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Review

# Vitamin D as an inducer of cathelicidin antimicrobial peptide expression: Past, present and future☆

John H. White[a,b,*]

[a] Department of Physiology, McGill University, Montreal, QC, Canada
[b] Department of Medicine, McGill University, Montreal, QC, Canada

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ABSTRACT

Vitamin D was discovered as the preventive agent of nutritional rickets, a defect in bone development due to inadequate uptake of dietary calcium. However, a variety of studies over the last several years has revealed that vitamin D controls much more than calcium homeostasis. For example, recent research has underlined the key role of vitamin D signaling in regulation of innate immunity in humans. Vitamin D is converted to 25-hydroxyvitamin D (25D), its major circulating form, and then to hormonal 1,25-dihydroxyvitamin D (1,25D) in target cells. We now know that when cells of the immune system such a macrophages sense a bacterial infection they acquire the capacity to convert circulating 25D into 1,25D. Moreover, 1,25D thus produced is a direct inducer of expression of genes encoding antimicrobial peptides, in particular cathelicidin antimicrobial peptide (CAMP). Antimicrobial peptides such as CAMP are vanguards of innate immune responses to bacterial infection and can act as signaling molecules to regulate immune system function. This review covers what we have learned in the past few years about the expression and function of CAMP under physiological and pathophysiological conditions, and addresses the potential future applications of vitamin D analogues to therapeutic regulation of CAMP expression.


**Contents**



## 1. Vitamin D and its molecular mechanisms of action

Vitamin D is obtained via two independent pathways; limited dietary sources, and UVB-induced photochemical and thermal conversion of 7-dehydrocholesterol in skin. Vitamin D was originally identified as the curative agent for nutrition rickets. Cod liver oil was discovered as an anti-rachitic activity in the 1820s, although it was not until several decades later that the active ingredient was identified as vitamin $D_3$. At around the same time, a connection was made between a lack of sun exposure and rickets, and in 1919 it was shown that artificial UV light cured rickets [1,2]. Vitamin D insufficiency or deficiency is quite widespread, due to inadequate dietary intake, and the fact that solar UVB is absorbed by atmospheric ozone leading to marked variations in surface intensity with latitude and time of year [3–5]. As a result, rates of vitamin D insufficiency or deficiency rise with increasing latitude, and fluctuate with the changing seasons.

More recent data from a variety of sources, ranging from molecular experiments to epidemiological studies, has provided evidence that vitamin D has a broad spectrum of actions. There is solid evidence for a role for vitamin D as a chemopreventive agent of a number of cancers, in particular cancers of the digestive tract [6,7]. In addition, it is now well established that vitamin D is an important

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* Department of Physiology, McGill University, McIntyre Bldg., 3655 Drummond St. Rm 1112, Montreal, Quebec, Canada H3R 2N2. Tel.: +1 514 398 8498; fax: +1 514 398 7452.
*E-mail address:* john.white@mcgill.ca.

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modulator of immune system function. It was first recognized in the 19th century that a number of factors including dry, warm, climates with plenty of sunshine were beneficial for patients suffering from tuberculosis (TB). Increasingly, recent work has established a direct association between vitamin D and TB susceptibility [9,10]. One such study has shown that 1,25D inhibits the growth of *Mycobacterium tuberculosis* in cultured human macrophages [8]. An epidemiological study of a Gujarati Asian immigrant population in the London area revealed an association between active TB and 25D deficiency, and an even stronger association of disease with undetectable serum 25D [11]. In addition, a double-blind randomized controlled trial revealed that a single dose of 100,000 U of vitamin $D_3$ (2.5 mg) enhanced anti-mycobacterial immunity in healthy tuberculin skin test-positive donors [12].

The term vitamin D refers collectively to vitamin $D_3$ and vitamin $D_2$, which is derived from irradiation of the yeast steroid ergosterol. Vitamin D undergoes hepatic 25-hydroxylation catalyzed by CYP2R1, CYP27A1, and possibly other enzymes to produce 25-hydroxvitamin D (25D; Refs. [5,13–16]), the major circulating vitamin D metabolite. 25D is then modified by 1α-hydroxylation catalyzed by CYP27B1 to produce hormonal 1,25-dihydroxyvitamin D (1,25D; Refs. [5,14,15]). Vitamin D compounds are catabolized via 24-hydroxylation by CYP24, whose expression is strongly inducible by 1,25D, constituting a negative feedback loop [5,14,15]. While the kidneys represent a major site of 1α-hydroxylation of 25D, recent studies have emphasized that generation of hormonal 1,25D in peripheral tissues contributes to the full scope of physiological actions of vitamin D. Indeed, studies detailed in this review revealed the importance of extra-renal 1α-hydroxylation of 25D in the function of 1,25D as an immune system regulator.

