VITAMIN D3: RESEARCH BREAKTHROUGHS AND THERAPEUTIC USE

Pohorila M.S., Martynov A.V., Romanova E.A., Igumnova N.I., Sidorenko T.A., Yukhimenko V.I., Shcherbak O.M.

Mechnikov Institute of Microbiology and Immunology

Introduction

Vitamin D3 (cholecalciferol), the natural form of vitamin D, is produced in the skin from 7-dehydrocholesterol. Upon irradiation, 7-dehydrocholesterol produces pre-vitamin D3 which undergoes a temperature-sensitive rearrangement of three double bonds to form vitamin D_3. The synthesis of vitamin D in the skin is the most important source of vitamin D. Vitamin D can also be taken through nutrition, in the diet, but it is present in only a few food sources, such as little number of fish species, containing relevant levels of vitamin D. Vegetarian diet is limited to the plant vitamin D_2 that is only present in some mushrooms. Commercially dark cultivated white button mushrooms contain low amounts of vitamin D_2, only wild mushrooms or sun-dried mushrooms contain elevated amounts of ergocalciferol [1].

Vitamin D_3 assumes its biological activity by binding with DBP (Vitamin D binding protein), which transports vitamin D and its metabolites from blood serum to the liver. Where vitamin D is hydroxylated at C-25 to produce its major circulating form – 25-hydroxyvitamin [2]. The 25-hydroxyvitamin D_3 needs to be further hydroxylated to form 1,25-dihydroxyvitamin D_3. Major sites for conversion of 25(OH)D3 to 1,25(OH)2D3 are kidney and placenta [3].

1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] is the hormonally active form of vitamin D. Novel researches show it generates a number of extraskeletal biological responses including inhibition of variety types cancer progression, effects on cardiovascular disorders and mediates a protection against a number of inflammatory, autoimmune and infection diseases [4].

The biological actions of $1,25(OH)_2D_3$ are mediated by the VDR. VDR is a polymorphic nuclear receptor and belongs to the steroid receptor family which includes receptors for retinoic acid, thyroid hormone, sex hormones, and adrenal steroids [5]. The heterogeneous loss of function mutations in the VDR caused the $1,25(OH)_2D_3$ organ resistance and leads to Hereditary vitamin D-resistant rickets [6]. The genomic mechanism of $1,25(OH)_2D_3$ action involves the direct binding of $1,25(OH)_2D_3$ activated VDR/RXR to specific DNA sequences [vitamin D response elements (VDREs)] in and around target genes resulting in either activation or repression of transcription [7]. In this way more than 1000 genes are regulated by Vitamin D through the VDR [8]. VDR modulates the expression of genes involved in immune function and cytokine production. The VDR and CYP27B1, the enzyme located in kidneys and target organs, are present in immune competent cells, bronchial and pulmonary epithelial cells, among others, and is up-regulated following the ligation of specific toll-like receptors by extracellular pathogens, implicating vitamin D in innate immunity [9-14].

By binding the VDR, calcitriol induces several endogenous antimicrobial peptides (AMP) in monocytes, neutrophils and epithelial cells including cathelicidin LL-37, α-defensin, β defensing and neutrophil gelatinase-associated lipocalin and up-regulates nitric oxide (NO) synthase [15-18]. AMPs inhibit infection caused by bacteria, viruses and fungi, while NO synthase augments bacterial killing by up-regulating the oxidative burst in activated macrophages [19-22]. Vitamin D may also induce a Th_2-based response, characterized by high Immunoglobulin IgE and eosinophilia, to combat extracellular infections caused by parasites, protozoa and fungi. In addition to its role in innate immunity, calcitriol suppresses pro-inflammatory cytokines *in vitro* and *in vivo*, and up-regulates anti-inflammatory cytokines, such as IL10 [23-27]. Since the inflammatory response associated with infections such influenza, pneumonia and sepsis increases both clinical severity and mortality, the ability to reduce inflammation may allow vitamin D to decrease mortality and disease burden in certain infections [28-31].

Notwithstanding the width of possible vitamin D application field, which being known now, large-scale clinical trials are still demanded.

Our review has the aim to summarize current scientific understanding of Vitamin D_3 effects on the immunological field with the focus on its capacity to enhance the anti-infection and anti-inflammatory immune reactivity.

Vitamin D – the innate and adoptive immunity modulator.

In a cross-sectional analysis serum 25(OH)D levels were found to be significantly lower in critically ill septic patients. This was associated with decreased concentrations of the antimicrobial protein cathelicidin [32]. This finding supports the theory that the vitamin D status regulates antimicrobial protein levels of innate immune cells and may be crucial in infection control. Dendritic cells (DC) are also important targets for the immune modulatory effects of vitamin D. Different studies have shown that calcitriol and its analogs can alter function and morphology of DC to induce a more tolerogenic, immature state [33-36]. Immature DC are characterized by decreased levels of MHC class II and co-stimulatory molecule expression (CD40, CD80, CD86), which leads to reduced antigen presentation accompanied by a lower IL12 secretion but an increased production of the tolerogenic interleukin IL10. Calcitriol has also been described to inhibit T cell cytokines such as IL2 and IL17 and toll like receptors on monocytes [37]. High-dose calcitriol supplementation in healthy humans (1 μg twice daily for 7 days) leads to a significant reduction of the proinflammatory cytokine IL6 produced by peripheral mononuclear cells [38]. It is likely that a combination of these effects results in the induction of potential regulatory T cells which are crucial for controlling immune responses [39]. *In vitro* data are also supported by results from VDR and CYP27B1 knockout

DOI: 10.5281/zenodo.1133763

mice which show significantly increased numbers of mature DC and abnormal DC chemotaxis [40].

