cascade. PAMPs of protozoa, viruses, bacteria and fungi comprise flagellin, viral RNA, bacteria DNA, lipoproteins and LPS. PAMPs may activate TLRs on various types of immune cells. Different TLR signalling activated by PAMPs induces the NF-κB pathway leading to upregulation of pro-inflammatory cytokine expression (Fig. 1A). Inhibitory proteins such as IκB are the regulators of NF-κB [106]. The upregulation of IκBα leads to decrease in NF-κB signalling *via* attaching of IκBα to NF-κB subunits and decrease in proinflammatory cytokines (Fig. 1B) which is observed in the airway epithelium in conditions during viral infection [107]. The incubation of cystic fibrosis respiratory epithelial cells with *Pseudomonas* LPS showed vitamin D-mediated upregulation of IκBα. Moreover, the total level of cellular IκBα increased in the cells treated with vitamin D, and thus, the level of pro-inflammatory cytokines produced *via* activation of NF-κB was reduced [108]. Figure 1B shows the mechanism of vitamin D-mediated upregulation of IκBα expression and decreasing proinflammatory cytokine expression.

Conclusion

Vitamin D is a crucial player in regulation of calcium and bone homoeostasis. The review showed that immune cells of both immune system arms are targets for the bioactive form of vitamin D and may activate circulating $25(OH)D_3$ which indicates not only classical endocrine but also paracrine and intracrine mechanisms of vitamin D activity. The pivotal concept is that the bioactive form of vitamin D may stimulate innate immunity and suppress adaptive immunity.

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