1,25D signals through the vitamin D receptor (VDR), a member of the nuclear receptor family of ligand-activated transcription factors [5,17]. The VDR contains highly conserved DNA binding and ligand binding domains [18]. Heterodimerization of the hormone-bound VDR with related retinoid X receptors (RXRs) is essential for high affinity DNA binding to cognate vitamin D response elements (VDREs) located in the regulatory regions of 1,25D target genes. High affinity VDREs are composed of direct repeats of PuG(G/T)TCA motifs separated by 3bp (DR3; Refs. [5,17,19–21]). While numerous VDREs have been identified in relatively promoter-proximal locations, recent work has provided evidence that the DNA-bound VDR can function at distances as great as 75 kb to regulate adjacent target gene transcription [22].

## 2. The *camp* (cathelicidin antimicrobial peptide) gene is a direct target of 1,25D signaling

1,25D signaling is ideally suited to analysis using genomic approaches, given that the VDR functions as a ligand-regulated gene switch. In our laboratory, we have used a combination of microarrays and *in silico* screens for VDREs to identify several hundred 1,25D target genes [23–26]. Our *in silico* screening for consensus VDREs revealed promoter-proximal elements in two genes encoding antimicrobial peptides (AMPs), CAMP (hCAP18, LL37) and DEFB2 (HBD2, DEFB4, β-defensin 2) [27]. Further analysis of the *camp* and *defb2* VDREs by chromatin immunoprecipitation assays revealed 1,25D-dependent binding of VDR/RXRs to both elements. Notably, *camp* expression was strongly stimulated by 1,25D in all cell types examined (epithelial cells, macrophages/monocytes, and neutrophils), whereas regulation of *defb2* expression more modest. The regulation of *camp* by 1,25D was subsequently observed by others in a range of cell types [28,29], including in 1,25D-treated or ultraviolet B-irradiated human skin biopsies [30], demonstrating that 1,25D is a primary inducer of the gene. AMPs are critical components of innate immune responses to bacterial, fungal and viral infections [31–33]. The identification of *camp* and *defb2* as VDR target genes revealed that 1,25D is a direct inducer of antimicrobial innate immunity in humans. The induction of AMP expression by 1,25D in humans provides further molecular evidence that a vitamin D replete state is protective against a range of bacterial and viral pathogens [34].

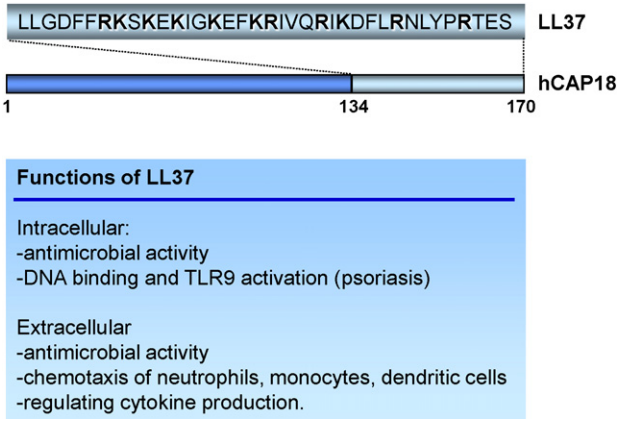


**Fig. 1.** Functions of LL37. A schematic representation of the hCAP18 precursor protein and the LL37 cleavage product is shown. Basic amino acids in the LL37 peptide are in bold. The functions of LL37 discussed in the text are listed below. See text for appropriate references.

## 3. Cathelicidins

Cathelicidins are present in a variety of species, and their name reflects the dual properties of members of the family. Cathelicidins are derived in part from the conserved N-terminal region known as the cathelin domain, which has the capacity to inhibit cathepsin-L cysteine protease activity. The suffix reflects the AMP activity of family members. Cathelicidin precursor proteins are proteolytically processed to release C-terminal peptides, whose sequences are poorly conserved among species [35]. Genes encoding cathelicidins are present in the genomes of invertebrates as well as a range of vertebrates, and gene numbers vary widely between species. The single human gene encodes a precursor protein called hCAP18, which is cleaved to release LL37, a cationic 37 a.a. AMP bearing tandem N-terminal leucine residues (Fig. 1), and further processing can occur in skin to release shorter peptides. hCAP18 is widely expressed in the immune system, and a variety of epithelial cells. Under physiological ionic conditions, LL37 is largely α-helical [36]. Although precise mechanisms vary among different AMPs, the cationic nature of LL37 (Fig. 1) is integral to its antimicrobial activity, as it is attracted to phospholipid head groups of capsular polysaccharides on membrane surfaces, ultimately leading to disruption of membrane integrity [35]. Cathelicidins are also chemotactic for neutrophils, monocytes and T cells (Fig. 1; Ref. [35]). Moreover, LL37 has signal transduction properties, stimulating chemokine and cytokine production by several cell types [35].

