Vitamin D and the Immune System: New Perspectives on an Old Theme

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- Cathelicidin • Regulatory T cells

HISTORICAL PERSPECTIVE

Nonclassic actions of vitamin D were first recognized 30 years ago when receptors for active 1,25-dihydroxyvitamin D_3 (1,25(OH)$_2D_3$) were detected in various neoplastic cells lines.[1,2] Other studies immediately following this showed that binding of 1,25(OH)$_2D_3$ to the vitamin D receptor (VDR) promoted antiproliferative and prodifferentiation responses in cancer cells,[3,4] highlighting an entirely new facet of vitamin D action. The spectrum of nonclassic responses to vitamin D was then extended to include actions on cells from the immune system.[5,6] This interaction was further endorsed by the observation that some patients with the granulomatous disease sarcoidosis present with increased circulating levels of 1,25(OH)$_2D_3$ and associated hypercalcemia.[7,8] In these patients the high serum level of 1,25(OH)$_2D_3$ is caused by increased activity of the enzyme 25-hydroxyvitamin D-1α-hydrooxylase (1α-hydroxylase). However, in contrast to normal subjects in whom 1α-hydroxylase is classically localized in the kidney, the increased synthesis of 1,25(OH)$_2D_3$ in patients with sarcoidosis involves 1α-hydroxylase activity in disease-associated macrophages.[9–11] Thus, it was concluded that the immune system had the potential to synthesize 1,25(OH)$_2D_3$ and elicit autocrine or paracrine responses from immune cells expressing the VDR.[12]

Despite these early advances, the precise nature of the interaction between vitamin D and the immune system remained unresolved for many years. Some pieces of the puzzle were easier to complete than others. For example, it became evident that dysregulation of 1,25(OH)$_2D_3$ was not restricted to sarcoidosis but was a common feature of many granulomatous disorders and some forms of cancer.[13] Likewise, at least in

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vitro, it was possible to potently regulate a range of immune cell functions using $1,25(OH)_2D_3$ or its synthetic analogs.[14,15] However, the key remaining question was whether or not vitamin D could act as a physiologic regulator of normal immune responses. Answers to this question began to appear about 5 years ago and new information on the fundamental nature of vitamin D sufficiency/insufficiency has provided a fresh perspective on nonclassic actions of vitamin D. As a consequence, there is now a much broader acceptance that vitamin D plays an active role in regulating specific facets of human immunity. Details of this are reviewed and the possible effect of vitamin D insufficiency and vitamin D supplementation on normal immune function and human disease are discussed in this article.

VITAMIN D AND INNATE IMMUNITY
Macrophages, Vitamin D, and Cathelicidin

Consistent with the earlier seminal observations of extrarenal 1α-hydroxylase activity in patients with sarcoidosis, the effects of vitamin D on macrophage function have been central to many of the new observations implicating vitamin D in the regulation of immune responses. In common with natural killer cells (NK) and cytotoxic T lymphocytes (cytotoxic T cells), macrophages, and their monocyte precursors play a central role in initial nonspecific immune responses to pathogenic organisms or tissue damage, so-called cell-mediated immunity. Their role is to phagocytose pathogens or cell debris and then eliminate or assimilate the resulting waste material. In addition, macrophages can interface with the adaptive immune system by using phagocytic material for antigen presentation to T lymphocytes (T cells).

For many years, the key action of vitamin D on macrophages was believed to be its ability to stimulate differentiation of precursor monocytes to more mature phagocytic macrophages.[3,5,16,17] This concept was supported by observations showing differential expression of VDR and 1α-hydroxylase during the differentiation of human monocytes macrophages.[18] The latter report also emphasized early studies showing that normal human macrophages were able to synthesize $1,25(OH)_2D_3$ when stimulated with interferon gamma (IFNγ).[19] Localized activation of vitamin D, coupled with expression of endogenous VDR was strongly suggestive of an autocrine or intracrine system for vitamin D action in normal monocytes/macrophages.

