Nonclassic Actions of Vitamin D

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Context: Vitamin D receptors are found in most tissues, not just those participating in the classic actions of vitamin D such as bone, gut, and kidney. These nonclassic tissues are therefore potential targets for the active metabolite of vitamin D, $1,25(OH)_2D$. Furthermore, many of these tissues also contain the enzyme CYP27B1 capable of producing $1,25(OH)_2D$ from the circulating form of vitamin D. This review was intended to highlight the actions of $1,25(OH)_2D$ in several of these tissues but starts with a review of vitamin D production, metabolism, and molecular mechanism.

Evidence Acquisition: Medline was searched for articles describing actions of $1,25(OH)_2D$ on parathyroid hormone and insulin secretion, immune responses, keratinocytes, and cancer.

Evidence Synthesis: Vitamin D production in the skin provides an efficient source of vitamin D. Subsequent metabolism to $1,25(OH)_2D$ within nonrenal tissues differs from that in the kidney. Although vitamin D receptor mediates the actions of $1,25(OH)_2D$, regulation of transcriptional activity is cell specific. $1,25(OH)_2D$ inhibits PTH secretion but promotes insulin secretion, inhibits adaptive immunity but promotes innate immunity, and inhibits cell proliferation but stimulates their differentiation.

Conclusions: The nonclassic actions of vitamin D are cell specific and provide a number of potential new clinical applications for $1,25(OH)_2D_3$ and its analogs. However, the use of vitamin D metabolites and analogs for these applications remains limited by the classic actions of vitamin D leading to hypercalcemia and hypercalcuria. (***J Clin Endocrinol Metab*** **94: 26–34, 2009)**

In the past few years, there has been growing appreciation for the many roles of vitamin D and its active metabolites in a large number of tissues. This has been stimulated by the appreciation that most tissues in the body have receptors for the active form of vitamin D, 1,25 dihydroxyvitamin D [$1,25(OH)_2D$] or calcitriol. These receptors are named appropriately vitamin D receptors (VDRs), and tissues with VDR are potential target tissues. Furthermore, many of these tissues also contain the enzyme, CYP27B1, responsible for converting the major circulating metabolite of vitamin D, 25 hydroxyvitamin D (25OHD), to $1,25(OH)_2D$. Regulation of CYP27B1 in these nonrenal tissues generally differs from that in the kidney and may be more substrate dependent. This has led to the concept that maintenance of adequate 25OHD levels in the blood is required for vitamin D regulation of a large number of physiologic functions beyond that of the classic actions involved with bone mineral metabolism. This review is intended first to cover the basics of vitamin D production, metabolism, and molecular mechanism of action and then examine the impact of vitamin D and its metabolites on tissues that are not principally concerned with regulation of bone mineral metabolism. Two forms of vitamin D exist: vitamin D_3 or cholecalciferol and vitamin D_2 or ergocalciferol. The former is produced in the skin under the influence of UVB radiation (UVR); the latter is produced by UVR in a variety of plant materials and yeast (Fig.1). Differences exist in their binding to the major transport protein in blood, vitamin D binding protein, and in their metabolism because of the differences in the chemistry of their side chains, with the result that single doses of D_2 lead to lower levels of circulating 25OHD than single doses of D_3 (1, 2), although daily administration of D_2 and D_3 maintains comparable levels of 25OHD (3). At the tissue level, these differences are minor in that the biologic activity of $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$ appear to be comparable at least with respect to binding to VDR. Therefore, references to vitamin D or D metabolites will refer to both forms unless otherwise indicated with a specific subscript.

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Abbreviations: D_3, Vitamin D_3 DRIP, vitamin D receptor interacting protein complex; FGF, fibroblast growth factor; HAT, histone acetyl transferase; Hr, hairless; IFN, interferon; 25OHD, 25 hydroxyvitamin D; $1,25(OH)_2D$, 1,25 dihydroxyvitamin D; SRC, steroid receptor activator complex; Th, T helper; TLR, toll-like receptor; Treg, regulatory T cells; UVR, UVB radiation; VDR, vitamin D receptor; VDRE, vitamin D response element.

