Vitamin D and Its Role in Cancer and Immunity: A Prescription for Sunlight

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Invited Review

Vitamin D and Its Role in Cancer and Immunity: A Prescription for Sunlight

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ABSTRACT: Vitamin D has been recognized for more than a century as essential for the normal development and mineralization of a healthy skeleton. More extensive roles for vitamin D were suggested by the discovery of the vitamin D receptor (VDR) in tissues that are not involved in calcium and phosphate metabolism. VDR has been discovered in most tissues and cells in the body and is able to elicit a wide variety of biologic responses. These observations have been the impetus for a reevaluation of the physiologic and pharmacologic actions of vitamin D. Here, we review the role of vitamin D in regulation of the immune system and its possible role in the prevention and treatment of cancer and immune-mediated diseases.

Vitamin D Overview

Most humans depend on sun exposure to satisfy their requirements for vitamin D. Brief exposure to direct sunlight on a bright summer day is all the body needs to produce sufficient cholecalciferol (or vitamin D_3).[1] Skin exposure to ultraviolet B light (wavelength 290–315 nm) initiates the photochemical conversion of 7-dehydrocholesterol to previtamin D_3 in the epidermis and dermis. Body heat then catalyzes the rapid isomerization of previtamin D_3 to vitamin D_3 (cholecalciferol), which binds to the vitamin-D-binding protein (VDBP) in the extracellular space.[2] Vitamin D_3 is transported to the liver, which uses 25-hydroxylase to produce 25-hydroxyvitamin D_3, or 25(OH)D_3.

Although endogenous production of vitamin D typically provides most of the vitamin D requirement, vitamin D can be ingested orally as either vitamin D_3 (cholecalciferol) or D_2 (ergocalciferol, derived from the irradiation of plant sterols). Very few foods naturally contain vitamin D: oily fish and fish products such as cod-liver oil, salmon, mackerel, and herring contain approximately 300–500 international units (IU) of vitamin D per serving.[3] Foods in the United States that are fortified with vitamin D include milk, orange juice, and some cereals, breads, yogurts, and cheeses. Most European countries permit margarine and some cereals to be fortified with vitamin D, and a few countries, including Sweden, also permit milk to be fortified with this vitamin.

25-Hydroxyvitamin D [25(OH)D] derived from endogenous production or exogenous sources is the major vitamin D metabolite in the human body's circulation system and is the best known indicator of vitamin D status. There is debate regarding cutoffs for vitamin D deficiency and insufficiency using 25(OH)D concentrations. The current thinking is that the traditional cutoff for deficiency, 10 ng/mL of 25(OH)D (above which protection from rickets or osteomalacia is conferred), is too low to indicate vitamin D adequacy. A low serum 25(OH)D concentration, often referred to as hypovitaminosis D, is not simply a biochemical abnormality. It is associated with physiologic, pathologic, and clinical evidence of vitamin D deficiency, including increased parathyroid hormone secretion, increased bone turnover, osteoporosis and mild osteomalacia, and an increased risk of hip and other fractures. To prevent more subtle or long-term effects of vitamin D insufficiency, circulating concentrations of at least 30 ng/mL 25(OH)D may be warranted.[3]

In the kidney, 25(OH)D is further hydroxylated to 1α,25-dihydroxyvitamin D [1α,25$(OH)_2D_3$, calcitriol], the most biologically active form of the vitamin, by renal 1α-hydroxylase under the control of parathyroid hormone. 1α,25$(OH)_2D_3$ principally targets the intestine, kidney, and bone to regulate calcium and phosphate homeostasis. Calcitriol is a lipid-soluble hormone that interacts with its vitamin D receptor (VDR) in the small intestine to increase the expression of an epithelial calcium channel, calcium-binding protein, and a variety of other proteins to help in transport of calcium from the intestinal lumen into circulation. Calcitriol also interacts

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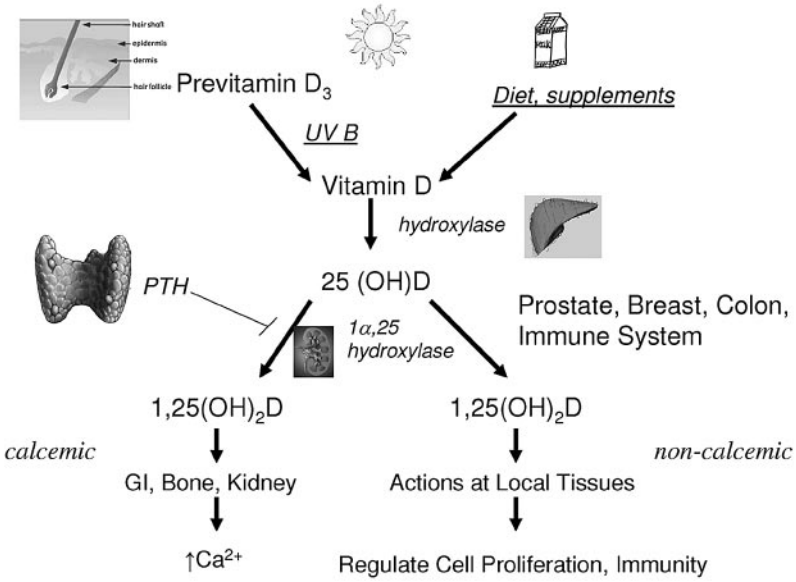

Figure 1. Calcemic and noncalcemic roles of vitamin D and their potential health consensequences.

with its VDR in the osteoblasts, which results in an increase in the mobilization of osteoclast precursors to become mature osteoclasts. The end result is mobilization of calcium stores from the skeleton to maintain calcium homeostasis.[1–6] These well-defined, calcemic functions of vitamin D are illustrated in Figure 1. It should be noted that $1\alpha,25(OH)_2D_3$ circulates at concentrations 1000-fold less than that of 25(OH)D, and that its half-life is hours rather than weeks, as is the case for 25(OH)D.

More recently, VDRs were found in cells of tissues not involved in calcium homeostasis, and extrarenal tissues were found to produce $1\alpha,25(OH)_2D_3$. These pathways are also indicated in Figure 1 and supported the hypothesis that vitamin D plays additional roles in cellular differentiation and the control of proliferation in a variety of cell types. These results have bolstered at a biochemical level what epidemiologists have been witnessing in observational studies: that vitamin D status may protect against certain cancers, as well as some autoimmune conditions.

Vitamin D and Cancer Prevention

Epidemiologic Studies

Geography, sunlight, and cancer risk. Most of the information about vitamin D and cancer prevention has been based on observational, case-control, or cohort studies linking sunlight exposure to cancer incidence or survival. The first of these studies was documented nearly a half century ago, when it was reported that individuals living in the northeast regions of the United States had an approximately 2-fold higher risk of dying of cancer than those living in southern regions.[7] Recently, several more studies found a similar association between latitude and the risk of developing prostate, breast, and colon cancer in the United States and Europe.[8–10] Colon cancer mortality was subsequently found to be higher among people from the Northeastern United States compared with people living in southern states.[11] It is now well established that the risk of developing and dying of prostate, breast, colon, ovarian, esophageal, non-Hodgkin's lymphoma, and a number of other lethal cancers correlated with living at higher latitudes.[11–24]

Several investigators have confirmed the reciprocal relationship of sunlight exposure and cancer. Most recently, Grant[24,25] reported that increased sun exposure decreases mortality from common cancers in both white men and women.[7] Grant[17] suggested that 25% of breast cancer mortality in Europe was related to living at a higher latitude and being vitamin D deficient.