Early studies of vitamin D effects on human adaptive immune cells demonstrated an expression of the nuclear VDR as well as vitamin D-activating enzymes in both T- and B cells [41]. Actually, VDR expression by these cells is very low in resting conditions but upon activation and proliferation, T- and B cells up-regulate VDR expression significantly, allowing regulation of up to 500 vitamin D responsive genes which influence differentiation and proliferation. In B cells, antiproliferative effects of vitamin D_3 such as inhibition of differentiation, proliferation, initiation of apoptosis and decreased immunoglobulin production were initially considered to be exclusively indirectly mediated by $CD4^+$ cells [42]. More recent studies confirmed additional direct effects of vitamin D_3 on B cell function, including inhibition of memory- and plasma-cell generation, as well as promotion of immunoglobulin-producing B cells apoptosis [43]. This control on B cell activation and proliferation may be clinically important in autoimmune diseases as B-cells producing autoreactive antibodies play a major role in the pathophysiology of autoimmunity.

T cells, is also thought to be an important target for the immune modulatory effects of vitamin D different forms. Four potential mechanisms by which vitamin D may influence T cell function have been proposed. There are direct, endocrine effects on T cells mediated via systemic calcitriol; direct, intracrine conversion of 25(OH)D to calcitriol by T cells; direct, paracrine effects of calcitriol on T cells following conversion of 25(OH)D to calcitriol by monocytes or dendritic cells and indirect effects on antigen presentation to T cells mediated via localized APC affected by calcitriol [44]. Vitamin D exposure leads to a shift from a proinflammatory to a more tolerogenic immune status, including very diverse effects on T cell subtypes. Thus, vitamin D_3 suppresses $CD4^+$ cell proliferation, differentiation and modulates their cytokine production [45]. In particular, treatment of T cells with calcitriol or analogs inhibits the secretion of proinflammatory Th1 (IL2, IFN-γ, TNFα), Th9 (IL9) and Th22 (IL22) cytokines [46-48], but promotes the production of more anti-inflammatory Th2 cytokines (IL3, IL4, IL5, IL10) [49]. IL17 producing Th17 cells are also affected by vitamin D. Recently, calcitriol was found to directly suppress IL17 production on a transcriptional level and activated human T-cells exposed to calcitriol produced significantly decreased levels of IL17, interferon-γ and IL21 [50, 51]. The same study also revealed a change towards a tolerogenic phenotype, including increased expression of genes typical for regulatory T cells (Tregs), by adding a combination of calcitriol and IL2 to human primary T cell cultures. Tregs act to suppress proinflammatory responses by other immune cells and aim to prevent exaggerated or autoimmune responses [52]. Tregs can be induced and stimulated by vitamin D in an indirect pathway, via APC, including the group of DC which stay in an immature state upon vitamin D treatment and therefore present less antigens. The direct pathway acts via systemic calcitriol effects or intracrine conversion of 25(OH)D to calcitriol by Tregs themselves. Administration of calcitriol to renal transplant recipients expanded the circulating Treg population [53]. In the subsequent randomized, placebo controlled trial in healthy subjects, the percentage of Tregs in peripheral blood increased significantly after supplementation with high doses of cholecalciferol [54, 55]. Using cholecalciferol supplementation as adjunctive therapy in new onset T1D patients, the percentage of peripheral Tregs increased, although there was no significant difference in Tregs percent between placebo and treatment group after 1 year of supplementation [56].

These results suggest that vitamin D may support the innate and adaptive immune system and could also provide a safe and useful future therapy to support immune tolerance in autoimmune diseases or following transplantation.

Vitamin D_3 supplementation

Though some prospective studies suggest that vitamin D plays a positive role in various infectious processes including tuberculosis, influenza and HIV, chronic obstructive pulmonary disease exacerbations and sepsis [57-59].

The last 10 years of vitamin D application trials are focused on its role in various pulmonary diseases. A majority of these researches studied respiratory tract infections and exacerbations linked to them, pneumonia and tuberculosis.

The placebo, randomized, double blind, single center trial (280 postmenopausal women) that started in 2007 and continued for 3 years shows that supplementation of vitamin D_3 in a daily dose 800 IU for 2 years and 2000 IU for 1 year decreased cold and influenza symptoms and self-reported respiratory tract infections [60].

Another placebo, randomized, double blind, but multicenter trial (430 healthy children) started in 2010 suggested that daily implication of vitD3 (1,200 IU) for 3 month leads to the decreasing of influenza A incidence and asthma attacks in children with asthma [61]. Majak in not very wide placebo, randomized, double blind, single center research (48 children with new asthma) shows that daily 500 IU of D_3 for 6 month helps to increase lung function, minimize the score of asthma symptoms and number of asthma exacerbations due to respiratory tract infection [62].