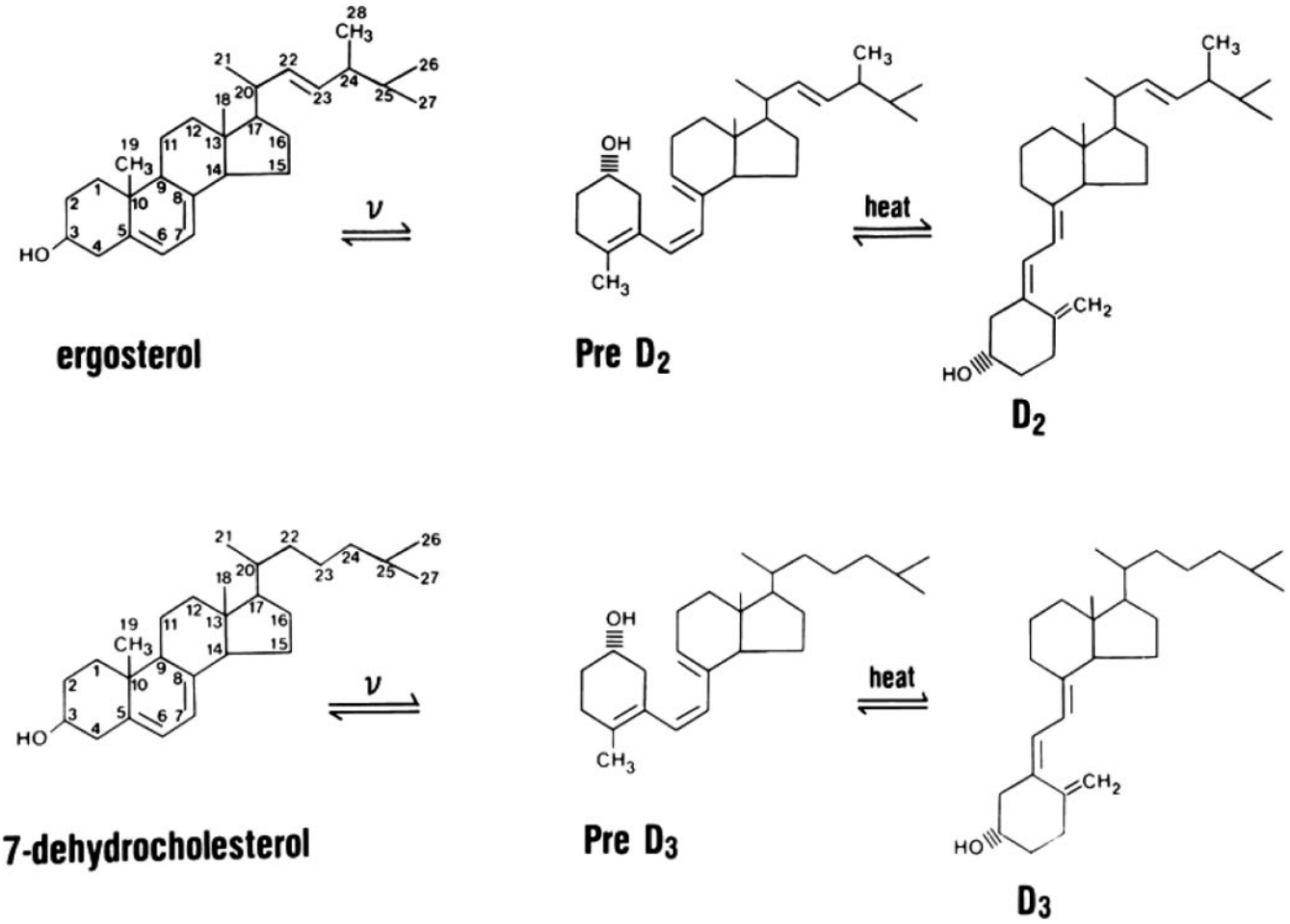

FIG. 1. Production of vitamin D_2 and vitamin D_3. Ergosterol in plants and 7-dehydrocholesterol in skin are the precursors for vitamin D_2 and vitamin D_3, respectively. UV light B breaks the B chain of each molecule to form the pre-D isomer, which then undergoes isomerization to D. D_2 and D_3 differ only in the side chain in which D_2 has a double bond between C22–C23 and a methyl group at C24. These differences alter somewhat its binding to DBP and metabolism.