However, confirmation of such a mechanism was only obtained in 2006 when Liu and colleagues[20] carried out DNA array analyses to define innate immunity genes that were specifically modulated in monocytes by *Mycobacterium tuberculosis*. In a seminal investigation both the VDR and the gene for 1α-hydroxylase (CYP27B1) were shown to be induced following activation of the principal pathogen recognition receptor for *M tuberculosis*, toll-like receptor 2/1 (TLR2/1). Subsequent experiments confirmed that precursor 25-hydroxyvitamin D_3 ($25OHD_3$) was able to induce intracrine VDR responses in monocytes that had been treated with a TLR2/1 activator. In particular, the TLR2/1-$25OHD_3$ combination stimulated expression of the antibacterial protein cathelicidin, so that vitamin D was able to promote monocyte killing of *M tuberculosis*.[20] Notably, the ability to promote expression of the antibacterial protein following a TLR2/1 challenge was directly influenced by the $25OHD_3$ status of the donor serum used for monocyte culture.[20] More recently, the authors have shown that vitamin D supplementation in vivo can also enhance TLR2/1-induced cathelicidin expression.[21] Cathelicidin was identified several years ago as a target for transcriptional regulation by $1,25(OH)_2D_3$-liganded VDR, in that its gene promoter contains a functional vitamin D response element (VDRE).[22,23] This VDRE occurs within a small interchangeable nuclear element (SINE) sequence which only seems to be present in

the cathelicidin gene promoter of higher primates, suggesting that vitamin D regulation of this facet of innate immunity is a relatively recent evolutionary development.[22]

Recent reports have underlined the importance of cathelicidin as a target for vitamin D but also suggest that this mechanism may be more complex than initially believed. As yet, the precise signal system by which TLR activation induces expression of VDR and 1α-hydroxylase remains unclear. Promoter-reporter analysis of the events involved in transcriptional regulation of CYP27B1 suggest that TLR4-mediated induction of the enzyme involves JAK-STAT, MAP kinase and nuclear factor kappaB (NF-κB) pathways, and that these synergize with IFNγ-mediated induction of CYP27B1.[24] However, other studies have proposed that TLR2/1 induction of 1α-hydroxylase occurs indirectly as a consequence of TLR2/1-induced interleukin (IL)-15, which is a potent inducer of CYP27B1 and 1α-hydroxylase activity.[25] In a similar fashion, IL-17A has been shown to enhance $1,25(OH)_2D_3$-mediated induction of cathelicidin, although this response does not seem to involve transcriptional regulation of 1α-hydroxylase or increased VDR sensitivity.[26] One pathway that has been poorly studied in this regard concerns the enzyme 24-hydroxylase, which is conventionally considered to function by inactivating $1,25(OH)_2D_3$. The gene for 24-hydroxylase (CYP24) is potently induced by $25OHD_3$ following TLR2/1 activation of monocytes[20] but, as yet, it is unclear whether this involves the nonmetabolic splice variant form of CYP24 known to be expressed by macrophages.[27]

Regulation of the antibacterial protein by $1,25(OH)_2D_3$ has been described for a wide variety of cell types other than macrophages, including keratinocytes,[23,28,29] lung epithelial cells,[30] myeloid cell lines,[22,23,29] and placental trophoblasts.[31] In some cases,[28,31] this seems to involve an intracrine response similar to that reported for monocytes. However, the mechanisms controlling local synthesis of $1,25(OH)_2D_3$ in these cells vary considerably. In keratinocytes, low baseline expression of 1α-hydroxylase is enhanced following epidermal wounding by transforming growth factor beta (TGFβ).[28] The resulting increase in $1,25(OH)_2D_3$ concentration up-regulates expression of TLR2 and TLR4 by keratinocytes, thereby priming these cells for further innate immune responses to pathogens or tissue damage.[28] By contrast, in trophoblasts, induction of cathelicidin and subsequent bacterial killing by $25OHD_3$ seems to be caused by constitutive 1α-hydroxylase activity, which is not further enhanced by TLR activation.[31] The latter may be a result of the rapid nonimmune induction of 1α-hydroxylase and VDR that occurs within the placenta during early gestation.[32]