Bergman in 2012 in placebo, randomized, double blind, single center trial proposed 4000 IU of D3 daily for 1 year to 140 participants with an antibody deficiency or participants who have had more than 4 respiratory tract infection episodes during the year. This scheme helps to decrease the rate of infection and number of days on antibiotics by 50 % [63]. Camargo in 2012 successfully used daily D3 in a dose 300 IU to 247 healthy ~ 10 years-old children for minimizing respiratory tract infection episodes [64]. In patients with moderate to very severe chronic obstructive pulmonary disease implication of 100,000 IU of vitamin D_3 with monthly intervals resulted in improvement in lung function, rates of exacerbation, morbidity and mortality, although positive results were limited to participants who were vitamin D deficient at baseline [65]. Cystic fibrosis

DOI: 10.5281/zenodo.1133763

patients who received a single 250,000 IU dose of vitamin D_3 while hospitalized experienced a significant increase in hospital-free days during the year and 50,4 % decrease in TNFα concentrations up to 12 weeks [43].

Vitamin D and Tuberculosis

Vitamin D has been widely studied in the prevention and treatment of tuberculosis. Current studies were focused on how calcitriol enhances the antimicrobial effects of macrophages and monocytes – important effector cells, fighting against pathogens such as *Mycobacterium tuberculosis* (MBT). Besides enhancing chemotaxis and phagocytic capabilities of innate immune cells [66], the complex of calcitriol, VDR, and retinoid X receptor directly activates the transcription of antimicrobial peptides such as defensin β2 (DEFB) and cathelicidin antimicrobial peptide (hCAP18) [67-69]. In detail, monocytes exposed to MBT show a strong induction of the 1α-hydroxylase CYP27B1 and the vitamin D receptor after recognizing pathogens by toll-like receptors, leading to a direct modulation of gene expression, favoring production of cathelicidin [70]. Besides TLR-signaling, other cytokines such as interferon-γ or IL-4 have been found to also effect CYP27B1 expression [71]. Human cathelicidin (hCAP18) which is cleaved from LL-37 (37-residue active cationic peptide) and then causes destabilization of microbial membranes, is up-regulated in response to infections in humans and also acts against bacteria, viruses and fungi [19].

Several studies tracked the impact of vitamin D on cytokines that promote anti-MTB activity and the resolution of infection. Suppression of antigen-stimulated pro-inflammatory cytokines, attenuation of anti-inflammatory cytokines, and a more rapid treatment-induced resolution of lymphopenia and monocytosis associated with TB infection occurred following 100,000 IU doses of vitamin D_3 given monthly for 4 months [72].The trial that studied tuberculosis (TB) prevention showed a single dose of 800 IU of vitamin D_3 to cause a significant increase in anthropometric measurements and a 59 % reduction in tuberculin skin test conversion rates when given to children for 6 weeks [73]. Some papers discussed vitamin D both as an adjunct to antibiotic treatment for TB and in its ability to direct killing of MBT [74-76]. Vitamin D implication led to clinical improvements in several studies, including: weight gains (in adults who received two doses of 600,000 IU of vitamin D_3 and children received daily doses of 1000 IU for 8 weeks [76, 77], less tissue involvement was observed after 1,000 IU daily use during 2 months and chest x-ray after 5,000 IU of vitamin D_3 daily for 3 months. Conversely, no improvement on x-ray was seen in children receiving daily doses of vitamin D (1,000 IU over 2 months [77] or adults receiving daily of 10,000 IU for 6 weeks [78] or monthly implication of 50,000 IU given twice doses of vitamin D_3 [79].

Conversion of sputum smear or sputum culture was used to measure response to treatment in several studies, though only sputum culture conversion is independently linked to long-term risk of treatment failure and relapse [80]. Also it was found [78] that 10,000 IU of vitamin D_3 given daily for 6 weeks to significantly increase sputum smear conversion (100 % in the treatment group vs. 76,7 % in the placebo group, p=0,002).

IFN-γ levels were impacted variably: 2 doses of vitamin D_3 (600,000 IU)) led to increasing of IFN-γ expression [76], while a single 100,000 IU dose of vitamin D_2 showed no change [75].

In the result of another trial (95 patients) was no effect on time to sputum culture conversion after adjusting for multiple baseline factors (35 vs 46,5 days, $p \leq 0,05$), but vitamin D accelerated normalization of erythrocyte sedimentation ratio and serum C-reactive protein in this population. Vitamin D also reduced chemokine production, but it had no effect on IFN-γ [72].

Two studies evaluated vitamin D in combination with another chemical thought to modulate activity against MTB; 5,000 IU of vitamin D_3 given for 4 days alone or in combination with phenylbutyrate induced both circulating levels and transcript expression of the anti-microbial peptide LL-37 [81], while 50,000 IU doses with or without l-arginine (for NO production promotion) showed no significant change in sputum culture conversion rate or x-ray involvement [79].