Vitamin D_3 production

Vitamin D_3 (D_3) is produced in the skin from 7-dehydrocholesterol through a two-step process in which the B ring is broken under UVR (*e.g.* sunlight), and the pre-D_3 so formed isomerizes to D_3 in a thermo-sensitive but noncatalytic process. Holick *et al.* (4–6) demonstrated that the formation of pre-D_3 is relatively rapid, reaching a maximum within hours. Both intensity of UVR and level of pigmentation in the skin regulate the rate of pre-D_3 formation but not the maximal level achieved. With continued UVR exposure, pre-D_3 is converted to the biologically inactive lumisterol. Tachysterol is also formed but, like pre-D_3, does not accumulate with extended UVR. The formation of lumisterol and tachysterol is reversible and can be converted back to pre-D_3 as pre-D_3 levels fall. Thus, prolonged exposure to sunlight will not produce toxic amounts of D_3 because of the photoconversion of pre-D_3 to lumisterol and tachysterol as well as the photoconversion of D_3 itself to suprasterols I and II and 5,6 transvitamin D_3 (4). Melanin in the epidermis, by absorbing UVR, reduces D_3 production. The intensity of UVR from sunlight varies according to season and latitude, so the farther one lives from the equator, the less time of the year one can rely on solar exposure to produce D_3. Clothing (7) and sunscreen (8) effectively prevent D_3 production in the covered areas.

Vitamin D metabolism

To be biologically active, vitamin D must first be converted to 25OHD. There are a number of cytochrome P450 enzymes, both mitochondrial and microsomal, capable of this function (9), although CYP27A1 has received the most study. These enzymes are principally but not exclusively found in the liver and have a high capacity for substrate vitamin D. The different enzymes have different substrate specificities for the two forms of vitamin D, but this has not proven to be physiologically significant. 25OHD production is primarily substrate dependent, so serum 25OHD is a reliable indicator of vitamin D status (10).

To be fully active, 25OHD must be further converted to $1,25(OH)_2D$ via CYP27B1, a mitochondrial P450 enzyme. Although the proximal renal tubule is the major source of $1,25(OH)_2D$ production for the body, the enzyme is also found in a number of extrarenal sites such as immune cells, epithelia of many tissues, bone, and parathyroid glands (11), in which it functions to provide $1,25(OH)_2D$ for local consumption as an intracrine or paracrine factor. Regulation of CYP27B1 in the proximal renal tubule is controlled by PTH and fibroblast growth factor (FGF)-23, which stimulate and inhibit, respectively, its expression (Fig. 2). CYP27B1 expression is also inhibited by $1,25(OH)_2D$ through a negative vitamin D response el-

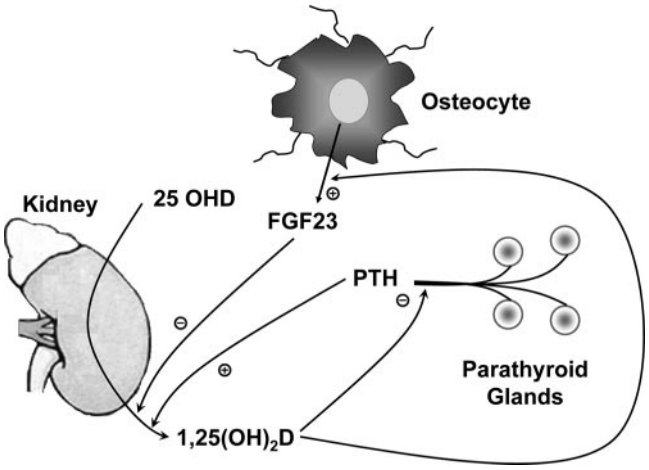

FIG. 2. Regulation of $1,25(OH)_2D$ production in the kidney. PTH stimulates and FGF23 inhibits $1,25(OH)_2D$ production in the kidney. In turn $1,25(OH)_2D$ inhibits PTH production and secretion from the parathyroid glands and stimulates FGF23 production from bone.

ement in its promoter to which VDR binds indirectly (12). Additionally $1,25(OH)_2D_3$ negatively regulates its own levels by inducing CYP24, like CYP27B1, a mitochondrial P450, that catabolizes both $1,25(OH)_2D$ and 25OHD (13).