Although most of the studies on vitamin D-mediated innate immunity have focused on the role of $1,25(OH)_2D_3$-bound VDR as a pivotal transcriptional regulator of cathelicidin, it is also important to recognize that other ligands may interact with the VDR.[33] For example, recent studies of biliary epithelial cells have shown that cathelicidin expression can be induced in a VDR-dependent fashion by bile salts.[34] This provides a mechanism for maintaining biliary sterility, although additive effects of $1,25(OH)_2D_3$ also highlight a novel therapeutic application for vitamin D in the treatment of primary biliary cirrhosis. Conversely, other compounds may act to disrupt normal $1,25(OH)_2D_3$-VDR-mediated immunity. The polycyclic aromatic hydrocarbon benzo[a]pyrene, a prominent product of cigarette smoking, has been shown to attenuate vitamin D-mediated induction of macrophage cathelcidin in a VDR-dependent fashion by stimulating expression of 24-hydroxylase and vitamin D catabolism.[35] The precise mechanism by which this occurs has yet to be determined but these data suggest that some toxic compounds are actively detrimental to vitamin D-mediated immunity.

The observations described earlier show clearly that vitamin D is a potent stimulator of mechanisms associated with pathogen elimination. In subsequent sections the clinical importance of this with respect to vitamin D insufficiency and immune-related

diseases is discussed in more detail. However, 1 key question that immediately arises from the current observations is why there is a need to involve the vitamin D system in the TLR-induction of innate immunity. As previously described, VDR-mediated transcriptional regulation of cathelicidin is a relatively recent evolutionary change and was presumably advantageous when primates (including early *Homo sapiens*) were exposed to abundant sunlight, thereby priming high serum levels of vitamin D. Other benefits of incorporating vitamin D into innate immune regulation include the fact that it is associated with key feedback control pathways. As already mentioned, vitamin D has its own catabolic enzyme in the form of 24-hydroxylase, which sensitively attenuates responses to $1,25(OH)_2D_3$ and, in the case of the CYP24 splice variant, may also attenuate synthesis of this vitamin D metabolite.[27] However, vitamin D may also provide feedback regulation of immune activation pathways in that $1,25(OH)_2D_3$ has been shown to potently down-regulate expression of monocyte TLR2 and TLR4, thereby suppressing inflammatory responses that are normally activated by these receptors.[36] Thus, by using CYP24 and TLR regulatory mechanisms, vitamin D may help to promote appropriate innate immune responses while preventing an over elaboration of innate immune responses and the tissue damage frequently associated with this.

Dendritic Cells and Antigen Presentation

In addition to the phagocytic acquisition and elimination of pathogens and cell debris, innate immunity also involves the presentation of resultant antigen to cells involved in the adaptive arm of the immune system (**Fig. 1**). Although several cells are able to do this, the most well-recognized group of professional antigen-presenting cells (APCs) are dendritic cells (DCs). Expression of VDR by purified tissue DCs was first reported in 1987.[37] Subsequent studies using populations of DCs isolated from skin (Langerhans cells) provided evidence that $1,25(OH)_2D_3$ could act to attenuate antigen presentation.[38] However, it was not until the later advent of in vitro monocyte-derived DC models that the effects of vitamin D metabolites on antigen presentation were fully elucidated. In 2000, parallel studies by the Adorini and Kumar groups showed that $1,25(OH)_2D_3$[39] and its synthetic analogs[40] inhibited the maturation of monocyte-derived DCs, thereby suppressing their capacity to present antigen to T cells. Based on these observations, it was proposed that vitamin D could act to promote tolerance and this was endorsed by studies of pancreatic islet transplantation in which lower rejection rates were observed in $1,25(OH)_2D_3$-treated mice.[41] Crucially this response to $1,25(OH)_2D_3$ appeared to be caused by decreased DC maturation and concomitant enhancement of suppressor or regulatory T cells (Treg).[41] Further studies have underlined the importance of Treg generation[42] as part of the interaction between vitamin D and the immune system, and this is discussed in greater detail in later sections of this article.