Negative results in some studies could be explained by variability of the Taq1 vitamin D receptor genotype polymorphism. It was shown that significantly accelerated conversion is appropriate of patients who have a *tt* genotype compared to those with the *Tt* or *TT* genotype.

Thus one study reported a significant benefit of vitamin D evident in 12 subjects with the *tt* Taq1 VDR genotype, with proportions culture positive at week 8 of 0 % and 57 % in the vitamin D and control arms, respectively [75]. There was no benefit of vitamin D in other VDR genotypes. These results were not confirmed by the study, where were founded no effect of VDR genotype on vitamin D in an analysis that included 30 *tt* genotype subjects. In this trial was carried out a subset analysis in 18 patients who were found after enrollment to have multidrug-resistant-tuberculosis. Treatment with second-line drugs began after a mean of 51 days for placebo recipients and 62 days for vitamin D recipients. Six of 10 recipients remained culture positive at week 8 in the placebo arm versus 1 of 8 in the vitamin D arm [75].

As a resume, vitamin D given largely as an adjunctive therapy with traditional anti-tuberculosis regimens in a variety of dose and dosing schedule has some impact on clearance of *M. tuberculosis* from sputum in the wide number randomized controlled multicenter trials of patients with active tuberculosis infection. Patients with infection of MBT with different strains of tuberculosis can take benefits from Vitamin D_3 consumption due to its effect on the clearance of MTB from sputum and on dampening the inflammatory response or anthropometric changes that may help tuberculosis patients recover. A significant microbiologic effect of vitamin D_3 was indicated in several trials that, also, sustained by in vitro tests, where its antimycobacterial effects in cultured macrophages

DOI: 10.5281/zenodo.1133763

was shown. Antimycobacterial effect is provided enhances the expression of the anti-microbial peptide human cathelicidin (hCAP18) in cultured macrophages [82]. The clinical benefit after high vitamin D_3 doses administrating to patients does not depend of their vitamin D_3 marked deficiency. The cause of this variation remains unexplained. The Role of genetic polymorphisms in the vitamin D receptor, or in the multiple enzymes involved in its metabolism in vitD_3 effectiveness remains unproved. Measurement of calcitriol-induced antimycobacterial activity in ex vivo whole blood culture in future studies may help in understanding the functional effects of specific genetic polymorphisms. So, big attention will be required in future studies to determine mechanism of vitamin effect on patients with tuberculosis.

References

1. Teichmann A, Dutta PC, Staffas A, Jägerstad M. Sterol and vitamin D_2 concentrations in cultivated and wild grown mushrooms: effects of UV irradiation // LWT Food Sci. Technol. 2007 Vol. 40. P. 815–822.
2. Bikle D, Adams J, Christakos S. Vitamin D: production, metabolism and clinical requirements. In: Primer Metab Bone Dis, edited by Rosen C, editor. Hoboken, NJ: Wiley. 2013. P. 235–245.
3. Zehnder D, Evans KN, Kilby MD. et al. The ontogeny of 25-hydroxyvitamin D(3) 1alpha-hydroxylase expression in human placenta and decidua // Am J Pathol. 2002. Vol. 161(1). P.105-114.
4. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects // Physiological Reviews. 2016. Vol. 96(1). P. 365-408.