## 4. Regulation of *camp* expression by 1,25D is human/primate-specific

Remarkably, neither of the VDREs in the *camp* and *defb2* genes is conserved in mice [27,28]. Gombart et al. [28] also found that *camp* was a 1,25D target gene and showed that the VDRE was embedded in an Alu repeat, a human/primate-specific SINE (short interspersed element) transposable element. In other studies, our genomics work revealed that Alu repeats in the human genome were a rich source of consensus DR2-type [PyG(G/T)TCAnnPyG(G/T)TCA] hor-

mone response elements recognized by retinoic acid receptors [37]. Consensus DR3 elements recognized by the VDR required a precise single nucleotide insertion in the Alu sequence and were far less common [37]. Recent work from Gombart et al. showed that the VDRE-containing Alu repeat in the *camp* gene originated in the primate lineage leading to humans and apes, as well as Old World and New World monkeys [38], dating the insertion event back at least 55–60 million years.

The broad conservation of the *camp* VDRE in primate and human genomes strongly suggests that the insertion event led to a selective advantage and was therefore of considerable functional significance in innate immune responses in these species. In other words, the conserved element pointed to an enhanced role for vitamin D signaling in regulating innate immunity in humans/primates. The VDR is present in most cells of the immune system, including T lymphocytes, neutrophils and, importantly in this regard, antigen presenting cells such as dendritic cells and macrophages [39–43].

The functional importance of the insertion event was underlined by the findings of Modlin and coworkers who have studied signaling through toll-like receptors (TLRs) for a number of years. TLRs are a family of pattern recognition receptors that recognize molecular motifs specific to pathogens and stimulate innate immune responses. Modlin's group established by 2001 that activation of TLR2, on either human or mouse macrophages, stimulates antimicrobial activity against TB infection [44,45]. Furthermore, Modlin's group showed that induction of AMPs in murine macrophages was dependent on inducible nitric oxide synthase (iNOS) activity, and iNOS inhibitors blocked induction of AMP activity in mouse macrophages. Remarkably, however, they had no such effect in human cells. However, Modlin and colleagues found in microarray studies that signaling through human macrophage TLR1/2 heterodimers stimulated with bacterial lipopeptides induced expression of both CYP27B1 and the VDR [29]. Most importantly, in TLR2/1-stimulated human macrophages cultured in human serum, downstream VDR-driven responses, in particular induction of *camp* expression, were strongly dependent on serum 25D concentrations. They were attenuated or absent in serum from vitamin D-deficient individuals; a defect that could be overcome by 25D supplementation. Moreover, in agreement with previous studies [46,47], 25D serum levels from African Americans were markedly lower than those of Caucasians [29]. This provided a clear demonstration of the dependence of innate immune responses on circulating 25D levels. Subsequent work by the group showed that ablation of CAMP expression in *M. tuberculosis*-infected macrophages blocked the antimicrobial action of 1,25D [48].

The above findings are important for two reasons: (i) they provide a clear demonstration of the importance of vitamin D signaling in regulating innate immune responses to common pathogens in humans, and (ii) they underline the important contributions of extra-renal 1α-hydroxylase (CYP27B1) activity to vitamin D physiology. Activated macrophages and dendritic cells express CYP27B1 [49–52], which, unlike renal 1α-hydroxylase, is not regulated by $Ca^{++}$ homeostatic signals, but rather by immune inputs. This expression renders the immune system responsive to circulating levels of 25D. The above studies also emphasize the importance of maintenance of vitamin D sufficiency for optimal innate immune function.

## 5. Regulation of CAMP expression under physiological and pathophysiological conditions *in vivo*

Our initial work [27], and that of Gombart et al. [28] showed that 1,25D-induced *camp* expression in a wide variety of cell human types *in vitro*, suggesting that the regulation is widespread *in vivo*. This has since been borne out in a number of publications. First and foremost, CAMP expression in skin is of considerable (patho)physiological significance. It has been speculated that regulation of *camp* transcription by 1,25D was retained in primates and humans because of the importance of cutaneous UVB-induced vitamin D synthesis in these organisms [53]. CAMP is strongly induced in human keratinocytes under conditions of epithelial wound healing [54–56], providing a molecular basis for the stimulatory role of vitamin D in the process. LL37 produced during wound healing induces cleavage and release of membrane bound heparin-binding-EGF (HB-EGF), which in turn stimulates the EGFR to enhance keratinocyte migration underlying re-epithelialization of the wound [57]. Notably, CYP27B1 expression is also induced in keratinocytes during wound healing, leading to induction of *camp* by locally produced 1,25D [58].

While the results described above represent one of the physiological benefits of the 1,25-CAMP pathway, the expression of cathelicidin in skin has emerged as a double-edged sword. AMP expression is decreased in atopic dermatitis (AD), leading to increased rates of infection of affected skin [59]. However, elevated 1,25D signaling in AD would not be beneficial because it would skew the T helper response towards a Th2 phenotype, due in part to the induction of thymic stromal lymphopoietin, which is a 1,25D target gene in human and mouse [25,60]. In contrast, the persistent inflammatory skin disorder Rosacea is characterized by elevated expression of CAMP, which is abnormally processed and contributes to inflammation [61]. It is possible that the 1,25D-induced CAMP expression may contribute to aggravation of Rosacea caused by exposure to UV light. It has been noted that azole antimycotics (ketoconazole, itraconazole, metronidazole), used in dermatology as antifungal agents in treatment of inflammatory conditions such as rosacea, also block cytochrome P450-driven vitamin D metabolism [62].