Although regulation of DC maturation represents a potential target for $1,25(OH)_2D_3$ and its synthetic analogs as treatment of autoimmune disease and host-graft rejection, another perspective was provided by the observation that DCs express 1α-hydroxylase in a similar fashion to macrophages.[43,44] Data from monocyte-derived DCs showed that 1α-hydroxylase expression and activity increases as DCs differentiate towards a mature phenotype.[44] Functional analyses showed that treatment with $25OHD_3$ suppresses DC maturation and inhibits T-cell proliferation, confirming the existence of an intracrine pathway for vitamin D similar to that observed for macrophages.[44] Mature DCs showed lower levels of VDR than immature DCs or monocytes.[44] This reciprocal organization of 1α-hydroxylase and VDR expression may be advantageous in that mature antigen-presenting DCs may be relatively insensitive to $1,25(OH)_2D_3$, thereby allowing induction of an initial T-cell response. However, the
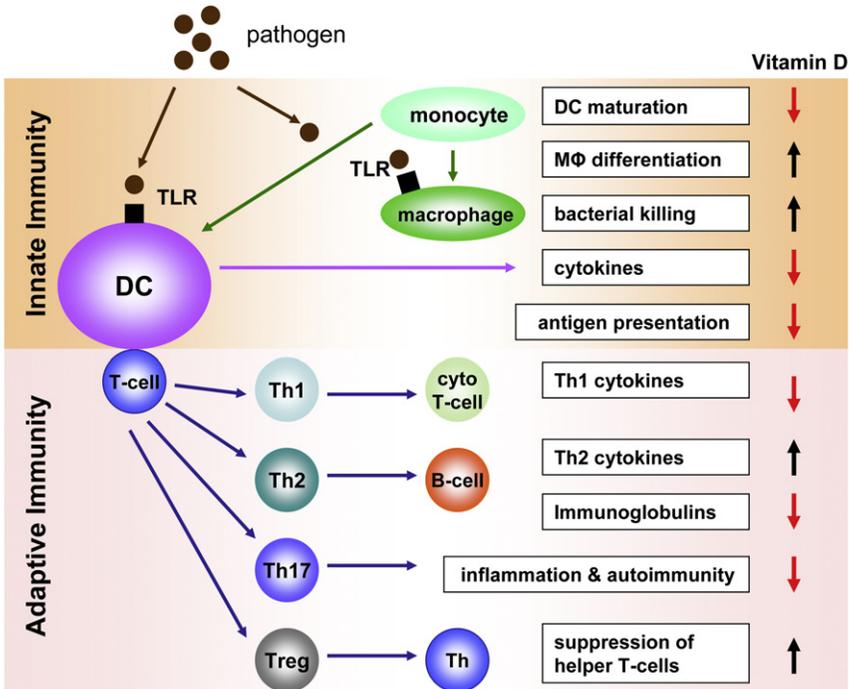
Fig. 1. Effects of vitamin D on innate and adaptive immunity. The principal innate and adaptive immune responses to a pathogenic challenge, and the positive or negative regulation of these responses by vitamin D. B-cell, B lymphocyte; cyto T-cell, cytotoxic T cell; DC, dendritic cell; MΦ, macrophage; T-cell, T lymphocyte; TLR, toll-like receptor; Treg, regulatory T cell.

high levels of $1,25(OH)_2D_3$ being synthesized by these cells can to act on VDR-expressing immature DCs and thus prevent their further development.[45] In this way, paracrine action of locally produced $1,25(OH)_2D_3$ allows initial presentation of antigen to T cells while preventing continued maturation of DCs and over stimulation of T cells.

Although DCs are heterogeneous in terms of their location, phenotype, and function, they are broadly divided into 2 groups based on their origin. Myeloid (mDCs) and plasmacytoid (pDCs) express different types of cytokines and chemokines and seem to exert complementary effects on T-cell responses, with mDCs being the most effective APCs[46] and pDCs being more closely associated with immune tolerance.[47] Therefore $1,25(OH)_2D_3$ preferentially regulates mDCs, suggesting that the key effect of vitamin D in this instance is to suppress activation of naive T cells. Although in this study pDCs showed no apparent immune response to $1,25(OH)_2D_3$, this does not preclude a role for vitamin D in the regulation of tolerogenic responses. One possibility is that local intracrine synthesis of $1,25(OH)_2D_3$ is more effective in achieving these responses. Alternatively, $1,25(OH)_2D_3$ synthesized by pDCs may regulate tolerance through paracrine effects on VDR-expressing T cells. This is discussed in further detail in the following section.