These observations are also supported by a report of men who had little sun exposure and developed prostate cancer 3–5 years earlier than men who had optimal sun exposure.[26] Vitamin D deficiency was reported to increase the risk for prostate cancer, and sunlight exposure is inversely proportional to prostate cancer mortality. Colon cancer incidence was evaluated in California because the state has a wide range of latitudes. Living in San Diego was reported to significantly decrease the risk of developing colon cancer compared with living in San Francisco or further north.[27]

There has been speculation that subjects living further north synthesized less vitamin D and that therefore vitamin D seems to be protective against

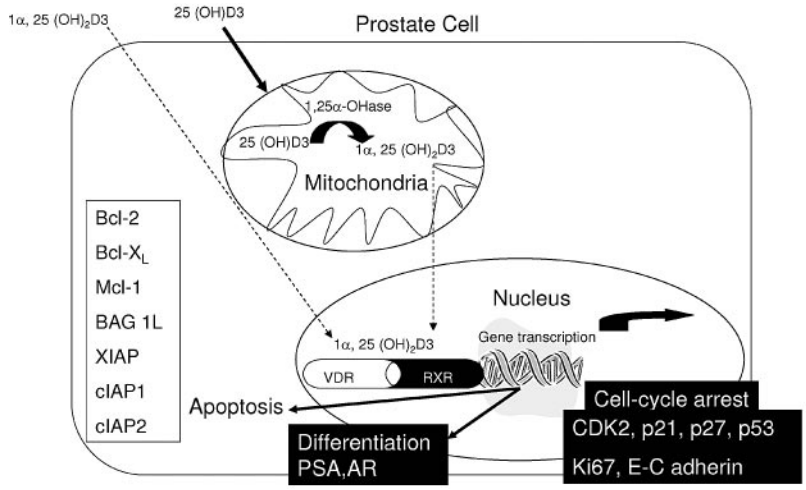

Figure 2. $1\alpha,25(OH)_2D_3$ regulates prostate cell growth. Vitamin D can influence the regulation of key genetic elements of cell differentiation and proliferation by either $1\alpha,25(OH)_2D_3$ entering the nucleus of the prostate cell or via conversion of 25 $(OH)D_3$ by 1,25α-OHase in the mitochondrion. Adapted from *Progress in Biophysics & Molecular Biology* Volume 62, Holick MF. Vitamin D: its role in cancer prevention and treatment, pp 49–59, © 2006 with permission from Elsevier. AR, androgen receptor; BAG 1L, Bcl-2 associated athanogene-1; BCL-2/BCL-X_L, B-cell leukemia/lymphoma family; CDK2, cyclin-dependent kinase 2; cIAP1 and cIAP2, cIAP1 and cIAP2 are members of a protein family; Mc1-1, Myeloid Cell Leukemia 1; p21, p27, and p53, tumor proteins 21, 27, and 53; PSA, prostate specific antigen; RXR, retinoic X receptors; VDR, vitamin D receptor; XIAP, X-linked mammalian inhibitor of apoptosis protein.

tumor development or growth. The sunlight effect therefore seems to attenuate the risk of numerous cancers, such as prostate, breast, colon, ovarian, non-Hodgkin's lymphoma, esophageal, stomach, pancreatic, rectal, kidney, corpus uteri, lung, and bladder. These studies, which controlled for other important variables such as smoking and lifestyle, provide indirect evidence of a possible causal relation between low vitamin D status and cancer risk.

Studies have looked more specifically at quantitative estimates of sunlight exposure and cancer risk or mortality. Men with elevated levels of sunlight exposure had a later onset of prostate cancer than those with low levels of sunlight exposure.[18] Solar ultraviolet (UV)-B exposure was inversely related to the risk of dying of cancer in men and women in the United States.[28]

Putative mechanisms for vitamin D protection against cancer. The original hypothesis for why exposure to sunlight decreased the risk of common cancers was that increased production of vitamin D_3 in the skin resulted in higher circulating levels of $25(OH)D_3$, which could be metabolized by the kidneys to $1\alpha,25(OH)_2D_3$. The discovery that VDRs existed in tissues that were not involved in calcium metabolism (ie, prostate, breast) reinforced the possibility that enhanced production of renal $1\alpha,25(OH)_2D_3$ could influence noncalcemic tissues. Studies demonstrated that $1\alpha,25(OH)_2D_3$ markedly inhibited a variety of genes responsible for cellular proliferation, including p21 and p27, and was also responsible for enhancing apoptotic activities and a variety of genes that regulate cellular differentiation.[29]

However, because the kidney's production of $1\alpha,25(OH)_2D_3$ is exquisitely regulated, it could not serve as the source of the active $1\alpha,25(OH)_2D_3$ responsible for the newly recognized cellular functions. Thus, another hypothesis to explain the observations that sunlight provided a protective effect *via* vitamin D was needed.[1–4] Another possibility was that the "target" cells of interest were themselves converting 25(OH)D to $1\alpha,25(OH)_2D_3$. Along these lines, cultured keratinocytes and prostate cells obtained from nondiseased prostate biopsies converted 25(OH)D to $1\alpha,25(OH)_2D_3$[18,27,30] (Figure 2). Following these observations, it has been noted that the colon, lung, breast, and other tissues all express the 25(OH)D-1α-hydroxylase (1-OHase; cyp 27 B1).[31] Thus, it has been suggested that raising blood levels of 25(OH)D provides an adequate substrate for the prostate, colon, and breast to produce their own local $1\alpha,25(OH)_2D_3$, which, in turn, is capable of regulating a variety of cellular processes that help control cellular growth and prevent malignancy.

Animal models. Although the epidemiologic evidence linking sun exposure to cancer risk seems strong, whether vitamin D deficiency *per se* promotes tumor growth remained unclear. To this end, a study evaluated the growth of a mouse colon cancer cell line MC-26 in Balb/c mice that were either vitamin D deficient or vitamin D sufficient.[32] The tumors grew much more rapidly in the vitamin D–deficient mice. By the end of the study on day 19, tumors in the vitamin D–deficient mice were on

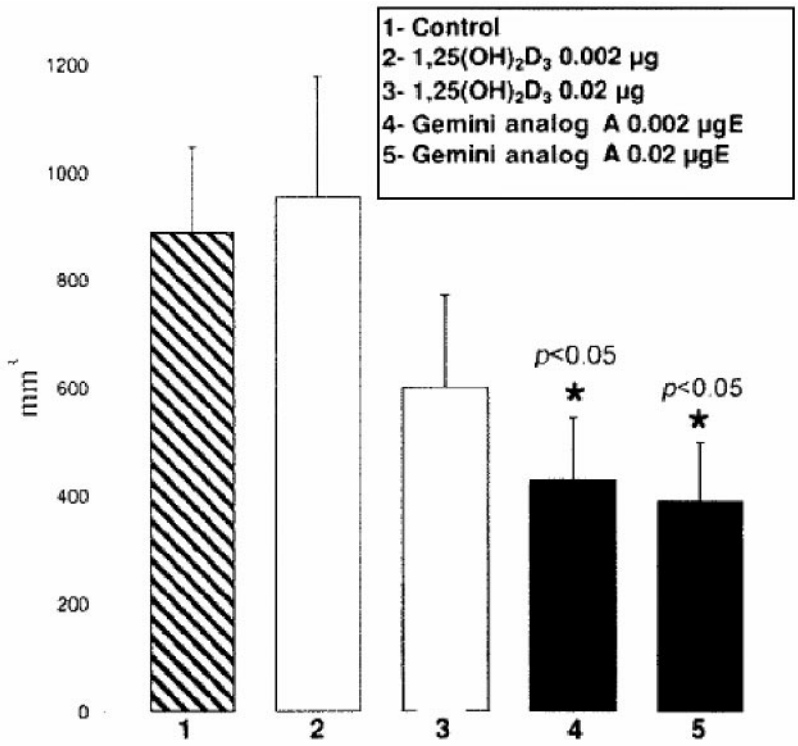

Figure 3. $1\alpha,25(OH)_2D_3$ and a novel vitamin D analog regulate colon cancer cell growth in mice. A novel Gemini analog was shown to diminish tumor volume when administered while a dose response reduction effect is shown for $1\alpha,25(OH)_2D_3$. Adapted with permission from Spina CS, Tangpricha V, Uskokovic M, Adorinic L, Maehr H, Holick MF. Vitamin D and cancer. *Anticancer Res*. 2006;26:2515–2524.

average 80% larger than in the vitamin D–sufficient mice. The 25(OH)D levels in the vitamin D–deficient mice at the end of the study were <5 ng/mL, whereas the vitamin D–sufficient mice maintained a 25(OH)D level of 35 ng/mL. This observation provides strong corroborating data supporting the concept that vitamin D sufficiency is important for reducing tumor cell growth. There is now emerging data that vitamin D may play a role in altering colon cancer cell growth. Spina et al[33] performed studies using murine models of colon cancer to demonstrate that those mice who were sufficient in vitamin D had a significantly lower ($p < .05$) tumor burden compared with vitamin D–deficient mice. Furthermore, using Gemini analog A in the same mouse model of colon cancer, these investigators demonstrated that the tumor burden was decreased by 50% in the treatment group when compared with placebo or $1\alpha,25(OH)_2D_3$ ($p < .05$; Figure 3).