5. Margolis RN, Christakos S, Ann NY. The nuclear receptor superfamily of steroid hormones and vitamin D gene regulation // Acad Sci. 2010. Vol. 1192. P. 208-214.
6. Feldman D, Malloy J, Bonekey P. Mutations in the vitamin D receptor and hereditary vitamin D-resistant rickets // Rep. 2014. Vol. 3. P. 510.
7. Pike JW, Meyer MB. Fundamentals of vitamin D hormone-regulated gene expression // J Steroid Biochem Mol Biol. 2014. Vol.144. Pt A. P. 5-11.
8. Carlberg C, Campbell MJ. Vitamin D receptor signaling mechanisms: integrated actions of a well-defined transcription factor // Steroids. 2013. Vol. 78(2). P.127-136.
9. Bhalla AK, Amento EP, Clemens TL, et al. Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation // J Clin Endocrinol Metab. 1983. Vol. 57(6). P.1308–1310.
10. Stumpf WE, Sar M, Reid FA, et al. Target cells for 1,25-dihydroxyvitamin D3 in intestinal tract, stomach, kidney, skin, pituitary, and parathyroid // Science. 1979. Vol. 206(4423). P.1188–1190.
11. Yim S, Dhawan P, Ragunath C, et al. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3) // J Cyst Fibros. 2007. Vol. 6(6). Vol. 403–410.
12. Hansdottir S, Monick MM, Hinde SL, et al. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense // J Immunol. 2008. Vol. 181(10). P.7090–7099.
13. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response // Science. 2006. Vol. 311(5768). P. 1770–1773.
14. White JH. Vitamin D signaling, infectious diseases, and regulation of innate immunity// Infect Immun. 2008. Vol. 76(9). P. 3837–3843.
15. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response // Science. 2006. Vol. 311(5768). P. 1770–1773.
16. Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression // J Immunol. 2004. Vol. 173(5). P. 2909–2912.
17. Ciornei CD, Sigurdardottir T, Schmidtchen A, et al. Antimicrobial and chemoattractant activity, lipopolysaccharide neutralization, cytotoxicity, and inhibition by serum of analogs of human cathelicidin LL-37 // Antimicrob Agents Chemother. 2005. Vol. 49(7). P. 2845–2850.
18. Thijssen HH, Janssen CA, Drittij-Reijnders MJ. The effect of S-warfarin administration on vitamin K 2,3-epoxide reductase activity in liver, kidney and testis of the rat // Biochem Pharmacol. 1986. Vol. 35(19). P. 3277–3282.
19. Ramanathan B, Davis EG, Ross CR, et al. Cathelicidins: microbicidal activity, mechanisms of action, and roles in innate immunity // Microbes Infect. 2002. Vol. 4(3). P.361–372.
20. Gropp R, Frye M, Wagner TO, et al. Epithelial defensins impair adenoviral infection: implication for adenovirus-mediated gene therapy // Hum Gene Ther. 1999. Vol. 10(6). P. 957–964.
21. Klotman ME, Chang TL. Defensins in innate antiviral immunity // Nat Rev Immunol. 2006. Vol. 6(6). P. 447–456.
22. Sly LM, Lopez M, Nauseef WM, et al. 1alpha,25-Dihydroxyvitamin D3-induced monocyte antimycobacterial activity is regulated by phosphatidylinositol 3-kinase and mediated by the NADPH-dependent phagocyte oxidase // J Biol Chem. 2001. P. 276(38). P. 35482–35493.
23. Rook GA, Hernandez-Pando R, Lightman SL. Hormones, peripherally activated prohormones and regulation of the Th1/Th2 balance // Immunol Today. 1994. Vol. 15(7). P.301–303.
24. Cox FE, Liew FY. T-cell subsets and cytokines in parasitic infections // Immunol Today. 1992. Vol. 13(11). P .445–448.
25. Thorne KJ, Mazza G. Eosinophilia, activated eosinophils and human schistosomiasis // J Cell Sci. 1991. Vol. 98(Pt 3). P. 265–270.
26. Diaz A, Allen JE. Mapping immune response profiles: the emerging scenario from helminth immunology // Eur J Immunol. 2007. Vol. 37(12). P. 3319–3326.