VITAMIN D AND ADAPTIVE IMMUNITY
Vitamin D and T-cell Function

Resting T cells express almost undetectable levels of VDR, but levels of the receptor increase as T cells proliferate following antigenic activation.[48–50] As

a consequence, initial studies of the effects of vitamin D on T cells focused on the ability of $1,25(OH)_2D_3$ to suppress T-cell proliferation.[48–50] However, the recognition that $CD4^+$ effector T cells were capable of considerable phenotypic plasticity, suggested that vitamin D might also influence the phenotype of T cells. Lemire and colleagues[51] first reported that $1,25(OH)_2D_3$ preferentially inhibited T helper 1 (Th1) cells, which are a subset of $CD4^+$ effector T cells closely associated with cellular, rather than humoral, immune responses. Subsequent studies confirmed this observation and demonstrated that the cytokine profile of $1,25(OH)_2D_3$-treated human T cells was consistent with Th2 cells, a subset of $CD4^+$ T cells associated with humoral (antibody)-mediated immunity.[52,53] The conclusion from these observations was that vitamin D promotes a T-cell shift from Th1 to Th2 and thus might help to limit the potential tissue damage associated with Th1 cellular immune responses. However, the validity of this generalization was called into question by studies using mouse T cells in which $1,25(OH)_2D_3$ was shown to inhibit cytokines associated with Th1 (IFNγ) and Th2 (IL-4). Subsequent analysis of immune cells from the VDR gene knockout mouse added further confusion by showing that these animals had reduced (rather than the predicted increased) levels of Th1 cells.[54] Thus, although in vitro vitamin D seems to broadly support a shift from Th1 to Th2 in $CD4^+$ cells, it seems likely that in vivo its effects on T cells are more complex.

The T-cell repertoire has continued to expand with the characterization of another effector T-cell lineage distinct from Th1 or Th2 cells, termed Th17 cells because of their capacity to synthesize IL-17.[55,56] Th17 cells play an essential role in combating certain pathogens but they have also been linked to tissue damage and inflammation.[57,58] The precise role of vitamin D as a regulator of Th17 cells has yet to be fully elucidated but studies of animal models of the gastrointestinal inflammatory disease colitis have shown that treatment with $1,25(OH)_2D_3$ reduces expression of IL-17,[59] and loss of $1,25(OH)_2D_3$ as a result of CYP27b1 gene ablation leads to increased levels of this cytokine.[60] Thus, it possible that vitamin D exerts some of its effects on inflammation and autoimmune disease through the regulation of Th17 cells.

A fourth group of $CD4^+$ T cells exert suppressor rather than effector functions and are known as regulatory T cells or Tregs. In view of its early recognition as a suppressor of T-cell proliferation, it was anticipated that vitamin D would have effects on Tregs, and indeed in 2002 Barrat and colleagues[61] showed that $1,25(OH)_2D_3$, in conjunction with glucocorticoids, potently stimulated the generation of IL-10–producing $CD4^+$/$CD25^+$ Tregs. Subsequent reports indicated that $1,25(OH)_2D_3$ alone can induce Tregs,[62] and it seems that preferential differentiation of Tregs is a pivotal mechanism linking vitamin D and adaptive immunity, with potential beneficial effects for autoimmune disease and host-graft rejection.[63–65] This immunosuppressive mechanism is likely to be mediated by the induction of tolerogenic DCs as described in the previous section of the review,[41,66,67] but direct effects on T cells may also be important.[68] In this latter study, it was notable that $1,25(OH)_2D_3$ increased IL-10 secretion and TLR9 expression by Tregs, suggesting a novel link between innate and adaptive immune responses.[68]

Relative to the amount of literature on $CD4^+$ effector cells, our understanding of the effects of vitamin D on $CD8^+$ suppressor T cells remains somewhat limited. In contrast to $CD4^+$ cells, $CD8^+$ cells show poor antiproliferative response to $1,25(OH)_2D_3$.[50,69,70] However, VDR expression seems to be abundant in $CD8^+$ cells suggesting that they are still potential targets for $1,25(OH)_2D_3$. Indeed subsequent reports have shown that $1,25(OH)_2D_3$ actively regulates cytokine production by $CD8^+$ cells,[71] and can also regulate the proliferation of $CD8^+$ cells following specific immune stimuli.[72] Despite

this, $1,25(OH)_2D_3$ does not seem to have a significant effect on animal disease models such as experimental autoimmune encephalomyelitis in which $CD8^+$ cells have been implicated.[73]