Dietary intake of vitamin D and cancer risk. Aside from sun exposure, others sources of vitamin D are in the form of diet and supplements. There are several epidemiologic studies of the association between vitamin D and breast cancer risk. Studies reporting dietary and supplemental intake demonstrate inconsistent results (Table 1).[20,35–38,41] Three case-controlled studies demonstrated no association between dietary vitamin D intake and breast cancer risk[35,39,40]; however, small sample size and selection bias may have skewed the results. On the other hand, the Nurses Health Study demonstrated an inverse association between vitamin D intake and breast cancer risk among premenopausal women but no association among postmenopausal women.[41] Along these lines, the Cancer Prevention Study II Nutrition Cohort found no correlation between dietary intake of vitamin D and development of breast cancer in postmenopausal women,[38] and 2 additional studies determined that the dietary intake of vitamin D in the female adolescent had no bearing in determining future breast cancer risk.[36,37] However, researchers provided additional preliminary evidence demonstrating a potential role of vitamin D in decreasing breast cancer risk. In a population-based, case-controlled study in Ontario, women with breast cancer (identified through the Ontario Cancer Registry) and controls were interviewed about their past and present diets and sun exposure.

The investigators' preliminary analysis suggests that earlier exposures to vitamin D during breast development in adolescence may be more significant for reducing breast cancer risk than recent exposures.[42,43] A recent study seeking to estimate the amount of vitamin D required to reduce the inci-

Table 1
Dietary or supplemental intake of vitamin D and breast cancer risk

Study design (reference)	No. cases/controls (cohort)	Comparison	RR (95% CL)
Cohort[20]	179/4747	Dietary, ≥200 *vs* ≤100 IU	0.85 (0.59–1.24)
		Supplemental, (daily *vs* never)	0.89 (0.60–1.32)
Hospital-based case control[35]	289/442	Dietary, (tertiles) T3 *vs* T1	1.43 (0.90–2.26)
Cohort[41]	3172/88,691	Total vitamin D, (IU/d)	0.72 (0.55–0.94)
		Premenopausal, >500 *vs* ≤150 IU	
		Postmenopausal, >500 *vs* ≤150 IU	0.94 (0.80–1.10)
Nested case control[36]	843/8430	Dietary vitamin D during adolescence, (quintiles) Q5 *vs* Q1	0.96 (Unknown)
Cohort[37]	361/47,355	Dietary vitamin D during adolescence, (quintiles) Q5 *vs* Q1	0.92 (0.66–1.27)
Cohort[38]	2855/68,567	Total vitamin D, (IU/day)	0.95 (0.81–1.13)
		Postmenopausal, >700 *vs* ≤100 IU	
		Postmenopausal, >300 *vs* ≤100 IU	0.89 (0.76–1.03)

Relative risk (RR) >1 imparts a greater risk of developing breast cancer, whereas RR <1 is associated with a decreased risk.
Adapted from Cui Y, Rohan TE. Vitamin D, calcium, and breast cancer risk: a review. *Cancer Epidemiol Biomarkers Prev.* 2006;15:1427–1437 with permission from the American Association for Cancer Research.
CL, confidence limit; IU, international unit.

dence of breast cancer was performed *via* a meta-analysis of 2 large previously published studies that measured vitamin D concentrations in the blood [as serum 25(OH)D] and subsequent breast cancer development. The results demonstrated that women who consumed 1000 IU a day of vitamin D in addition to the normal background amount had a 10% reduced risk of breast cancer.[43] Garland and colleagues[44] plotted a dose-response gradient and found that as quintiles of serum 25(OH)D (0–11 ng/mL, 12–25 ng/mL, 26–31 ng/mL, 32–42 ng/mL, and >42 ng/mL) increased, the risk of breast cancer significantly decreased. A serum 25(OH)D concentration exceeding 52 ng/mL (which would require intake of >2700 IU/d of vitamin D_3 in an individual weighing 70 kg) was associated with 50% lower risk of breast cancer compared with a serum concentration of <12 ng/mL. The authors noted that the US median intake of 320 IU/d of vitamin D is only about one-tenth of the amount found to be associated with a 50% reduction of breast cancer incidence. They concluded that increasing daily intake of vitamin D, perhaps by fortification of foods, should be considered. Studies analyzing endogenous circulating vitamin D levels and breast cancer risk are shown in Table 2.[12,19,34,45,46]

Table 2
Endogenous vitamin D levels and breast cancer risk

Study design (reference)	No. cases/controls (cohort)	Comparison	RR (95% CL)
Nested case control[45]	96/96	Serum $1\alpha,25\text{-}(OH)_2D_3$ (pg/mL) (≥51 *vs* <32)	1.0 (0.2–3.4)
Hospital-based case control[19]	131/149	Blood $1\alpha,25\text{-}(OH)_2D_3$ (pmol/mL) (≤33.61 *vs* >62.94)	5.3 (2.1–13.4)
		Blood 25-(OH)D	No association
Hospital-based case control[46]	179/179	Plasma 25-(OH)D (nmol/L) <50 *vs* >150	5.83 (2.31–14.7)
Nested case control[12]	701/724	Plasma 25(OH)D (quintiles) Q5 *vs* Q1	0.73 (0.49–1.07)

Relative risk (RR) >1 imparts a greater risk of developing breast cancer, whereas RR <1 is associated with a decreased risk.
Adapted from Cui Y, Rohan TE. Vitamin D, calcium, and breast cancer risk: a review. *Cancer Epidemiol Biomarkers Prev.* 2006;15:1427–1437 with permission from the American Association for Cancer Research.
CL, confidence limit; IU, international unit.

Biochemical indicators of vitamin D status and cancer risk. There has been considerable debate recently about concentrations of circulating 25(OH)D that can be viewed as optimal for preventing vitamin D deficiency and, in particular, promoting health. Prospective and retrospective studies have shown that circulating levels of 25(OH)D above 50 nmol/L (20 ng/mL) were associated with a 30%–50% decreased risk of developing prostate, breast, and colon cancer.[47]