It is intriguing that cathelicidin expression is also elevated in psoriasis, another inflammatory skin condition [59], where vitamin D analogs have proven therapeutically effective. Lande et al. [63] showed that CAMP triggers an autoimmune response in psoriasis by activating TLR signaling in plasmatoid dendritic cells (pDCs) of the skin, which are specialized for sensing viral and certain microbial infections. CAMP binds directly to DNA in pDCs of psoriatic skin forming condensed aggregates that are delivered to TLR9 receptors, triggering an immune response. The DNA binding capacity of CAMP arises from its cationic, amphipathic structure. It is likely that that anti-psoriatic activity of vitamin D analogues result from their antiproliferative activities and their capacity to suppress Th1-driven immune responses.

Skin is not the only site of vitamin D-regulated cathelicidin expression in epithelial cells. A remarkable recent study by D'Aldebert et al. showed that both the VDR and CAMP were highly expressed in biliary epithelial cells in human liver [64]. In a healthy individual the biliary tract is free of microbes, unlike the lumen of the intestines, consistent with robust antimicrobial defenses. Importantly, D'Aldebert et al. showed that CAMP expression in bilary epithelial cells was regulated by physiological and therapeutic bile salts via signaling through the VDR and the related farnesoid X receptor (FXR). FXR is a physiological bile acid receptor. However, there is also strong evidence that the VDR can function as a bile acid sensor [65]. Indeed, the secondary bile acid lithocholic acid was found to be an efficacious inducer of CAMP mRNA and protein in normal human keratinocytes [66]. Notably, CAMP expression induced by bile acids could be further enhanced by 1,25D in the D'Aldebert et al. study, and therapeutic bile acid treatment of inflammatory biliary disease enhanced expression of both the VDR and CAMP [64].

Similarly, antimicrobial defenses are essential for maintaining the integrity of the epithelial lining of the lungs. In our studies,

we found that 1,25D strongly induced *camp* expression in human Calu-3 lung carcinoma cells, a line that retains characteristics of upper airway epithelial cells [27]. We also found that Calu-3 cells treated with 1,25D released antimicrobial activity against *Pseudomonas aeruginosa*, a pathogen responsible for chronic infections in patients with cystic fibrosis (CF). Yim et al. took these findings a step further by showing that 1,25D-induced *camp* transcription 10-fold in normal human bronchial epithelial (NHBE) cells [67]. This regulation was conserved in bronchial epithelial cells derived from CF patients homozygous for inactivating mutation of the *cftr* gene. Additionally, Yim et al. showed that NHBE cells could be induced to release antimicrobial activity against *P. aeruginosa* that could be partially blocked by preincubation with an anti-LL37 antibody [67].

Other active sites of active vitamin D metabolism and signaling signaling exist in the endometrium and placenta during pregnancy. Placental CYP27B1 is produced early in gestation, prior to the onset of skeletal development [68], consistent with vitamin D metabolism contributing to physiological responses other than calcium homeostasis. Hewison and colleagues showed that 25D is metabolized to 1,25D in cultured maternal decidual cells from first-trimester human pregnancies, and drives induction of CAMP expression [69]. More recently, the group showed that CAMP is also robustly induced from 1,25D produced *in situ* in placental trophoblast cells and promotes antibacterial responses [70]. CYP27B1 expression in trophoblasts in this study appeared to be independent of TLR signaling pathways. Collectively, these results show that CAMP expression can be induced in both maternal and fetal cells during gestation by intracrine vitamin D signaling. There is some evidence that trophoblasts can be phagocytic, and Liu et al. [70] hypothesized that intracrine production of CAMP may lead to its delivery to phagocytic vesicles, similar to what is observed in macrophages [29].

## 6. A therapeutic role for vitamin D analogues in regulation of cathelicidin expression

As described above, cathelicidin expression has turned out to be a double-edged sword in skin pathophysiology. One the one hand, cathelicidin is an inducer of epithelial wound healing, which is beneficial. On the other, its elevated expression contributes to the pathogenesis of inflammatory conditions such as psoriasis and rosacea [61,63]. The beneficial effects of vitamin D analogues in psoriasis likely arise from their antiproliferative activities and capacity to suppress inflammatory immune responses. Conversely, augmenting CAMP expression in the absence of stimulation of Th2-driven immune responses may be beneficial in treatment of atopic dermatitis.