Although many of the studies linking $1,25(OH)_2D_3$ with adaptive immunity have focused on changes in T-cell proliferation and phenotype, it is important to recognize that other facets of T-cell function may also be affected by the hormone. In particular, recent studies have shown that vitamin D can exert powerful effects on the homing of T cells to specific tissues. Initial studies suggested that $1,25(OH)_2D_3$ acts to inhibit migration of T cells to lymph nodes.[74] However, more recent reports have shown an active role for vitamin D in promoting homing of T cells to the skin via up-regulation of chemokine receptor 10 (CCR10), the ligand for which, CCL27, is expressed by epidermal keratinocytes.[75] This T-cell homing response was induced by $25OHD_3$ as well as $1,25(OH)_2D_3$ and the author suggested that DCs and T cells were possible sources of the local 1α-hydroxylase activity.[75] In contrast to its positive effect on epidermal T-cell homing, vitamin D seems to exert a negative effect on chemokines and chemokine receptors associated with the gastrointestinal tract.[75] However, it seems likely that this is highly T-cell selective as newer studies using the VDR gene knockout mouse have shown aberrant gastrointestinal migration of a subset of $CD8^+$ cells, and this effect seems to be closely linked to the increased risk of colitis in VDR knockout mice.[76]

Vitamin D and B-cell Function

Like T cells, active but not inactive B cells express the VDR.[77] Consequently, initial studies indicated that $1,25(OH)_2D_3$ could directly regulate B-cell proliferation[78] and immunoglobulin (Ig) production.[77] Subsequent work contradicted this, suggesting instead that the ability of $1,25(OH)_2D_3$ to suppress proliferation and Ig production was caused by indirect effects mediated via helper T cells.[79] However, more recent reports have shown that $1,25(OH)_2D_3$ does indeed exert direct effects on B-cell homeostasis.[80] In addition to confirming direct VDR-mediated effects on B-cell proliferation and Ig production, this study also highlighted the ability of $1,25(OH)_2D_3$ to inhibit the differentiation of plasma cells and class-switched memory cells, suggesting a potential role for vitamin D in B-cell–related disorders such as systemic lupus erythematosus. Expression of CYP27b1 was also detected in B cells, indicating that B cells may be capable of autocrine/intracrine responses to vitamin D.[80] Indeed, this may be common to lymphocytes in general as CYP27b1 expression has also been detected in T cells.[75]

VITAMIN D, THE IMMUNE SYSTEM AND HUMAN HEALTH

For many years vitamin D status was defined simply by whether or not the patient had symptoms of the bone disease rickets (osteomalacia in adults). However, an entirely new perspective on vitamin D status has arisen from the observation that serum levels of the main circulating form of vitamin D ($25OHD_3$) as high as 75 nM correlate inversely with parathyroid hormone.[81] This, has prompted the introduction of a new term, vitamin D insufficiency, defined by serum levels of $25OHD_3$ that are suboptimal (<75 nM) but not necessarily rachitic (<20 nM).[82] Unlike serum concentrations of $1,25(OH)_2D_3$, which are primarily defined by the endocrine regulators of the vitamin D–activating enzyme, 1α-hydroxylase, circulating levels of $25OHD_3$ are a direct reflection of vitamin D status, which for any given individual depends on access to vitamin D either through exposure to sunlight or through dietary intake. The net effect of this is that vitamin D status can vary significantly in populations depending on geographic,

social, or economic factors. As a result of these new parameters for vitamin D status, a consensus statement from the 13th Workshop on Vitamin D concluded that vitamin D insufficiency was a worldwide epidemic. Moreover, recent studies have shown that in the last 10 years alone, serum vitamin D levels have on average fallen by 20%.[83] The key question now being considered is what is the physiologic and clinical effect of global vitamin D insufficiency beyond classic bone diseases such as rickets? Epidemiologic studies have highlighted possible links between vitamin D insufficiency and a wide range of human diseases.[82] The final section of the article describes 4 of the key clinical problems that have been linked to the immunomodulatory properties of vitamin D.