Control of $1,25(OH)_2D_3$ production (and levels) by nonrenal tissues differs. When macrophages are activated via specific toll-like receptors (TLRs), CYP27B1 is induced (14). In these cells $1,25(OH)_2D_3$ production appears to be governed primarily by the availability of substrate (14). PTH and FGF23 do not regulate CYP27B1 in these cells due presumably to lack of their cognate receptors. Furthermore, macrophages may express a nonfunctional alternatively spliced form of CYP24 located in the cytoplasm that potentially interferes with substrate access to the mitochondrial CYP24 (15), thus reducing 25OHD and $1,25(OH)_2D$ catabolism in these cells. The keratinocyte also contains CYP27B1, which like the macrophage enzyme can be induced by activation of specific TLRs (16). Both TNF-α and interferon (IFN)-γ stimulate $1,25(OH)_2D_3$ production by keratinocytes (17, 18), suggesting that the keratinocyte like the macrophage uses $1,25(OH)_2D$ for important host defense mechanisms. Unlike the macrophage, the keratinocyte has a fully functional CYP24, and its induction by $1,25(OH)_2D$ is the major means by which $1,25(OH)_2D$ limits its own levels in the epidermis (19).

Mechanism of action

The mechanism of action of the active form of $1,25(OH)_2D$ is similar to that of other steroid hormones and is mediated by its binding to VDR. VDR is a member of the superfamily of nuclear hormone receptors including receptors for steroid and thyroid hormones and retinoic acid. VDR functions as a heterodimer generally with the retinoid X receptor for regulation of vitamin D target genes. These heterodimeric complexes interact with specific DNA sequences [vitamin D response elements (VDREs)], generally within the promoter of target genes, resulting in either activation or repression of transcription (20–23). The control of transcription requires the additional recruitment of coregulators (24). These VDREs can be many thousand nucleotides away from the transcription start site, however (25). For activation, two major coactivator complexes have been identified: the steroid receptor activator complex (SRC) comprised of the p160 family of SRC1, SRC2, and SRC3 coactivators (26) and the vitamin D receptor interacting protein complex (DRIP) or mediator complex (22). These coactivator complexes interact with the C-terminal (activation function-2 or AF-2) domain of VDR after ligand binding and recruit cAMP response element-binding (CREB) protein-binding protein and other histone acetyl transferases (HATs) and methyltranferases to the VDR, resulting in a multisubunit complex (20–23). The HAT and methyltranferase activity of the SRC complex is thought to destabilize the interaction between DNA and the histone core, enabling transcription to occur. The DRIP complex does not have HAT activity but functions, at least in part, through recruitment of RNA polymerase II to the transcription start site. These complexes do not bind to the VDR at the same time (27). It is not clear whether these different complexes shuttle in and out of the transcription machinery, act sequentially, or act on different genes. In skin we have found that DRIP is more abundant in the proliferating keratinocyte, whereas SRC3 is more abundant in differentiated keratinocytes (28). Furthermore, different genes regulated by VDR in these cells require different coactivators (29, 30), indicating that at least in the keratinocyte, these coactivator complexes serve different functions and different genes.

Corepressors block VDR-mediated transcriptional activity. Well-studied corepressors include nuclear corepressor (NCoR) and silencing mediator of retinoic acid and thyroid receptor (SMRT). These corepressors typically bind VDR in the absence of $1,25(OH)_2D$ and are displaced when $1,25(OH)_2D$ binding recruits the coactivators to the VDR. One recently discovered corepressor with more limited tissue distribution and function is hairless (Hr). Hr is found primarily in brain, epidermis, hair follicles, and other epithelia, although trace levels of expression have been found elsewhere (31). Lack of Hr like lack of VDR results in failure of hair follicle cycling (32). Hr binds to VDR and like other corepressors inhibits its transcriptional activity in a manner relieved by $1,25(OH)_2D$ (33). Other transcription factors also modulate the activity of VDR. β-Catenin binds to VDR and regulates its ability to induce a number of genes (and vice versa) (34). Similarly, YY1 and CCAAT enhancer binding proteins-β and -δ modulate VDR-mediated transcription (35–37). These coregulators differ in their tissue distribution, providing for substantial tissue specificity in the actions of $1,25(OH)_2D$ and VDR.

Nonclassic target tissues

The nonclassic actions of vitamin D can be categorized into three general effects: regulation of hormone secretion, regulation of immune function, and regulation of cellular proliferation and differentiation. These categories are somewhat artificial, and the effects of $1,25(OH)_2D$ on any given tissue may involve actions in more than one of these categories. Nevertheless, the categories serve a pedagogic purpose.