DOI: 10.5281/zenodo.1133763

27. Romani L. Cell mediated immunity to fungi: a reassessment // Med Mycol. 2008. Vol. 46(6). P. 515–529.
28. Kobasa D, Takada A, Shinya K, et al. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus // Nature. 2004. P. 431(7009). P.703–707.
29. Cheung CY, Poon LL, Lau AS, et al. Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? // Lancet. 2002. Vol. 360(9348). P. 1831–1837.
30. Kellum JA, Kong L, Fink MP, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study // Arch Intern Med. 2007. Vol. 167(15). P. 1655–1663.
31. Yende S, D'Angelo G, Kellum JA, et al. Inflammatory markers at hospital discharge predict subsequent mortality after pneumonia and sepsis // Am J Respir Crit Care Med. 2008. Vol. 177(11). P. 1242–1247.
32. Jeng L., Yamshchikov AV, Judd SE, Blumberg HM, Martin GS, Ziegler TR, Tangpricha V. Alterations in vitamin D status and anti-microbial peptide levels in patients in the intensive care unit with sepsis // J. Transl. Med. 2009. Vol. 7. P. 28.
33. Grimnes G, Figenschau Y, Almås B, Jorde R. Vitamin D, insulin secretion, sensitivity, and lipids: results from a case-control study and a randomized controlled trial using hyperglycemic clamp technique // Diabetes 2011. Vol. 60. P. 2748-2757.
34. Shab-Bidar S, Neyestani TR, Djazayery A, Eshraghian MR, Houshiarrad A, Kalayi A, Shariatzadeh N, Khalaji N, Gharavi A. Improvement of vitamin D status resulted in amelioration of biomarkers of systemic inflammation in the subjects with type 2 diabetes // Diabetes Metab Res Rev 2012. Vol. 28. P. 424-430.
35. Kampmann U, Mosekilde L, Juhl C, Moller N, Christensen B, Rejnmark L, Wamberg L, Orskov L. Effects of 12weeks high dose vitamin D3 treatment on insulin sensitivity, beta cell function, and metabolic markers in patients with type 2 diabetes and vitamin D insufficiency - a double-blind, randomized, placebo-controlled trial // Metabolism 2014. Vol. 63. P. 1115-1124.
36. Bucharles S, Barberato SH, Stinghen AE, et al. Impact of cholecalciferol treatment on biomarkers of inflammation and myocardial structure in hemodialysis patients without hyperparathyroidism // J Ren Nutr 2012. Vol. 22. P. 284-291.
37. Sokol SI, Srinivas V, Crandall JP, et al. The effects of vitamin D repletion on endothelial function and inflammation in patients with coronary artery disease // Vasc Med. 2012. Vol. 17. P. 394-404.
38. Alvarez JA, Zughaier SM, Law J, et al. Effects of high-dose cholecalciferol on serum markers of inflammation and immunity in patients with early chronic kidney disease // Eur J Clin Nutr 2013. Vol. 67. P. 264-269.
39. Breslavsky A, Frand J, Matas Z, Boaz M, Barnea Z, Shargorodsky M. Effect of high doses of vitamin D on arterial properties, adiponectin, leptin and glucose homeostasis in type 2 diabetic patients // Clin Nutr. 2013. Vol. 32. P.970-975.
40. Witham MD, Crighton LJ, Gillespie ND, Struthers AD, McMurdo ME. The effects of vitamin D supplementation on physical function and quality of life in older patients with heart failure: a randomized controlled trial // Circ Heart Fail 2010. Vol. 3. P.195-201.
41. Mahon BD, Gordon SA, Cruz J, Cosman F, Cantorna MT. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation // J Neuroimmunol. 2003. Vol. 134. P.128-132
42. Pappa HM, Mitchell PD, Jiang H, et al. Maintenance of optimal vitamin D status in children and adolescents with inflammatory bowel disease: a randomized clinical trial comparing two regimens // J Clin Endocrinol Metab. 2014. Vol. 99. P.3408-3417.
43. Grossmann RE, Zughaier SM, Liu S, Lyles RH, Tangpricha V. Impact of vitamin D supplementation on markers of inflammation in adults with cystic fibrosis hospitalized for a pulmonary exacerbation // Eur J Clin Nutr. 2012. Vol. 66. P. 1072-1074.
44. Hewison M. An update on vitamin D and human immunity // Clin. Endocrinol. 2012. Vol. 76. P.315–325.
45. Lemire JM, Adams JS, Kermani-Arab V, Bakke AC, et al. 1,25-Dihydroxyvitamin D_3 suppresses human T helper/inducer lymphocyte activity *in vitro* // J. Immunol. 1985. Vol. 134. P. 3032–3035.
46. Cantorna MT. Mechanisms underlying the effect of vitamin D on the immune system // Proc. Nutr. Soc. 2011. Vol. 69. P. 286–289.
47. Baeke F, Korf H, Overbergh L, Verstuyf A, et al. The vitamin D analog, TX527, promotes a human CD4+CD25highCD127low regulatory T cell profile and induces a migratory signature specific for homing to sites of inflammation // J. Immunol. 2011. Vol. 186. P.132–142.
48. Palmer MT, Lee YK, Maynard CL, Oliver JR, et al. Lineage-specific effects of 1,25-dihydroxyvitamin D_3 on the development of effector CD4 T cells // J. Biol. Chem. 2011. Vol. 286. P. 997–1004.
49. Boonstra A, Barrat FJ, Crain C, et al. 1α,25-dihydroxyvitamin D_3 has a direct effect on naive CD4+T cells to enhance the development of Th2 cells // J. Immunol. 2001. Vol. 167. P. 4974–4980.
50. Joshi S, Pantalena L, Liu XK, Sarah L, et al. 1,25-Dihydroxyvitamin D_3 ameliorates Th17 autoimmunity via transcriptional modulation of interleukin-17A // Mol. Cell. Biol. 2011. Vol. 31. P. 3653–3669.
51. Jeffery LE, Burke F, Mura M, Zheng Y, et al. 1,25-Dihydroxyvitamin D_3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3 // J. Immunol. 2009. Vol. 183. P.5458–5467.
52. Rudensky AY. Regulatory T cells and foxP3 // Immunol. Rev. 2011. Vol. 241. P. 260–268.
53. Ardalan MR, Maljaei H, Shoja MM, Piri AR, et al. Calcitriol started in the donor, expands the population of $CD4^+$ $CD25^+$ T cells in renal transplant recipients // Transplant. Proc. 2007. Vol. 39. P.951–953.
54. Prietl B., Pilz S., Wolf M., Tomaschitz A., Obermayer-Pietsch B., Graninger W., Pieber T.R.