A recent study by Dr Walter Willett's group from Harvard University found that higher serum vitamin D levels correlated with reduced risk for certain forms of cancers in a population of middle-aged to elderly men in a retrospective analysis of data from a major study of health professionals.[48] Giovannucci and colleagues[48] analyzed prospective data from Harvard's Health Professionals Follow-up Study, which includes >50,000 men aged 40–75 years. Higher serum levels of vitamin D were associated with a significantly lower incidence of colorectal, pancreatic, esophageal, and oral/pharyngeal cancers. An increase of 25 nmol/L in serum vitamin D level was associated with a 17% decline in overall cancer incidence and a 29% drop in total cancer deaths. For cancers involving the digestive system, a 25 nmol/L increase in vitamin D reduced cancer incidence by 43% and mortality by 45%. This study was the first to examine total cancer incidence and mortality by using a comprehensive assessment of factors that determine 25(OH)D levels. The authors estimated that cancer mortality for US men could be reduced by 29% if serum vitamin D levels were increased by 25 nmol/L throughout the male population. The findings from this cohort study are the latest of several[49–52] linking vitamin D status with reduced cancer risk, and are some of the most compelling yet. The results, with lower risks of most (but not all) forms of cancer, are also some of the most broad-based, and they indicate that vitamin D may have a role in most human tumors. Factors associated with higher serum levels of vitamin D were white race, residence in the southern United States, higher intakes of vitamin D, body mass index <22 kg/m^2, and more physical activity. Vitamin D supplements (as opposed to dietary vitamin D) had only a slight effect on serum vitamin D. The investigators noted that increasing one's serum vitamin D by 25 nmol/L (the basis of their relative-risk calculation) might mean adding at least 1500 IU/d of vitamin D, far exceeding the US Department of Agriculture's recommended dietary allowance (RDA) of vitamin D currently set at 200–400 IU for people aged 1–70 years. A glass of milk contains only 100 IU and would increase serum vitamin D by only 2–3 nmol/L, they noted, but "a fair-skinned individual can produce 20,000 IU of vitamin D in the skin through 20–30 minutes of sun exposure." A recent editorial also noted that "the amount of sun needed to produce adequate levels of vitamin D, at least for bone health, is modest and can be obtained in a light-skinned person by a brief summertime afternoon stroll."[53] Other previous reports support a role for vitamin D in the prevention of colorectal cancer.[15,22,54] Giovannucci[55] has recently published a review of the data on colon cancer risk and vitamin D.

Other cancers where vitamin D plays a protective role. Polesel et al[56] reported that vitamin D also serves a protective role against the development of non-Hodgkin's lymphoma. Vitamin D (chiefly contained in fish) provided protection against non-Hodgkin's lymphoma that was stronger in women, whereas no differences emerged according to age (odds ratio [OR] = 0.4; 95% confidence interval [CI], 0.2–0.9). Vitamin D also seems to play a protective role in pancreatic cancer.[57] In 2 US cohorts, increased intakes of vitamin D were associated with reduced risks for pancreatic cancer, suggesting a potential role for vitamin D in the pathogenesis and prevention of this malignancy.

Melanoma, on the other hand, has been linked to sun exposure. Thus, the risk of excessive sun exposure needs to be weighed against the benefit of sun-derived vitamin D. Li et al[58] reported that genetic variants (ie, TaqI t protective allele and FokI f risk allele) in VDR may alter risk of cutaneous melanoma. Thus, genetic alternations in VDRs may in part determine the risk for developing cutaneous melanoma from sun exposure.

Mechanisms Involved in the Anticancer Effects of Vitamin D

Over the past quarter century, evidence for the anticancer properties of vitamin D and its analogs has been emerging in the literature. The volume of data supports a multipronged attack that involves growth arrest at the G1 phase of the cell cycle, apoptosis, tumor-cell differentiation, disruption of growth-factor-mediated cell survival signals, and inhibition of angiogenesis and cell adhesion (Figure 2). Processes involved in the tumor suppressive activity of vitamin D analogues include inhibition of cell proliferation, induction of apoptosis, inhibition of cell adhesion, G_1-phase cell-cycle arrest, promotion of cell differentiation, inhibition of angiogenesis, alteration of growth factors, and inhibition of metastasis.[59,60] Thus, VDR-mediated pathways constitute potential therapeutic targets for cancer prevention and treatment.

Proposed Cellular Mechanisms

Cell cycle regulators. Direct regulation of the cell cycle by $1\alpha,25(OH)_2D_3$ has been studied predominantly in *in vitro* systems. The $1\alpha,25(OH)_2D_3$-VDR system arrests the cancerous cell cycle at the G_0-G_1 transition through multiple mechanisms.[59] In the monomyelocytic cell line U937, $1\alpha,25(OH)_2D_3$-activated VDR directly binds to the promoter of p21 and induces its expression. Furthermore,

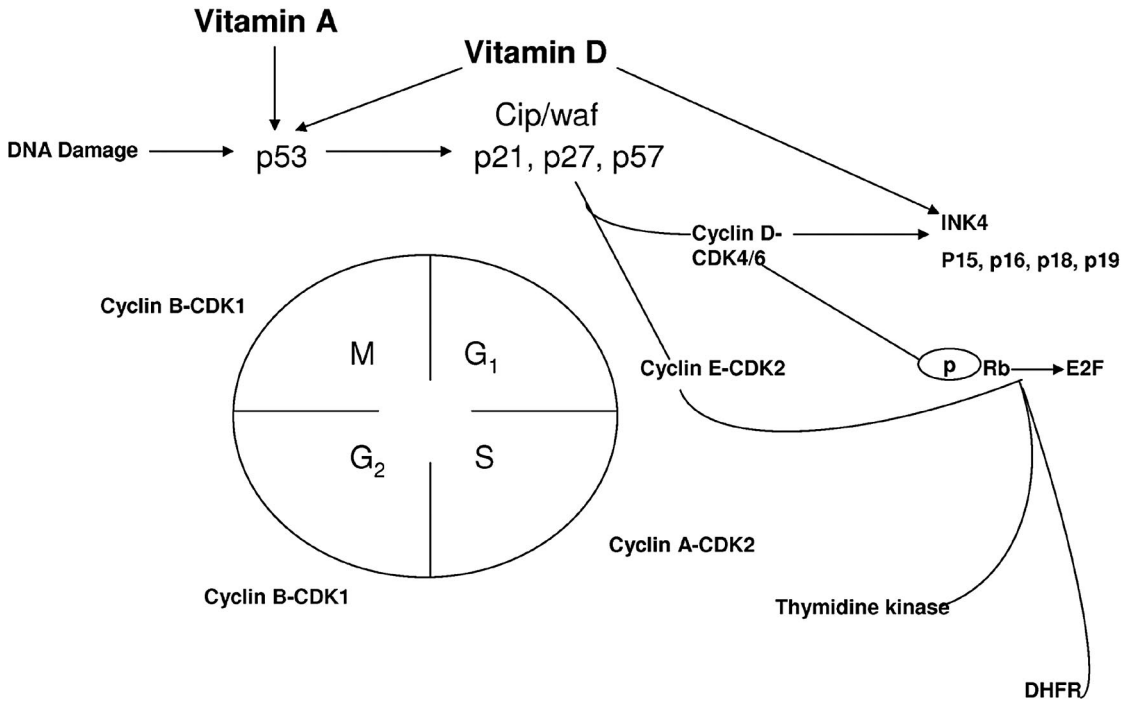

Figure 4. Nutrient regulation of cell cycle proteins. Vitamin D has broad effects in regulating the cell cycle by regulating the expression of p53, Cip/waf p21, p27, p57, p15, p16, and p18, which effectively inhibits the G1-to-S phase transition. Adapted with permission from Bohnsack BL, Hirschi KK. Red light, green light: signals that control endothelial cell proliferation during embryonic vascular development. *Cell Cycle*. 2004;3:1506–1511.

$1\alpha,25(OH)_2D_3$ increases the expression of p27 as well as p15, p16, and p18, which effectively inhibits the G1-to-S phase transition (Figure 4).[60] Furthermore, in the MCF-7 breast cancer cell line, treatment with $1\alpha,25(OH)_2D_3$ inhibits cellular proliferation, and microarray analysis demonstrated an up-regulation of a number of cell cycle regulatory genes, including p21-activated kinase 1 and p53.[61] Thus, vitamin D is a potent regulator of cellular differentiation and proliferation through direct regulation of cell cycle proteins.