Collectively, the above findings strongly suggest that there are therapeutic roles for vitamin D analogs that separate regulation of *camp* induction from broader immune system regulation, or from the antiproliferative functions of 1,25D. A recent study [71] showed differential regulation of *camp* and other AMP genes by 1,25D and some of its analogs in keratinocytes. In our laboratory, we have also synthesized vitamin D analogs that are efficacious inducers of *cyp24*, have 1,25D-like antiproliferative activity, but weakly regulate expression of *camp* (unpublished results), supporting the idea that such compounds can be generated. Conversely, a compound that functioned as an efficacious inducer of CAMP and other AMPs, but weakly induced expression of TSLP and Th2 responses might be beneficial for treatment of AD. Finally, the results of D'Aldebert et al., strongly suggest that non-calcemic, efficacious inducers of CAMP expression may also be beneficial in treatment alone, or in combination with, therapeutic bile acids in management of inflammatory biliary disease, particular given that fact that therapeutic bile acids were found to enhance expression of the VDR [64].

## References


[1] R. Lin, J.H. White, The pleiotropic actions of vitamin D, BioEssays 26 (2004) 21–28.
[2] Unraveling the enigma of vitamin D. Beyond Discovery, National Academy of Sciences, USA. http://www.beyonddiscovery.org/Includes/DBLink.asp?ID=1176.
[3] M.F. Holick, Resurrection of vitamin D deficiency and rickets, J. Clin. Invest. 116 (2006) 2062–2072.
[4] M.F. Holick, High prevalence of vitamin D inadequacy and implications for health, Mayo Clin. Proc. 81 (2006) 353–373.
[5] M.F. Holick, Vitamin D deficiency, New Engl. J. Med. 357 (2007) 266–281.
[6] E. Giovannucci, Y. Liu, E.B. Rimm, B.W. Hollis, C.S. Fuchs, M.J. Stampfer, W.C. Willett, Prospective study of predictors of vitamin D status and cancer incidence and mortality in men, J. Natl. Cancer Inst. 98 (2006) 451–459.
[7] K.K. Deeb, D.L. Trump, C.S. Johnson, Vitamin D signalling pathways in cancer: potential for anticancer therapeutics, Nat. Rev. Cancer 7 (2007) 684–700.
[8] G.A. Rook, J. Steele, L. Fraher, S. Barker, R. Karmali, J. O'Riordan, J. Stanford, Vitamin D3, interferon, and control of proliferation of Mycobacterium tuberculosis by human monocytes, Immunology 57 (1986) 159–163.
[9] P.D.O. Davies, R.C. Brown, J.S. Woodhead, Serum concentrations of vitamin D metabolites in untreated tuberculosis, Thorax 40 (1985) 187–190.
[10] J.M. Grange, P.D.O. Davies, R.C. Brown, J.S. Woodhead, T. Kardjito, A study of vitamin D levels in Indonesian patients with untreated pulmonary tuberculosis, Tubercle 66 (1985) 187–191.
[11] R.J. Wilkinson, M. Llewelyn, Z. Toossi, P. Patel, G. Pasvol, A. Lalvani, D. Wright, M. Latif, R.N. Davidson, Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis amongst Gujarati Asians in West London: a case-control study, Lancet 355 (2000) 618–621.
[12] A.R. Martineau, R.J. Wilkinson, K.A. Wilkinson, S.M. Newton, B. Kampmann, B.M. Hall, G.E. Packe, R.N. Davidson, S.M. Eldridge, Z.J. Maunsell, S.J. Rainbow, J.L. Berry, C.J. Griffiths, A Single Dose of Vitamin D Enhances Immunity to Mycobacteria, Am. J. Resp. Crit. Care Med. 176 (2007) 208–213.