Regulation of hormone secretion

The ability of $1,25(OH)_2D$ to regulate hormone secretion plays an important role in maintaining normal bone mineral

homeostasis (Fig. 2) and in the case of insulin secretion illustrates an important nonclassical action of therapeutic importance.

PTH

$1,25(OH)_2D$ inhibits the synthesis and secretion of PTH (38) and prevents the proliferation of the parathyroid gland (38, 39). The parathyroid gene contains a negative VDRE through which $1,25(OH)_2D$ exerts its suppression (38). $1,25(OH)_2D$ also upregulates the calcium-sensing receptor (40), which by sensitizing the parathyroid gland to calcium inhibition provides an additional means by which $1,25(OH)_2D$ regulates PTH production and secretion. Because PTH stimulates $1,25(OH)_2D$ production in the kidney, this inhibition of PTH production and secretion provides an important feedback loop. These actions of $1,25(OH)_2D$ are exploited clinically through the use of $1,25(OH)_2D$ and analogs to control secondary hyperparathyroidism in renal failure. Furthermore, the ability of the parathyroid gland to make its own $1,25(OH)_2D$ provides an explanation for the reciprocal relationship between 25OHD and PTH levels, but not between $1,25(OH)_2D$ and PTH levels, in the blood of subjects with vitamin D insufficiency (41).

Insulin

$1,25(OH)_2D$ stimulates insulin secretion, although the mechanism is not well defined (42, 43). VDR and calbindin-D_{28k} are found in pancreatic β-cells (44, 45), and studies using calbindin-D_{28k} null mice have suggested that calbindin-D_{28k}, by regulating intracellular calcium, can modulate depolarization-stimulated insulin release (46). Furthermore, calbindin-D_{28k}, by buffering calcium, can protect against cytokine mediated destruction of β-cells (47). A number of mostly case control and observational studies have suggested that vitamin D deficiency contributes to increased risk for type 2 diabetes mellitus (48).

FGF23

FGF23 is produced primarily by bone, and in particular by osteoblasts and osteocytes. $1,25(OH)_2D_3$ stimulates this process, but the mechanism is not clear (49). Inasmuch as FGF23 inhibits $1,25(OH)_2D$ production by the kidney, this feedback loop like that for PTH secretion maintains a balance in the levels of these important hormones. Mutations in the phosphate-regulating gene with hololologies to endopeptidases on the X chromosome (PHEX) or FGF23 itself (which prevent its proteolysis) or conditions such as McCune-Albright disease and tumor-induced osteomalacia in which FGF23 is overexpressed in the involved tissue lead to hypophosphatemia and inappropriately low $1,25(OH)_2D$ accompanied by osteomalacia. The role of (PHEX), which was originally thought to cleave FGF23, in regulating FGF23 levels is no longer clear. In contrast mutations in UDP-*N*-acetyl-α-D galactosamine-polypeptide *N*-acetylgalactosaminyl transferase (GALNT3), which glycosylates FGF23, or in FGF23, which blocks this glycosylation result in inhibited FGF23 secretion leading to hyperphosphatemia, increased $1,25(OH)_2D$ and tumoral calcinosis (50).

Regulation of immune function

The potential role for vitamin D and its active metabolite $1,25(OH)_2D$ in modulating the immune response was first appreciated 25 yr ago with three important discoveries: 1) the presence of VDRs in activated human inflammatory cells (51), 2) the ability of $1,25(OH)_2D$ to inhibit T cell proliferation (52), and 3) the ability of disease activated macrophages to produce $1,25(OH)_2D$ (*i.e.* express CYP27B1) (53). Vitamin D and CYP27B1 play important roles in both innate and adaptive immunity, which impact a number of clinical conditions. For example, vitamin D deficiency is a well-known accompaniment of various infectious diseases such as tuberculosis (54), and $1,25(OH)_2D_3$ has long been recognized to potentiate the killing of mycobacteria by monocytes (55). The mechanism underlying these observations has recently been determined by the observation that the monocyte, when activated by mycobacterial lipopeptides, expresses CYP27B1, producing $1,25(OH)_2D$ from circulating 25OHD and in turn inducing cathelicidin, an antimicrobial peptide that enhances killing of the mycobacterium. Inadequate 25OHD levels fail to support this process (14). As a second example, it has been observed that vitamin D deficiency and/or living at higher latitudes (with less sunlight) are associated with a number of autoimmune diseases including type 1 diabetes mellitus, multiple sclerosis, and Crohn's disease (56). In a large Finnish study, providing infants with 2000 IU vitamin D for their first year of life reduced the incidence of type 1 diabetes mellitus by 80% (57). Other studies have linked vitamin D deficiency to increased risk of multiple sclerosis (58), asthma (59), and other immunologic diseases. A discussion of the mechanisms by which $1,25(OH)_2D$ regulates adaptive and innate immunity follows (Fig. 3).