Role of $1\alpha,25(OH)_2D_3$ in Regulating Cell Growth and Terminal Differentiation

$1\alpha,25(OH)_2D_3$ regulates proliferation and differentiation of different kinds of cells, including keratinocytes, osteoblasts, and hematopoietic cells.[2] $1\alpha,25(OH)_2D_3$ has been shown to inhibit cellular growth and induced differentiation of M-1 leukemic cells and HL-60 leukemic cells expressing VDR.[62] $1\alpha,25(OH)_2D_3$ inhibits proliferation and induces differentiated function in osteoblasts, keratinocytes, and hematopoietic cells.[63] $1\alpha,25(OH)_2D_3$ is generally associated with inhibiting proliferation and inducing differentiation. The growth inhibition of cancer cells by $1\alpha,25(OH)_2D_3$ is associated with growth factor signaling through transforming growth factor-β (TGF-β), which is a potent inhibitor of proliferation of many cell types and is involved in cell cycle control and apoptosis. Vitamin D analogs induce an autocrine TGF-β activity through increasing expression of TGF-β isoforms or TGF-β receptors in nonmalignant and malignant cells.[64] Insulin-like growth factor (IGF)-binding protein 3 induction by $1\alpha,25(OH)_2D_3$ seems to contribute to its antiproliferative and proapoptotic actions in primary cancer cells.[65–67] Recent microarray studies of gene expression profiles in cancer cells have further highlighted the capacity of $1\alpha,25(OH)_2D_3$ analogs to drive malignant cells to a more differentiated state.[68]

Role of $1\alpha,25(OH)_2D_3$ in Apoptosis

$1\alpha,25(OH)_2D_3$-induced apoptosis is an important contributor to its growth-suppressing and anticancer properties. $1\alpha,25(OH)_2D_3$ analogs have been shown to induce apoptosis in cancer cells by modulating B-cell leukemia/lymphoma-2 genes (Bcl-2) and Bax proteins (which is a proapoptotic member of the Bcl-2 protein family), tumor necrosis factor (TNF)-α, and caspase-dependent and independent mechanisms.[69]

Role of $1\alpha,25(OH)_2D_3$ in Controlling Tumor Invasion and Metastasis

Aside from growth inhibition, vitamin D and its analogs decrease the invasiveness of several cell

Table 3
Distribution of vitamin D receptor (VDR) in normal human tissues

Tissue	Immunocytochemical staining
Liver	+/++
Kidney	++/+++
Thyroid	++/+++
Adrenal	+/++
Stomach	+/++
Duodenum	++
Jejunum	++
Colon	+++
Skin	++
Breast epithelium	++
Skeletal muscle	−

+, Weak; ++, moderate; +++, strong; −, negative.

lines *in vitro*, and they inhibit angiogenesis and metastasis in xenograft and transgenic mouse models *in vivo*.[70] In cultured malignant cells, $1\alpha,25(OH)_2D_3$ and its analogs down-regulate cell-invasion-associated proteases, including matrix metalloproteinases 2 and 9 and serine proteinases.[71] In prostate and colon cells, $1\alpha,25(OH)_2D_3$ and its analogs increase the expression of E-cadherin, a tumor suppressor associated with the metastatic potential of cells, and inhibit the oncogenic β-catenin signaling.[72] $1\alpha,25(OH)_2D_3$ and its analogs inhibit the proliferation of some tumor-derived endothelial cells, inhibit the expression of vascular endothelial cell growth factor that induces angiogenesis in tumors, and suppress tenascin-C, which promotes growth, invasion, and angiogenesis during tumorigenesis.[73]

Breast, colon, prostate, skin, lung, and a variety of other cell lines, when exposed to $1\alpha,25(OH)_2D_3$, showed marked inhibition of cellular growth and induction of terminal differentiation.[74] Although vitamin D is not currently used as a chemotherapeutic agent, it shows promise for drug development in the treatment of certain cancers. However, at this point, it is far from a certainty.

Vitamin D Analogs for Cancer Treatment

As discussed earlier, the nuclear VDR has been isolated from a variety of target cells and tissues (Table 3), suggesting that vitamin D compounds may have therapeutic potential throughout several body systems. The major drawback of $1\alpha,25(OH)_2D_3$, however, is its effect on calcium metabolism, which results in hypocalcaemia and hypercalciuria. Newly developed vitamin D analogs with lower calcemic activity have been shown to retain many therapeutic properties of $1\alpha,25(OH)_2D_3$.[75–106] MC 903 is a vitamin D analog with low (2/0.05) ratio of cell growth inhibition to calcemic activity. In contrast, CB 966 (6/0.2), CB 1093 (160/0.27) and KH 1060 (1000/1.3) have much higher ratios making them potentially effective therapeutic agents without the potential risk of toxicity.[75] More than 2000 synthetic analogs of the biologic form of vitamin D ($1\alpha,25(OH)_2D_3$) are presently known. These analogs interfere with the molecular switch of nuclear $1\alpha,25(OH)_2D_3$ signaling.

Most $1\alpha,25(OH)_2D_3$ analogs have been identified as agonists, a few are antagonists (ie, ZK 159222), and only Gemini analogs (analogs having additional side-chains attached) and some of its derivatives act under restricted conditions as nonagonists.

Five vitamin D analogs have been approved for clinical use in a variety of disorders: calcipotriol (Dovonex; Leo Pharmaceuticals, Copenhagen, Denmark) for the treatment of psoriasis, 19-nor-$1,25(OH)_2D_2$ (Zemplar; Abbott Laboratories, Abbott Park, IL) for secondary hyperparathyroidism, doxercalciferol (Hectorol; Bone Care Int, Madison, WI) for reduction of elevated parathyroid hormone levels, 22-oxacalcitriol (Maxacalcitol; Chugai Pharmaceuticals, Tokyo, Japan), and alfacalcidol. Thus, these synthetic analogs which maintain antiproliferative effects but do not have strong calcemic activity have been developed and show promising results (Table 4). Studies using the human osteosarcoma cell line MG-63 demonstrated that 3 of these analogs (KH1060, EB1089, and CB1093), have a greater antiproliferative effect than $1,25(OH)_2D_3$. Treatment with these analogs increases p27 protein levels by increasing expression and decreasing degradation. The analogs cause decreased levels of cyclin E, decreased CDK2 kinase activity and hypophosphorylation of Rb, and inhibition of the G1-to-S phase transition.[105] Similar results have been seen in neuroblastoma cell lines treated with a 20-epi-$1\alpha,25(OH)_2D_3$ analog,[106] suggesting that in the future, these $1\alpha,25(OH)_2D_3$ analogs may be useful in chemotherapeutic regimens.

Several other analogs are currently being tested in preclinical and clinical trials for the treatment of various types of cancer and osteoporosis, as well as immunosuppression.[80] Vitamin D analogs are effective treatments and are widely used as drugs for hyperproliferative skin disorders such as psoriasis, and in suppression of secondary hyperparathyroidism and parathyroid hyperplasia resulting from chronic renal insufficiency.[107–109] For instance, Gemini analogs have been recently found to be 100–1000 times more potent in their antiproliferative activity than the natural hormone. Studies in mice suggest that they may be useful in the treatment of some cancers, including colon cancer.[27] Understanding how analogs exert their selective actions may allow for the design of more effective and safer vitamin D compounds for the treatment of a wide range of clinical disorders. The promising vitamin D analogs under development for the treatment of cancer are shown in Table 4[76–88]; the *in vivo* effects of vitamin D analogs in animal models for breast cancer, prostate cancer, and colon cancer are shown in Table 5[89–106]; and the molecular targets

Table 4
Clinical development of 1α,25(OH)$_2$D$_3$ and its analogues for cancer treatment