[13] J.B. Cheng, M.A. Levine, N.H. Bell, D.J. Mangelsdorf, D.W. Russell, Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase, Proc. Natl. Acad. Sci. USA 101 (2004) 7711–7715.
[14] G. Jones, S.A. Strugnell, H.F. DeLuca, Current understanding of the molecular actions of vitamin D, Physiol. Rev. 78 (2005) 1193–1231.
[15] D.E. Prosser, G. Jones, Enzymes involved in the activation and inactivation of vitamin D, Trends Biochem. Sci. 29 (2004) 664–673.
[16] R. Shinkyo, T. Sakaki, M. Kamakura, M. Ohta, K. Inouye, Metabolism of vitamin D by human microsomal CYP2R1, Biochem. Biophys. Res. Commun. 324 (2004) 451–457.
[17] A. Chawla, J. Repa, R.M. Evans, D.J. Mangelsdorf, Nuclear receptors and lipid physiology: Opening the X-files, Science 294 (2001) 1866–1870.
[18] N. Rochel, J.M. Wurtz, A. Mitschler, B. Klaholz, D. Moras, The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand, Mol. Cell 5 (2000) 173–179.
[19] L. Drocourt, J.C. Ourlin, J.M. Pascussi, P. Maurel, M.J. Vilarem, Expression of CYP3A4, CYP2B6, and CYP2C9 is regulated by the vitamin D receptor pathway in primary human hepatocytes, J. Biol. Chem. 277 (2002) 25125–25132.
[20] P.D. Thompson, P.W. Jurutka, G.K. Whitfield, S.M. Myskowski, K.R. Eichhorst, C.E. Dominguez, C.A. Haussler, M.R. Haussler, Liganded VDR induces CYP3A4 in small intestinal and colon cancer cells via DR3 and ER6 vitamin D responsive elements, Biochem. Biophys. Res. Commun. 299 (2002) 730–738.
[21] K.E. Thummel, C. Brimer, K. Yasuda, J. Thottassery, T. Senn, Y. Lin, H. Ishizuka, E. Kharasch, J. Schuetz, E. Schuet, Transcriptional control of intestinal cytochrome P-4503A by 1alpha,25-dihydroxyvitamin D3, Mol. Pharmacol. 60 (2001) 1399–1406.
[22] S. Kim, M. Yamazaki, L.A. Zella, N.K. Shevde, J.W. Pike, Activation of receptor activator of NF-kappa B ligand gene expression by 1,25-dihydroxyvitamin D-3 is mediated through multiple long-range enhancers, Mol. Cell. Biol. 26 (2006) 6469–6486.
[23] N. Akutsu, R. Lin, Y. Bastien, A. Bestawros, D.J. Enepekides, M.J. Black, J.H. White, Regulation of gene expression by 1α,25-dihydroxyvitamin $D_3$ and its analog eb1089 under growth inhibitory conditions in squamous carcinoma cells, Mol. Endocrinol. 15 (2001) 1127–1139.
[24] R. Lin, Y. Nagai, R. Sladek, Y. Bastien, J. Ho, K. Petrecca, G. Sotiropoulou, E.P. Diamandis, T. Hudson, J.H. White, Expression profiling in squamous carcinoma cells reveals pleiotropic effects of vitamin D3 signaling on cell proliferation, differentiation and immune system regulation, Mol. Endocrinol. 16 (2002) 1243–1256.
[25] T.T. Wang, L. Tavera-Mendoza, D. Laperriere, Y. Nagai, N. Burton MacLeod, E. Libby, R. Zhang, V. Bourdeau, A. Konstorum, B. Lallement, S. Mader, J.H. White, Large-scale in silico and microarray-based genomic screening of 1,25-dihydroxyvitamin $D_3$ target genes, Mol. Endocrinol. 19 (2005) 2685–2695.
[26] J.H. White, Profiling 1,25-dihydroxyvitamin D3-regulated gene expression by microarray analysis, J. Ster. Biochem. Mol. Biol. 89–90C (2004) 239–244.
[27] T.T. Wang, F. Nestel, V. Bourdeau, Y. Nagai, Q. Wang, J. Wu, L. Tavera-Mendoza, R. Lin, J.W. Hanrahan, S. Mader, J.H. White, Cutting Edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression, J. Immunol. 173 (2004) 2909–2912.
[28] A.F. Gombart, N. Borregaard, H.P. Koeffler, 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 D-3, FASEB J. 19 (2005) 1067–1077.