Adaptive immunity

The adaptive immune response involves the ability of T and B lymphocytes to produce cytokines and immunoglobulins, respectively, to specifically combat the source of the antigen presented to them by cells such as macrophages and dendritic cells. Vitamin D exerts an inhibitory action on the adaptive immune system. In particular, $1,25(OH)_2D$ suppresses proliferation and immunoglobulin production and retards the differentiation of B cell precursors into plasma cells (60). In addition $1,25(OH)_2D$ inhibits T cell proliferation (52), in particular the T helper (Th)-1 cells capable of producing IFN-γ and IL-2 and activating macrophages (61). These actions prevent further antigen presentation to and recruitment of T lymphocytes (role of IFN-γ), and T lymphocyte proliferation (role of IL-2). In contrast IL-4, IL-5, and IL10 production can be increased (62), shifting the balance to a Th2 cell phenotype. CD4+/CD25+ regulatory T cells (Treg) are also increased by $1,25(OH)_2D_3$ (63) as shown by increased FoxP3 expression and IL-10 production (64). The IL-10 so produced is one means by which Treg block Th1 development. At least in part, these actions on T cell proliferation and differentiation stem from actions of $1,25(OH)_2D$ on dendritic cells to reduce their antigen presenting capability. The impact of $1,25(OH)_2D_3$ on Th17 development and function is more recently discovered, and many of the effects of $1,25(OH)_2D$ on various autoimmune diseases previously ascribed to inhibition of Th1 development and function are now being ascribed at least in part to inhibition of Th17 develop-