Study	Treatment	Diagnosis	Protocol	Reference
Phase II	1α,25(OH)$_2$D$_3$	Prostate cancer before prostatectomy	Weekly p.o.	76
Phase II	1α,25(OH)$_2$D$_3$ + docetaxel	Prostate cancer	Weekly p.o.	77
Phase II	1α,25(OH)$_2$D$_3$ + carboplatin	Prostate cancer	Weekly p.o.	78
Phase II	1α,(OH)D$_2$	Prostate cancer	Daily p.o.	79
Phase II	EB1089	Liver cancer	Daily p.o.	80
Phase II	EB1089	Pancreatic cancer	Daily p.o.	81
Phase I/II	1α,25(OH)$_2$D$_3$ + docetaxel + estramustine	Prostate cancer	High-dose pulse p.o.	82
Phase I	1α,25(OH)$_2$D$_3$ + paclitaxel	Advanced solid tumors	Days 1–3/wk p.o.	83
Phase I	1α,25(OH)$_2$D$_3$	Liver cancer	Daily hepatic infusion	84
Phase I	1α,25(OH)$_2$D$_3$	Advanced malignancy	Weekly p.o.	85
Phase I	1α,25(OH)$_2$D$_3$	Advanced malignancy	Every other day s.c.	86
Phase I	16-Ene-23-yne-1α,25 (OH)$_2$D$_3$	Advanced malignancy	Daily p.o.	87
Phase I	1α(OH)D$_2$	Prostate cancer	Daily p.o.	88
Phase I	EB1089	Breast and colorectal cancer	Daily p.o.	89

for vitamin D compounds in cancer are shown in Table 6. Optimal administration of vitamin D analogs is only being realized, with high-dose intermittent administration overcoming bioavailability and hypercalcemic problems. Combination therapy with cytotoxic agents (taxols and cisplatins), antiresportive agents (biphosphonates), agents blocking vitamin D metabolism (ketoconazole), ionizing radiation, antiproliferative agents (retinoic acids), antihypercalcemic agents (dexamethasone), histone deacetylase inhibitors, or cytochrome P450 inhibitors may achieve better results through synergy, as shown *in vitro*.[110–115] Understanding how analogs exert their tissue-specific selective actions may

Table 5
In vivo *effects of vitamin D analogues in animal models of cancer*

Tumor, model	Analogue	Effect	Reference
Breast			
N-methyl-*N*′ nitrosourea induced	EB1089	Tumor suppression	89
	Ro24–5531	Reduced tumor incidence	90
	1α(OH)D$_5$	Reduced tumor incidence	91
MCF-7 xenografts	22-Oxa-1α,25(OH)$_2$D$_3$	Tumor suppression	92
	TX522	Tumor suppression	93
	TX527	Tumor suppression	93
MDA-MB-231 xenografts	EB1089	Inhibited skeletal metastasis	94
Prostate			
LNCaP xenografts	EB1089	Tumor suppression	95
	EB1089	Tumor suppression	95
	LG190119	Tumor suppression	96
	LG190119	Reduced tumor incidence	96
PC-3 xenografts	Ro23–7553	Tumor suppression	97
MAT LyLu tumors in rats	EB1089	Inhibited lung metastasis	98
MDA-PCa 2b xenografts	JK-1626–2	Inhibited metastatic bone lesions	99
Colon			
1,2-Dimethylhydrazine induced	22-Oxa-1α,25(OH)$_2$D$_3$	Reduced tumor incidence	100
	Ro25–5317	Reduced tumor incidence	101
	Ro25–9022	Reduced tumor incidence	101
Azoxymethane induced	Ro24–5531	Reduced tumor incidence	102
	1α(OH)D$_5$	Reduced aberrant crypt foci	103
LoVo xenografts	EB1089	Tumor suppression	103
HT-29 xenografts	Ro25–6760	Tumor suppression	104
MC-26 xenografts	RO-4383561	Reduced tumor growth	100

Table 6
The molecular targets for vitamin D compounds in cancer

Class	Genes	Regulation
Cell cycle/apoptosis	Bax-mitochondrial translation	↑
	Hypophosphorylated Rb	↑
	$P21^{Waf1}$, $p27^{Kip1}$	↑
	Cyclin D1, A, E	↓
	Telomerase	↓
	Bcl-2	↓
	CDK-2, -4, -6	
Inflammatory	Cox-2	↓
	TNFα	↑
Kinases	P13-K, p38, EKR activities	↑
	PKC levels	↑
Oncogenes	c-myc	↓
	Dek1	↓
	Fli	↓
Transcription factors	C/EBPβ, C/EBPγ	↑
Tumor suppressors	PTEN	↑
	P53	↑

allow for the design of more effective and safer vitamin D compounds for the treatment of hyperproliferative disorders, including cancer.

Vitamin D and the Immune System

T Cells as the Main Target

$1,25(OH)_2D_3$ seems to modulate immunity principally *via* regulating T-cell function. VDR has been found to be expressed on virtually every type of cell involved in immunity (Table 3).[110–113] The immunomodulatory actions of vitamin D are elicited through its direct action on T-cell and antigen-presenting cell (APC) functions. Thus, $1,25(OH)_2D_3$ may have an important physiologic role in immunoregulation and therapeutic target in immune-mediated diseases.

Effects on T Cells and T Helper 1 Cytokine Profiles: Relevance to Autoimmunity

In autoimmune patients, T cells target tissues such as the central nervous system (multiple sclerosis [MS]), the gut (Crohn's disease, inflammatory bowel diseases [IBD]), the joints (rheumatoid arthritis) and the pancreas (type-1 diabetes). The common denominator between these diseases is that T-helper 1 (Th1) cells secrete a proinflammatory profile of cytokines (TNF-α, interferon [INF], IFNγ) at the site of pathology, which drives the disease process. In general, if a treatment for Th1-mediated autoimmunity works, it suppresses the number or activity of Th1 cells or antagonizes the cytokines (TNF-α in particular) that they produce. In addition, treatments that work for one Th1-driven disease are likely to suppress other Th1-driven autoimmune diseases (eg, infliximab used to treat Crohn's disease and rheumatoid arthritis).

Vitamin D regulates T cells both directly and indirectly *via* APCs.[120,121] When vitamin D is deficient or signals through the VDR are weakened, Th1 cell actions are intensified, whereas regulatory T cells and Th2 cells are diminished, thus favoring an autoimmune Th1 response.[122,123] $1\alpha,25(OH)_2D_3$ increases regulatory T cells and Th2 cells (anti-inflammatory cytokines) while suppressing Th1 cell activities.[124] VDRs are required to maintain a physiologic balance of Th1 and Th2 cell responses, and furthermore in the absence of the VDR, Th2 cell functions are diminished.[125] $1\alpha,25(OH)_2D_3$ suppresses production of the Th1 proinflammatory cytokines interleukin-2 (IL-2) by binding to the distal nuclear factor of activated T cells (NF-AT) binding site in the promoter of the human IL-2 gene and IFNγ through the interaction of VDRs, with a vitamin D response element (VDRE) in the promoter region of the IFNγ gene.[126,127]

Because vitamin D deficiency favors a proinflammatory Th1 immune response, does supplementation rebalance immunity*? In vitro* addition of $1\alpha,25(OH)_2D_3$ has recently been shown to enhance and promote differentiation of type 2 T-helper (Th2) lymphocytes through a direct effect on naïve $CD4^+$ T cells. $1\alpha,25(OH)_2D_3$ has been shown *in vitro* to inhibit the development of Th1 cells while promoting the development of Th2 cells.[121,128,129] Thus, $1\alpha,25(OH)_2D_3$ seems to modulate T-cell differentiation, driving cells toward the Th2 phenotype and inhibiting Th1 development.

Thus, the action of $1\alpha,25(OH)_2D_3$ in antagonizing Th1 cytokine production while promoting Th2 function may have an important therapeutic role in diseases whereby Th1 cytokine responses drive the immunopathology.