[29] P.T. Liu, S. Stenger, H.Y. Li, L. Wenzel, B.H. Tan, S.R. Krutzik, M.T. Ochoa, J. Schauber, K. Wu, C. Meinken, D.L. Kamen, M. Wagner, R. Bals, A. Steinmeyer, U. Zugel, R.L. Gallo, D. Eisenberg, M. Hewison, B.W. Hollis, J.S. Adams, B.R. Bloom, R.L. Modlin, Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response, Science 311 (2006) 1173–1770.
[30] G. Weber, J.D. Heilborn, C.I. Chamorro Jimenez, A. Hammarsjö, H. Törmä, M. Ståhle, Vitamin, D induces the antimicrobial protein hCAP18 in human skin, J. Invest. Dermatol. 124 (2005) 1080–1082.
[31] R.E.W. Hancock, H.G. Sahl, Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies, Nature Biotechnol. 24 (2006) 1551–1557.
[32] H. Jenssen, P. Hamill, R.E.W. Hancock, Peptide antimicrobial agents, Clin. Microbiol. Rev. 19 (2006) 491–511.
[33] A. Patil, A.L. Hughes, G. Zhang, Rapid evolution and diversification of mammalian α-defensins as revealed by comparative analysis of rodent and primate genomes, Physiol. Genomics 20 (2004) 1–11.
[34] J.H. White, Vitamin D signaling, infectious diseases and regulation of innate immunity, Infect. Immun. 76 (2008) 3837–3843.
[35] Y.P. Lai, R.L. Gallo, AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense, Trends Immunol. 30 (2009) 131–141.
[36] M.H. Braff, A. Bardan, V. Nizet, R.L. Gallo, Cutaneous defense mechanisms by antimicrobial peptides, J. Invest. Dermatol. 125 (2005) 9–13.
[37] D. Laperriere, T.T. Wang, J.H. White, S. Mader, Genome-wide mapping and function of consensus DR-type nuclear receptor hormone response elements in Alu repeats, BMC Genomics 8 (2007) 23, doi:10.1186/1471-2164-8-23.
[38] A.F. Gombart, T. Saito, H.P. Koeffler, Exaptation of an ancient Alu short interspersed element provides a highly conserved vitamin D-mediated innate immune response in humans and primates, BMC Genomics 10 (2009) 321, doi:10.1186/1471-2164-10-321.
[39] D.M. Provvedini, C.D. Tsoukas, L.J. Deftos, S.C. Manolagas, 1,25-Dihydroxyvitamin D3 receptors in human leukocytes, Science 221 (1983) 1181–1183.
[40] A.K. Bhalla, E.P. Amento, B. Serog, L.H. Glimcher, 1,25-Dihydroxyvitamin D3 inhibits antigen-induced T cell activation, J. Immunology 133 (1984) 1748–1754.
[41] A. Brennan, D.R. Katz, J.D. Nunn, S. Barker, M. Hewison, L.J. Fraher, J.L. O'Riordan, Dendritic cells from human tissues express receptors for the immunoregulatory vitamin D3 metabolite, dihydroxycholecalciferol, Immunology 61 (1987) 457–461.
[42] L. Adorini, Intervention in autoimmunity: The potential of vitamin D receptor agonists, Cell. Immunol. 233 (2005) 115–124.
[43] A.W. Norman, Minireview: vitamin D receptor: New assignments for an already busy receptor, Endocrinology 147 (2006) 5542–5548.
[44] H. Brightbill, D.H. Libraty, S.R. Krutzik, R.B. Yang, J.T. Belisle, J.R. Bleharski, M. Maitland, M.V. Norgard, S.E. Plevy, S.T. Smale, P.J. Brennan, B.R. Bloom, P.J. Godowski, R.L. Modlin, Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors, Science 285 (1999) 732–736.
[45] S. Thoma-Uszynski, S. Stenger, O. Takeuchi, M.T. Ochoa, M. Engele, P.A. Sieling, P.F. Barnes, M. Rollinghoff, P.L. Bolcskei, M. Wagner, S. Akira, M.V. Norgard, J.T. Belisle, J.T. Godowski, B.R. Bloom, R.L. Modlin, Induction of direct antimicrobial activity through mammalian toll-like receptors, Science 291 (2001) 1544–1547.
[46] S. Nesby-O'Dell, K.S. Scanlon, M.E. Cogswell, C. Gillespie, B.W. Hollis, A.C. Looker, C. Allen, C. Doughertly, E.W. Gunter, E.A. Bowman, Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: Third National Health and Nutrition Examination Survey, 1988–1994, Am. J. Clin. Nutr. 76 (2002) 187–192.
[47] W.W. Stead, J.W. Senner, W.T. Reddick, J.P. Lofgren, Racial differences in sensitivity to infection by mycobacterium tuberculosis, N. Engl. J. Med. 322 (1990) 422–427.
[48] P.T. Liu, S. Stenger, D.H. Tang, R.L. Modlin, Cutting Edge: Vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin, J. Immunol. 179 (2007) 2060–2063.
[49] J.S. Adams, O.P. Sharma, M.A. Gacad, F.R. Singer, Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis, J. Clin. Invest. 72 (1983) 1856–1860.
[50] L. Overbergh, B. Decallonne, D. Valckx, A. Verstuyf, J. Depovere, J. Laureys, O. Rutgeerts, R. Saint-Arnaud, R. Bouillon, C. Mathieu, Identification and immune regulation of 25-hydroxyvitamin D-1-alpha-hydroxylase in murine macrophages, Clin. Exp. Immunol. 120 (2000) 139–146.
[51] L. Overbergh, K. Stoffels, M. Waer, A. Verstuyf, R. Bouillon, C. Mathieu, Immune regulation of 25-hydroxyvitamin D-1 alpha-hydroxylase in human monocytic THP1 cells: Mechanisms of interferon-gamma-mediated induction, J. Clin. Endo. Metab. 91 (2006) 3566–3574.