DOI: 10.5281/zenodo.1133763

Vitamin D supplementation and regulatory T cells in apparently healthy subjects: Vitamin D treatment for autoimmune diseases? // Isr. Med. Assoc. J. 2010. Vol. 12. P.136–139.
55. Bock G, Prietl B, Mader JK, Höller E, et al. The effect of vitamin D supplementation on peripheral regulatory T cells and β cell function in healthy humans: A randomized controlled trial // Diabetes Metab. Res. Rev. 2011. Vol. 25. P. 942–945.
56. Gabbay MAL, Sato MN, Finazzo C, Duarte AJS, Dib SA. Effect of cholecalciferol as adjunctive therapy with insulin on protective immunologic profile and decline of residual β-cell function in new-onset type 1 diabetes mellitus // Arch. Pediatr. Adolesc. Med. 2012. Vol. 166. P. 601–607.
57. Ferrari M, Schenk K, Papadopoulou C, et al. Serum 25-hydroxy vitamin D and exercise capacity in COPD // Thorax. 2011. Vol. 66(6). P. 544–545.
58. Wilkinson RJ, Llewelyn M, Toossi Z, et al. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. Lancet. 2000;355(9204):618–21.
59. Holick MF. High prevalence of vitamin D inadequacy and implications for health // Mayo Clin Proc. 2006. Vol. 81(3). P.353–373.
60. Aloia JF, Li-Ng M. Re: epidemic influenza and vitamin D // Epidemiol Infect. 2007. Vol. 135(7). P.1095–1096.
61. Urashima M, Segawa T, Okazaki M, et al. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren // Am J Clin Nutr. 2010. Vol. 91(5). P.1255–1260.
62. Majak P, Olszowiec-Chlebna M, Smejda K, et al. Vitamin D supplementation in children may prevent asthma exacerbation triggered by acute respiratory infection // J Allergy Clin Immunol. 2011. Vol. 127(5). P. 1294–1296.
63. Bergman P, Norlin AC, Hansen S, et al. Vitamin D3 supplementation in patients with frequent respiratory tract infections: a randomised and double-blind intervention study // BMJ Open. 2012.2(6)
64. Camargo CA, Ganmaa D, Frazier AL, et al. Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia // Pediatrics. 2012 . Vol. 130(3). P.561–567.
65. Lehouck A, Mathieu C, Carremans C, et al. High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease: a randomized trial // Ann Intern Med. 2012. Vol. 156(2). P.105–114.
66. Baeke F., Takiishi T., Korf H., Gysemans C., Mathieu C. Vitamin D: Modulator of the immune system. Curr. Opin. Pharmacol. 2010. Vol. 10. P. 482–496.
67. Wang T, Nestel FP, Bourdeau V, Nagai Y, et al. Cutting edge: 1,25-Dihydroxyvitamin D_3 is a direct inducer of antimicrobial peptide gene expression // J. Immunol. 2004. Vol. 173. P. 2909–2912.
68. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3 // FASEB J. 2005. Vol. 19. P.1067–1077.
69. White JH. Vitamin D metabolism and signaling in the immune system // Rev. Endocr. Metab. Disord. 2012. Vol. 13. P.21–29.
70. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response // Science. 2006. Vol. 311. P. 1770–1773.
71. Edfeldt K, Liu PT, Chun R, Fabri M, Schenk M, et al. T-cell cytokines differentially control human monocyte antimicrobial responses by regulating vitamin D metabolism // Proc. Natl. Acad. Sci. 2010. Vol. 107. P. 22593–22598.
72. Coussens AK, Wilkinson RJ, Hanifa Y, et al. Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment // Proc Natl Acad Sci. 2012. Vol. 109(38). P.15449–15454.
73. Ganmaa D, Giovannucci E, Bloom BR, et al. Vitamin D, tuberculin skin test conversion, and latent tuberculosis in Mongolian school-age children: a randomized, double-blind, placebo-controlled feasibility trial // American Journal of Clinical Nutrition. 2012. Vol. 96(2). P. 391–396.
74. Kota SK, Jammula S, Kota SK, et al. Effect of vitamin D supplementation in type 2 diabetes patients with pulmonary tuberculosis // Diabetes Metab Syndr. 2011. Vol. 5(2). P. 85–9.
75. Martineau AR, Timms PM, Bothamley GH, et al. High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial // Lancet. 2011. Vol. 377(9761). P. 242–250.
76. Salahuddin N, Ali F, Hasan Z, et al. Vitamin D accelerates clinical recovery from tuberculosis: results of the SUCCINCT Study [Supplementary Cholecalciferol in recovery from tuberculosis]. A randomized, placebo-controlled, clinical trial of vitamin D supplementation in patients with pulmonary tuberculosis // BMC Infect Dis. 2013. Vol. 13. P. 22.
77. Morcos MM, Gabr AA, Samuel S, et al. Vitamin D administration to tuberculous children and its value // Boll Chim Farm. 1998. Vol. 137(5). P. 157–164.
78. Nursyam EW, Amin Z, Rumende CM. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion // Acta Med Indones. 2006. Vol. 38(1) P. 3–5.
79. Ralph AP, Waramori G, Pontororing GJ, et al. L-arginine and vitamin D adjunctive therapies in pulmonary tuberculosis: a randomised, double-blind, placebo-controlled trial // PLoS One. 2013. Vol. 8(8):e70032.
80. Benator D, Bhattacharya M, Bozeman L, et al. Rifapentine and isoniazid once a week versus rifampicin and isoniazid twice a week for treatment of drug-susceptible pulmonary tuberculosis in HIV-negative patients: a randomised clinical trial // Lancet. 2002. Vol. 360(9332). P. 528–534.
81. Mily A, Rekha RS, Kamal SM, et al. Oral intake of phenylbutyrate with or without vitamin D3 upregulates the cathelicidin LL-37 in human macrophages: a dose

DOI: 10.5281/zenodo.1133763

finding study for treatment of tuberculosis // BMC Pulm Med. 2013. Vol. 13. P. 23.
82. Crowle AJ, Ross EJ, May MH. Inhibition by 1,25(OH)2-vitamin D3 of the multiplication of virulent tubercle bacilli in cultured human macrophages // Infect Immun. 1987. Vol. 55(12). P. 2945-2950.

VITAMIN D3: RESEARCH BREAKTHROUGHS AND THERAPEUTIC USE

Pohorila M.S., Martynov A.V., Romanova E.A., Igumnova N.I., Sidorenko T.A., Yukhimenko V.I., Shcherbak O.M.