Effects on APCs

The secretion of cytokines by APCs such as macrophages and dendritic cells (DCs) is crucial for the recruitment and activation of T lymphocytes. The actions of APCs on T cells are also influenced by $1\alpha,25(OH)_2D_3$. Interleukin-12 (IL-12) is the principal APC-derived cytokine that determines the direction (Th1 *vs* Th2) of the immune response. Thus, Th1-stimulating cytokines are inhibited by $1\alpha,25(OH)_2D_3$ in DCs, as well as in other APCs.[124] IL-12 stimulates the development of $CD4^+$ Th1 lymphocytes and inhibits the development of $CD4^+$ Th2 lymphocytes. By inhibiting IL-12, $1\alpha,25(OH)_2D_3$ shifts the immune response away from a Th1 and toward a Th2 profile. Finally, $1\alpha,25(OH)_2D_3$ stimulates DC production of the immunosuppressive cytokine IL-10, which antagonizes the Th1 driving effects of IL-12.[130] These observations provide a rationale for use of vitamin D and its analogs in the prevention and treatment of autoimmune disease.

Autoimmune Disease

Autoimmune diseases occur because of an inappropriate immune-mediated attack against self-tissue, resulting in tissue injury and disease. Both environmental and genetic factors play a vital role in disease development. Vitamin D availability *via* sun exposure or diet may contribute to the development of both MS and IBD. In support of this view, vitamin D and signaling through the VDR have been shown in mice to dictate the outcome of experimental MS and IBD. Vitamin D seems to regulate T-cell development and function, which may influence the outcome of the immune response either toward or away from autoimmunity.[131,132] For example, in the absence of vitamin D and signals delivered through the VDR, autoreactive T cells develop, whereas in the presence of active $1\alpha,25(OH)_2D_3$ and a functional VDR, the balance in the T-cell response is restored and autoimmunity avoided.[133]

Vitamin D from sunlight exposure is lower in areas where IBD occurs most often, as IBD is most prevalent in northern climates such as North America and Northern Europe.[134,135] Vitamin D deficiency is common in patients with IBD even when the disease is in remission.[136,137] Why vitamin D deficiency occurs more frequently in IBD is unclear. It is probably due to the combined effects of low vitamin D intake, malabsorption of many nutrients including vitamin D, and decreased outdoor activities in climates that are not optimal for vitamin D synthesis in the skin.

The strongest evidence that the VDR and its ligand have important roles in the pathogenesis of IBD comes from studies in mouse models. IL-10 knockout mice, model for the Crohn's disease form of IBD, spontaneously develop enterocolitis within 5–8 weeks of birth due to an uncontrolled immune response to resident intestinal flora in conventional animal facilities.[138] Approximately 30% of mice die subsequent to the development of severe anemia and weight loss. By contrast, IL-10 knockout mice raised in pathogen-free facilities develop a milder form of enterocolitis that does not result in death. Experimental IBD is induced by TNF-α- and IFNγ-secreting Th1 cells. Th2 or T regulatory cells inhibit both the development and function (cytokine secretion) of Th1 cells. Cantorna[123] was the first to establish an experimental link between vitamin D status and IBD. The author showed that vitamin D deficiency exacerbates the symptoms of enterocolitis and increases morbidity and mortality in IL-10 knockout mice, whereas supplementation with vitamin D ameliorates IBD symptoms, reduces inflammation, and improves histologic scores and mortality.[123] They noted that 100% of the vitamin D–deficient IL-10 KO mice expressed symptoms of IBD (diarrhea, rectal bleeding) and 60% died before 9 weeks of age from complications of severe IBD. In contrast, vitamin D–sufficient mice showed no outward symptoms of IBD at the same time point.[134]

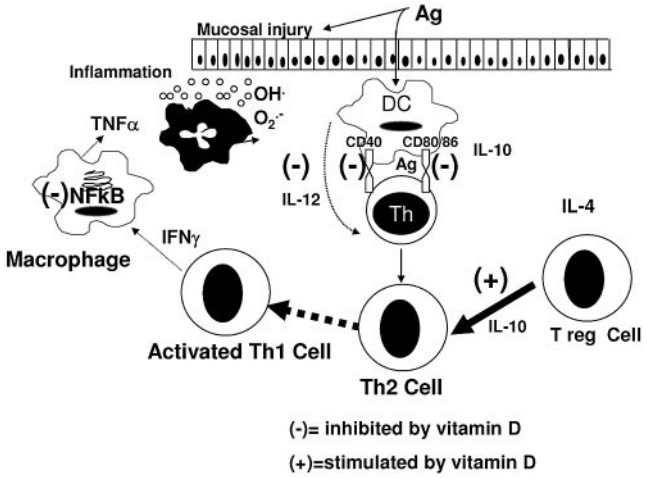

Figure 5. Potential role of vitamin D in Crohn's disease. In Crohn's disease, bacterial antigens drive antigen-presenting cells (DCs) to produce cytokines such as interleukin-12 (IL-12) which drive a T-helper 1 (Th1) proinflammatory response to induce macrophages, which produce TNFα and neutrophil chemoattractive agents, ultimately resulting in the production of noxious agents and tissue injury. The damaged intestinal tissue is more permeable to antigens that drive the vicious cycle of antigen-presentation, local immune activation, and tissue injury. Anti-inflammatory cytokines such as interleukin-10 (IL-10), made by regulatory T cells (T regs), anatagonize Th1 proinflammatory processes by stimulating T-helper 2 function. Vitamin D anatagonizes Th1 proinflammatory responses by interfering with antigen presentation and Th1 activation, up-regulating Th2 cytokines and down-regulating NFκB in macrophages.
Ag, antigen; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

Supporting the vitamin D deficiency data, mice that are both IL-10 and VDR deficient (double VDR/IL-10 KO) develop a fulminating form of experimental IBD that leads to 100% mortality by 7 weeks of age.[123] Interestingly, the severity of IBD in the VDR/IL-10 knockout mice is the same regardless of whether or not disease-causing microorganisms are present in the colony. Both vitamin D deficiency and VDR deficiency render experimental IBD more severe. These observations provide strong evidence that establishes vitamin D and VDR as a physiologic regulator of intestinal inflammation in IBD. The accumulating evidence for the immunomodulatory effects of VDR ligands certainly provides a rationale for further investigation of their potential in the treatment of IBD. A schematic depicting the potential role of vitamin D in Crohn's disease is shown in Figure 5.

A large epidemiologic study showed an inverse relationship between vitamin D status and the development of MS. The study showed that women with the highest vitamin D intakes (including supplements) had a 40% reduction in the risk of developing disease.[139] Like IBD, vitamin D deficiency is common in patients with MS.[122] The cause of low

vitamin D levels in MS patients is also likely to be due to a combination of low vitamin intakes and decreased outdoor activities in climates that are not optimal for vitamin D synthesis in the skin. *In vivo*, the immune targets of vitamin D have been defined primarily in Th1-driven autoimmune diseases. Vitamin D deficiency accelerates the development of experimental MS and type-1 diabetes.[139–142] Conversely, $1,25(OH)_2D_3$ treatment suppressed the development of these Th1-mediated autoimmune diseases.[143] In addition, $1\alpha,25(OH)_2D_3$ treatment of mice with ongoing MS symptoms halted the disease progression in these mice, showing that vitamin D altered the immune response even after the disease had been established. $1\alpha,25(OH)_2D_3$ has been shown to inhibit Th1-driven responses in a number of different models.[142]

Vitamin D may also play a role in preventing type I diabetes mellitus. Autoimmune type 1 diabetes can be prevented in nonobese diabetic mice by $1\alpha,25(OH)_2D_3$ and its analogs.[144,145] Treatment of NOD mice from weaning until old age not only prevented clinical diabetes, it also prevented the histologic lesion insulitis.[146] In this model of autoimmune diabetes, up-regulation of regulatory immune cells and a shift from Th1 toward Th2 lymphocytes locally in the pancreases of treated mice can be observed. This protective Th2 population is induced not only at the site of the β cell attack but also in the peripheral immune system.[147] After immunization of $1\alpha,25(OH)_2D_3$-treated NOD mice with a diabetes-specific autoantigen (a peptide of GAD65), lymphocytes of the draining lymph nodes showed an increased IL-4 and decreased IFNγ production *in vitro* and *in vivo*. Strikingly, this immune deviation induced by $1\alpha,25(OH)_2D_3$ is limited to pancreatic autoantigens and could not be seen after immunization with the β cell-irrelevant protein ovalbumin.