[52] K. Stoffels, L. Overbergh, A. Giulietti, L. Verlinden, R. Bouillon, C. Mathieu, Immune regulation of 25-hydroxyvitamin-D-3-1 alpha-hydroxylase in human monocytes, J. Bone Min. Res. 21 (2006) 37–47.
[53] S. Segaert, Vitamin D regulation of cathelicidin in the skin: toward a renaissance of vitamin D in dermatology? J. Invest. Dermatol. 128 (2008) 773–775.
[54] M. Carretero, M.J. Escamez, M. Garcia, B. Duarte, A. Holguin, L. Retamosa, J.L. Jorcano, M. del Rio, F. Larcher, In vitro and in vivo wound healing-promoting activities of human cathelicidin LL-37, J. Invest. Dermatol. 128 (2008) 223–236.
[55] R.A. Dorschner, V.K. Pestonjamasp, S. Tamakuwala, T. Ohtake, J. Rudisill, V. Nizet, B. Agerberth, G.H. Gudmundsson, R.L. Gallo, Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A Streptococcus, J. Invest. Dermatol. 117 (2001) 91–97.
[56] J.D. Heilborn, M.F. Nilsson, G. Kratz, G. Weber, O. Sorensen, N. Borregaard, M. Stahle-Backdahl, The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium, J. Invest. Dermatol. 120 (2003) 379–389.
[57] S. Tokumaru, K. Sayama, Y. Shirakata, H. Komatsuzawa, K. Ouhara, Y. Hanakawa, Y. Yahata, M. Dai, H. Tohyama, L. Nagai, S. Yang, A. Higashiyama, M. Yoshimura, Sugai, K. Hashimoto, Induction of keratinocyte migration via transactivation of the epidermal growth factor receptor by the antimicrobial peptide LL-37, J. Immunol. 175 (2005) 4662–4668.
[58] J. Schauber, R.A. Dorschner, A.B. Coda, A.S. Büchau, P.T. Liu, D. Kiken, Y.R. Helfrich, S. Kang, H.Z. Elalieh, A. Steinmeyer, U. Zügel, D.D. Bikle, R.L. Modlin, R.L. Gallo, Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D–dependent mechanism, J. Clin. Invest. 117 (2007) 803–811.
[59] M. Zasloff, Sunlight, vitamin D, and the innate immune defenses of the human skin, J. Invest. Dermatol. 125 (2005), pp. xvi–xvii.
[60] M. Li, P. Hener, Z. Zahang, S. Kato, D. Metzger, P. Chambon, Topical vitamin D3 and low-calcemic analogs induce thymic stromal lymphopoietin in mouse keratinocytes and trigger an atopic dermatitis, Proc. Natl. Acad. Sci. USA 103 (2006) 11736–11741.
[61] K. Yamasaki, A. Di Nardo, A. Bardan, M. Murakami, T. Ohtake, A. Coda, R.A. Dorschner, C. Bonnart, P. Descargues, A. Hovnanian, V.B. Morhenn, R.L. Gallo, Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea, Nature Med. 13 (2007) 975–980.
[62] S. Segaert, T. Simonart, The epidermal vitamin D system and innate immunity: some more light shed on this unique photoendocrine system? Dermatology 217 (2008) 7–11.
[63] R. Lande, J. Gregorio, V. Facchinetti, B. Chatterjee, Y.H. Wang, B. Homey, W. Cao, Y.H. Wang, B. Su, F.O. Nestle, T. Zal, I. Mellman, J.M. Schröder, Y.J. Liu, M. Gilliet, Plasmacytoid dendritic cells sense self-DNA coupled with anti-microbial peptide, Nature 449 (2007) 563–568.
[64] E. D'Aldebert, M.J. Biyeyeme, BiX.X. Mve, M. Mergey, D. Wendum, D. Firrincieli, A. Coilly, L. Fouassier, C. Corpechot, R. Poupon, C. Housset, N. Chignard, Bile salts control the antimicrobial peptide cathelicidin through nuclear receptors in the human biliary epithelium, Gastroenterology 136 (2009) 1435–1443.
[65] M. Makishima, T.T. Lu, W. Xie, G.K. Whitfield, H. Domoto, R.M. Evans, M.R. Haussler, D.J. Mangelsdorf, Vitamin D receptor as an intestinal bile acid sensor, Science 296 (2002) 1313–1316.
[66] M. Peric, S. Koglin, Y. Dombrowski, K. Groß, E. Bradac, T. Ruzicka, J. Schauber, VDR and MEK-ERK dependent induction of the antimicrobial peptide cathelicidin in keratinocytes by lithocholic acid, Mol. Immunol. (2009), doi:10.1016/j.molimm.2009.08.010.
[67] S. Yim, P. Dhawan, C. Ragunath, S. Christakos, G. Diamond, Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3), J. Cyst. Fibros. 6 (2007) 403–410.
[68] D. Zehnder, K.N. Evans, M.D. Kilby, J.N. Bulmer, B.A. Innes, P.M. Stewart, M. Hewison, The ontogeny of 25-hydroxyvitamin D(3) 1alpha-hydroxylase expression in human placenta and decidua, Am. J. Pathol. 161 (2002) 105–114.
[69] K.N. Evans, L. Nguyen, J. Chan, B.A. Innes, J.N. Bulmer, M.D. Kilby, M. Hewison, Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells, Biol. Reprod. 75 (2006) 816–822.
[70] N. Liu, A.T. Kaplan, J. Low, L. Nguyen, G.Y. Liu, O. Equils, M. Hewison, Vitamin D induces innate antibacterial responses in human trophoblasts via an intracrine pathway, Biol. Reprod. 80 (2009) 398–406.
[71] M. Peric, S. Koglin, Y. Dombrowski, K. Gross, E. Bradac, A. Büchau, A. Steinmeyer, U. Zügel, T. Ruzicka, J. Schauber, Vitamin D analogs differentially control antimicrobial peptide/"alarmin" expression in psoriasis, PLoS One 4 (July 7) (2009) e6340.