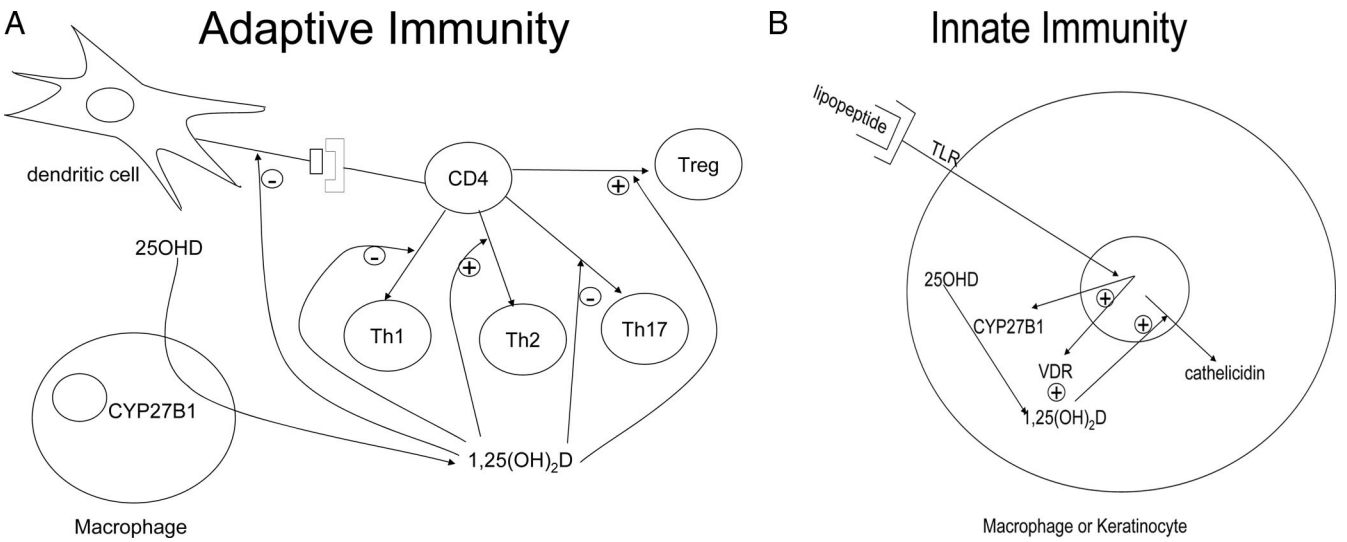

FIG. 3. Regulation of immune function by $1,25(OH)_2D$. $1,25(OH)_2D$ suppresses adaptive immunity (A) by inhibiting the maturation of dendritic cells, reducing their capacity to present antigen to CD4 cells. $1,25(OH)_2D$ further inhibits the proliferation and differentiation of CD4 cells into Th1 and Th17 cells and promotes the production of Th2 and Treg cells. On the other hand $1,25(OH)_2D$ promotes innate immunity (B) in that when the macrophage is activated by TLRs, VDR and CYP27B1 are induced enabling the macrophage to produce $1,25(OH)_2D$, which then induces cathelicidin, a potent antimicrobial peptide.

ment and function (64). The ability of $1,25(OH)_2D$ to suppress the adaptive immune system appears to be beneficial for a number of conditions in which the immune system is directed at self, *i.e.* autoimmunity. In a number of experimental models (65, 66) including inflammatory arthritis, autoimmune diabetes, experimental allergic encephalitis (a model for multiple sclerosis), and inflammatory bowel disease, $1,25(OH)_2D_3$ administration has prevented and/or treated the disease process. As indicated previously, studies in humans also show promise. However, suppression of the adaptive immune system may come at a price if such suppression leads to decreased response to infectious agents or decreased immune surveillance.

Innate immunity

Innate immune responses involve the activation of toll like receptors (TLRs) in polymorphonuclear cells, monocytes, and macrophages as well as in a number of epithelial cells including those of the epidermis, gingiva, intestine, vagina, bladder, and lungs. TLRs are transmembrane pathogen recognition receptors that interact with specific membrane patterns shed by infectious agents that trigger the innate immune response in the host (67). Activation of TLRs leads to the induction of antimicrobial peptides and reactive oxygen species, which kill the organism. Among those antimicrobial peptides is cathelicidin. The expression of this antimicrobial peptide is induced by $1,25(OH)_2D$ in both myeloid and epithelial cells (68, 69). As noted previously, both macrophages (53) and epithelial cells (70) are capable of responding to and producing $1,25(OH)_2D$ (*i.e.* they both have VDR and CYP27B1). Stimulation of TLR2 by an antimicrobial peptide in macrophages (14) or stimulation of TLR2 in keratinocytes by wounding the epidermis (16) results in increased expression of CYP27B1, which in the presence of adequate substrate (25OHD) stimulates the expression of cathelicidin. Lack of substrate (25OHD), VDR, or CYP27B1 blunts the ability of these cells to respond to a challenge with respect to cathelicidin production (14, 16, 69). As mentioned, the innate immune system is widely distributed and operates not only in cells within the lymphopoietic system but also within epithelia of those tissues facing the outside environment in which it contributes to the protective barrier of those tissues. Therefore, it seems that it is no accident of nature that both VDR and CYP27B1 can be found in those tissues.

Regulation of proliferation and differentiation

Epidermis and hair follicle

The epidermis is unique in that under physiological conditions it is capable of not only making vitamin D but also converting it to $1,25(OH)_2D$ in the same cell that is also fully capable of responding to the $1,25(OH)_2D$ produced. As noted previously, $1,25(OH)_2D$ enables the keratinocyte to mount the innate immune response and suppress the autoimmune mechanisms that contribute at least in part to psoriasis. However, $1,25(OH)_2D$ also promotes the differentiation of keratinocytes and inhibits their proliferation (71, 72). In the epidermis proliferation occurs in the basal layer, and as the keratinocytes move out of the basal layer, differentiation is initiated. As the keratinocyte moves from one layer of epidermis to the next, differentiation proceeds in a sequential fashion, ultimately resulting in the enucleated corneocyte enmeshed in a lipid-rich matrix that provides the barrier function. $1,25(OH)_2D$ is involved in all steps of this process in that it limits proliferation in the basal layer and induces in a sequential pattern the expression of genes whose products ultimately produce the permeability barrier. The ability of $1,25(OH)_2D$ to act sequentially on gene expression as the differentiation process unfolds is due to the differential distribution of coactivators (DRIP205 and SRC3) within the epidermis as a function of differentiation (28, 73) and the differential use of these coactivators by genes involved in the early and late stages of differentiation (30, 74).