Other effects on the immune system of NOD mice have been described, the most important being a restoration of the defective apoptosis sensitivity of lymphocytes, leading to a more efficient elimination of potentially dangerous autoimmune effector cells.[148] This increased apoptosis induced by $1\alpha,25(OH)_2D_3$ and its analogs in DCs and T lymphocytes of NOD mice has been described after treatment with different apoptosis-inducing signals, such as corticosteroids, and could help to explain why an early short-term treatment with these agents, before onset of autoimmunity, confers long-term protection and promotes tolerance restoration.

Besides preventing the onset of autoimmune diseases, $1\alpha,25(OH)_2D_3$ and its analogs are also able to treat ongoing autoimmune diseases. Treatment of NOD mice with analogs of $1\alpha,25(OH)_2D_3$ can prevent the progression of an initial β cell attack (reflected by the presence of insulitis) to clinical overt diabetes.[149] Interestingly, in this model of ongoing autoimmune destruction, no induction of suppressor cells by $1\alpha,25(OH)_2D_3$ could be demonstrated. Nevertheless, within the pancreases of protected mice, again a shift from Th1 toward Th2 cytokines is noted. Further, analogs of $1\alpha,25(OH)_2D_3$ are able to inhibit the recurrence of autoimmune diabetes after syngeneic islet transplantation in NOD mice.[150,151]

Development of noncalcemic vitamin D analogs could permit sustained systemic administration without causing significant hypercalcemia, thus allowing wider clinical applications in the future. Vitamin D supplementation, which is an inexpensive and efficient way to prevent vitamin D deficiency, might also help reduce the risk of autoimmune disease.

VDR Polymorphisms and Disease

Most of the biologic activities of $1\alpha,25(OH)_2D_3$ are mediated by a high-affinity receptor that acts as a ligand-activated transcription factor. The major steps involved in the control of gene transcription by the VDR include ligand binding, heterodimerization with retinoid X receptor (RXR), binding of the heterodimer to VDREs, and recruitment of other nuclear proteins into the transcriptional preinitiation complex. Thus, genetic alterations of the VDR gene could lead to important defects on gene activation, affecting calcium metabolism, cell proliferation, and immune function. For example, VDR gene mutations cause vitamin D–resistant rickets, a rare monogenetic disease.[152] A polymorphism is a genetic variant that appears in at least 1% of the population. These changes can occur in noncoding parts of the gene (introns), so they would not be seen in the protein product. Changes in these regulatory parts of the gene would then affect the degree of expression of the gene and thus the levels of the protein. The discovery of genetic variants linked with susceptibility of diseases can be the key to advances in preventive medicine. In general, association studies can be used to test whether a polymorphism occurs more frequently in the cases studied than in the controls. If a relationship with the disease emerges from association studies, this finding would strongly support the idea that the candidate gene is in some way involved in the disease. The diseases associated with VDR polymorphisms are summarized in Table 7.[153]

Carling et al[154] reported a relationship between the *Bsm*I polymorphism and primary hyperparathyroidism. Recently, 2 meta-analyses performed by Thakkinstian et al[155,156] demonstrated a positive association between the *b* allele and bone mass density. Furthermore, it was shown that the haplotypes *Bat* and *BAt* were significantly associated with osteoporosis.

An association has been described between VDR polymorphisms and susceptibility to and outcome of some cancers, like breast, prostate, and colon cancers. In 1997, Ingles et al[157] published one of the first reports finding a relationship between the

Table 7
Association between VDR polymorphisms and disease

Malignancy	Autoimmune disease
Prostate cancer	Diabetes mellitus (type 1)
Colon cancer	Systemic lupus erythematous
Malignant melanoma	Multiple sclerosis
Bone mineral density	Crohn's disease
Early postnatal growth	Graves disease
Primary hyperparathyroidism	Autoimmune hepatitis
Nephrolithiasis	Primary biliary cirrhosis
Tuberculosis infection	Diabetes mellitus (type 2)
Hepatitis B infection	Psoriasis

polyA polymorphism of the VDR gene and prostate cancer in the US population. Taylor et al[158] showed the relationship between the *Taq*I polymorphism and an increased risk of prostate cancer, whereas the restriction site (*tt*) was associated with a lower risk with higher levels of $1\alpha,25(OH)_2D_3$.[159] However, other investigators found no association between *Taq*I or polyA and prostate cancer.[160–162] In the case of other VDR polymorphisms (*Fok*I, *Bsm*I), conflicting results can be found as well.[159,162–165] In addition, a recent meta-analysis of 28 different studies was performed, and no relationship was found between any of the former VDR polymorphisms and prostate cancer susceptibility.[166]

As with prostate cancer, conflicting results have been reported regarding the possible relationship between breast cancer and VDR polymorphisms. The majority of the reports present in the literature found no relationship between the risk of breast cancer and *Taq*I polymorphism, but some of them presented a link between *Taq*I polymorphism and risk of metastases.[167–171]

The *Bsm*I shows an opposite pattern, with a relationship detected in 3 reports.[172–174] In the case of *Fok*I the consensus is higher, and most of the reports showed no association with increased risk of breast cancer.[171,173,174] To date, studies investigating the relationship between polyA or *Apa*I[170,171] and the risk of breast cancer showed a link between these VDR polymorphisms and the possibility of presenting a tumor. The number of papers analyzing VDR polymorphisms in other cancer types is significantly lower. Regarding colon carcinoma, there are reports showing an association with *Bsm*I polymorphism,[175,176] whereas conflicting results are found regarding *Fok*I.[177,178]

VDR polymorphisms have been described in a number of autoimmune diseases. A positive relationship between the *B* allele of the *Bsm*I polymorphism and a lack of a relationship between the *Fok*I polymorphism and the incidence of systemic lupus erythematosus has been observed.[179–181] In the case of Crohn's disease, a link has been suggested between the *Taq*I, *Apa*I and *Fok*I polymorphisms and disease susceptibility.[182] Furthermore, a link between *Bsm*I and *Fok*I with primary biliary cirrhosis and autoimmune hepatitis has also been found.[183,184] In MS patients, a higher presence of the *bA* haplotype has been detected,[185,186] whereas *Taq*I[187] and *Fok*I[188] polymorphisms were observed.

In summary, a vast amount of information has been collected through the years regarding the association of vitamin D polymorphisms with susceptibility to contract different diseases. Unfortunately, the results obtained so far are conflicting, and the role of VDR polymorphisms remains obscure. Therefore, the use of VDR polymorphisms as diagnostic tools, or even as markers for a higher propensity toward some diseases, is still a matter of debate.

Recommendations and Conclusion

Vitamin D deficiency is a common clinical problem in the United States.[189–194] Because most patients with mild deficiency are asymptomatic, physicians should have a high index of suspicion in populations at highest risk for deficiency. Identification and treatment of patients with vitamin D deficiency are important for optimal bone development and muscle strength. The major source of vitamin D for both children and adults comes from reasonable sun exposure. In the absence of sun exposure, most experts now agree that 1000 IU of vitamin D is needed daily in the absence of sun exposure to maintain a healthy blood level of 25(OH)D of between 75 and 125 nmol/L (30–50 ng/mL).[194–200] However, vitamin D supplementation is not widely practiced and most supplements only contain 400 IU of vitamin D. As reviewed in this manuscript, in addition to bone health, there is mounting scientific evidence that implicates vitamin D deficiency with an increased risk of autoimmune disease and many common deadly cancers. Children over the age of 1 year and all adults should receive 1000 IU of vitamin D per day, or have judicious sun exposure to satisfy their vitamin D requirement. Measurement of 25(OH)D should be encouraged. The risks and potential benefits of vitamin D supplementation should be further studied for use in patients who either have or are at high risk for breast, colon, and prostate cancer and autoimmune diseases.

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