Vitamin D3 (cholecalciferol), the natural form of vitamin D, is produced in the skin from 7-dehydrocholesterol. The synthesis of vitamin D in the skin is the most important source of vitamin D. Vitamin D can also be taken through nutrition, in the diet, but it is present in only a few food sources, containing relevant levels of vitamin D. 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] is the hormonally active form of vitamin D. Novel researches show it generates a number of extraskeletal biological responses including inhibition of variety types cancer progression, effects on cardiovascular disorders and mediates a protection against a number of inflammatory, autoimmune and infection diseases The biological actions of $1,25(OH)_2D_3$ are mediated by the VDR. The genomic mechanism of $1,25(OH)_2D_3$ action involves the direct binding of $1,25(OH)_2D_3$ activated VDR/RXR to specific DNA sequences in and around target genes resulting in either activation or repression of transcription [7] VDR modulates the expression of genes involved in immune function and cytokine production. The VDR and CYP27B1, the enzyme located in kidneys and target organs, are present in immune competent cells, bronchial and pulmonary epithelial cells, among others, and is up-regulated following the ligation of specific toll-like receptors by extracellular pathogens, implicating vitamin D in innate immunity. By binding the VDR, calcitriol induces several endogenous antimicrobial peptides (AMP) in monocytes, neutrophils and epithelial cells including cathelicidin LL-37, α-defensin, β defensing and neutrophil gelatinase-associated lipocalin and up-regulates nitric oxide (NO) synthase. Since the inflammatory response associated with infections such influenza, pneumonia and sepsis increases both clinical severity and mortality, the ability to reduce inflammation may allow vitamin D to decrease mortality and disease burden in certain infections. Notwithstanding the width of possible vitamin D application field, which being known now, large-scale clinical trials are still demanded. Our review has the aim to summarize current scientific understanding of Vitamin D_3 effects on the immunological field with the focus on its capacity to enhance the anti-infection and anti-inflammatory immune reactivity. **Vitamin D and Tuberculosis.** Vitamin D has been widely studied in the prevention and treatment of tuberculosis. Current studies were focused on how calcitriol enhances the antimicrobial effects of macrophages and monocytes – important effector cells, fighting against pathogens such as *Mycobacterium tuberculosis* (MBT). Several studies tracked the impact of vitamin D on cytokines that promote anti-MTB activity and the resolution of infection. Suppression of antigen-stimulated pro-inflammatory cytokines, attenuation of anti-inflammatory cytokines, and a more rapid treatment-induced resolution of lymphopenia and monocytosis associated with TB infection occurred following 100,000 IU doses of vitamin D_3 given monthly for 4 months. Conversion of sputum smear or sputum culture was used to measure response to treatment in several studies, though only sputum culture conversion is independently linked to long-term risk of treatment failure and relapse. Also it was found that 10,000 IU of vitamin D_3 given daily for 6 weeks to significantly increase sputum smear conversion (100 % in the treatment group vs. 76,7 % in the placebo group, p=0,002). IFN-γ levels were impacted variably: 2 doses of vitamin D_3 (600,000 IU)) led to increasing of IFN-γ expression , while a single 100,000 IU dose of vitamin D_2 showed no change . Negative results in some studies could be explained by variability of the Taq1 vitamin D receptor genotype polymorphism. It was shown that significantly accelerated conversion is appropriate of patients who have a *tt* genotype compared to those with the *Tt* or *TT* genotype. But these results were not confirmed by another study, where were founded no interaction between VDR genotype effectiveness of vitamin D. Several trials show vitamin D given largely as an adjunctive therapy with traditional anti-tuberculosis regimens in a variety of dose and dosing schedule has some impact on clearance of MBT from sputum in the wide number randomized controlled multicenter trials of patients with active tuberculosis infection. Patients with infection of MBT with different strains of tuberculosis can take benefits from Vitamin D_3 consumption due to its effect on the clearance of MTB from sputum and on dampening the inflammatory response or anthropometric changes that may help tuberculosis patients recover. A significant microbiologic effect of vitamin D_3 was indicated in several trials that, also, sustained by in vitro tests, where its antimycobacterial effects in cultured macrophages was shown. Antimycobacterial effect is provided enhances the expression of the anti-microbial peptide human cathelicidin (hCAP18) in cultured macrophages. The clinical benefit after high vitamin D_3 doses administrating to patients does not depend of their vitamin D_3 marked deficiency. The cause of this variation remains unexplained. The role of genetic polymorphisms in the vitamin D receptor, or in the multiple enzymes involved in its metabolism in vitD_3 effectiveness remains unproved. Measurement of calcitriol-induced antimycobacterial activity in ex vivo whole blood culture in future studies may help in understanding the functional effects of specific genetic polymorphisms. So, big attention will be required in future studies to determine mechanism of vitamin effect on patients with tuberculosis.

DOI: 10.5281/zenodo.1133763

Keywords*:* Vitamin D_3, cholecalciferol, VDR, infection, pulmonary disease, tuberculosis, innate immunity, adoptive immunity, antimicrobial effect

DOI: 10.5281/zenodo.1133763