Hair follicle cycling is the best example of a role for VDR independent of $1,25(OH)_2D$, as clearly illustrated by the development of alopecia in VDR-mutated animals (75) includ-

ing humans (76) but not in CYP27B1 mutated animals (77) and humans (78). The mechanism by which VDR regulates hair follicle cycling remains unclear, but the alopecia phenotype of the VDR null animal is similar to that seen in animals with mutations in Hr (79, 80) and β-catenin (81, 82) that render these proteins transcriptionally inactive. As noted previously, Hr and β-catenin bind to VDR and are capable of regulating its transcriptional activity. What is not known in the hair follicle is the identity of the target genes. The VDR is found in the keratinocytes of the outer root sheath as well as in cells of the bulge in which the stem cells are also located, suggesting an important role for the VDR in regulating the proliferation and differentiation of these cells during the process of hair follicle cycling (83).

Psoriasis is a chronic, generalized, and scaly erythematous dermatosis thought to be due to a Th1- or Th17-mediated immune reaction to as-yet-unidentified antigens in the skin that may cause or at least is accompanied with increased proliferation and decreased differentiation of the keratinocytes in the epidermis. Analogs of $1,25(OH)_2D$, including calcipotriol, tacalcitol, and maxicalcitol as well as calcitriol itself have proved effective therapy for moderate forms of this disease (84). This form of therapy likely works by inhibiting the inflammatory component via a direct action on the T cells (85) as well as by reducing keratinocyte proliferation and enhancing their differentiation (86).

Cancer

$1,25(OH)_2D$ has been evaluated for its potential anticancer activity in animal and cell studies for approximately 25 yr (87). The list of malignant cells that express VDR is now quite extensive. The accepted basis for the promise of $1,25(OH)_2D$ in the prevention and treatment of malignancy includes its antiproliferative, prodifferentiating effects on most cell types. In particular $1,25(OH)_2D$ stimulates the expression of cell cycle inhibitors p21 and p27 (88) and the expression of the cell adhesion molecule E-cadherin (89) and inhibits the transcriptional activity of β-catenin (89–91). In keratinocytes, $1,25(OH)_2D$ has been shown to promote the repair of DNA damage induced by UVR (92), reduce apoptosis and increase survival after UVR (93), and increase p53 (94). Epidemiological evidence supporting the importance of adequate vitamin D nutrition (including sunlight exposure) for the prevention of a number of cancers (95–99) is extensive. Although numerous types of cancers show reduction (100), most attention has been paid to cancers of the breast, colon, and prostate. A recent report from the Women's Health Initiative failed to find a reduction in colon cancer in women receiving 400 IU vitamin D plus 1000 mg calcium (101), although this study has been criticized for using too low a dose of vitamin D, poor compliance, and failure to control for vitamin D and calcium supplementation in the placebo group. 25OHD levels were not recorded at the end of the study to show differences between the treated and nontreated groups. In contrast a prospective 4-yr trial with 1100 IU vitamin D and 1400–1500 mg calcium showed a 77% reduction in cancers after excluding the initial year of study (102), including a reduction in both breast and colon cancers. In this study, vitamin D supplementation raised the 25OHD levels from a mean of 28.8 to 38.4 ng/ml with no changes in the placebo or calcium only arms of the study. However, this was a relatively small study in which cancer prevention was not the primary outcome variable.

Trials of $1,25(OH)_2D$ and its analogs for the treatment of cancer have been disappointing. In a small study involving seven subjects with prostate cancer treated with doses of $1,25(OH)_2D$ up to 2.5 μg for 6–15 months, six of seven showed a decrease in the rise of prostate-specific antigen, a marker of tumor progression (103), and one patient showed a decline. However, hypercalciuria was common and limiting. A preliminary report of a larger study involving 250 patients with prostate cancer using 45 μg $1,25(OH)_2D$ weekly in combination with docetaxel demonstrated a nonsignificant decline in prostate-specific antigen, although survival was significantly improved (hazard ratio 0.67) (104). The incidence of either hypercalcemia or hypercalciuria was not reported. A larger follow-up study has recently been reported (30th annual meeting of the American Society for Bone and Mineral Research, 2008) but not yet published, which failed to confirm the improved survival. Most likely until an analog of $1,25(OH)_2D$ is developed that is both efficacious and truly nonhypercalcemic, treatment of cancer with vitamin D metabolites will remain problematic.

Acknowledgments

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