Vitamin D and Tuberculosis

The observation that vitamin D acts to promote innate immune responses to TLR activation by *M tuberculosis*[20] has provided a new perspective on observations made many decades ago on the beneficial effects of ultraviolet light exposure on tuberculosis (TB). As a consequence this has become the most well-studied facet of the interaction between vitamin D and innate immunity.[84] Initial studies to assess the effects of 25OHD status on ex vivo macrophage function have shown that supplementation with a single oral dose of 2.5 mg of vitamin D enhances the ability of recipient macrophages to combat Bacille Calmette Guérin infection in vitro.[85] The potential benefits of vitamin D as treatment of TB have been further endorsed by a study that showed that adjunct vitamin D supplementation (0.25 mg vitamin D/d) of TB patients receiving conventional therapy for the disease reduced the time for sputum smear conversion from acid-fast bacteria (AFB)–positive to AFB-negative status.[86] A recent, double-blind, randomized, placebo-controlled trial showed that vitamin D supplementation had no effect on clinical outcomes or mortality amongst TB patients, although it should be emphasized that none of the supplemented patients in this study showed a significant increase in serum vitamin D levels.[87]

Vitamin D and Multiple Sclerosis

Several epidemiology studies have reported an association between vitamin D insufficiency and the incidence and/or severity of the autoimmune disease multiple sclerosis (MS) (reviewed in Ref.[88]). These observations have been supported by analysis of animal models, such as the experimental autoimmune encephalomyelitis (EAE) mouse, which show increased disease severity under dietary vitamin D restriction.[89] Conversely, administration of $1,25(OH)_2D_3$ to EAE mice confers disease protection through effects on cytokine synthesis and apoptosis of inflammatory cells.[90,91] Some effects of $1,25(OH)_2D_3$ on EAE seem to be dependent on IL-10 activity.[65]

Vitamin D and Type 1 Diabetes

In common with MS, published reports suggest that there is a link between vitamin D deficiency and another autoimmune disease, type 1 diabetes (reviewed in Ref.[92]). Low circulating levels of $25OHD_3$ have been reported in adolescents at the time of diagnosis of type 1 diabetes,[93] and other data have documented the beneficial effects of vitamin D supplementation in protecting against type 1 diabetes.[94] Another strand of evidence linking vitamin D with type 1 diabetes stems from extensive genetic analyses on the physiologic effect of inherited variations in the genes for various components of the vitamin D metabolic and signaling system. Previous studies have indicated that some VDR gene haplotypes confer protection against diabetes,[95] and more recently this has been expanded to show that genetic variants of the CYP27b1 gene also affect susceptibility to type 1 diabetes.[96] Similar to animal model

studies for MS, in vivo use of the nonobese diabetic (NOD) mouse as a model for type 1 diabetes has shown increased disease severity under conditions of dietary vitamin D restriction.[97]

Vitamin D and Crohn Disease

Several strands of evidence have linked vitamin D to the dysregulated immune responses observed with inflammatory bowel diseases such as Crohn disease. First, epidemiology suggests that patients with Crohn disease have decreased serum levels of $25OHD_3$.[98–100] Second, studies in vivo using various animal models indicate that $1,25(OH)_2D_3$ plays a crucial role in the pathophysiology of experimentally induced forms of inflammatory bowel disease.[60,101–103] Third, expression of 1α-hydroxylase has been detected in the human colon,[104] with the vitamin D–activating enzyme being up-regulated in disease-affected tissue from patients with Crohn disease.[105] In the case of the latter, dysregulated colonic expression of 1α-hydroxylase was associated with increased circulating levels of $1,25(OH)_2D_3$ indicating that, as with sarcoidosis, localized synthesis of this vitamin D metabolite can spill over into the general circulation under conditions of persistent disease.[105] Current studies have implicated aberrant innate immune handling of enteric microbiota as an initiator of the adaptive immune damage associated with Crohn disease.[106] It is thus tempting to speculate that effects of vitamin D on this disease may involve the activation of innate immunity, together with the suppression of adaptive immunity and associated inflammation.

SUMMARY

It is almost 30 years since an interaction between vitamin D and the immune system was first documented. Although this was initially proposed as a nonclassic effect of vitamin D associated with granulomatous diseases, our current view is now changed considerably. Recent studies have shown a potential physiologic role for vitamin D in regulating normal innate and adaptive immunity. Future studies now need to focus on the clinical implications of vitamin D–mediated immunity and, in particular, the possible beneficial effects of supplementary vitamin D with respect to infectious and autoimmune diseases.

REFERENCES

1. Eisman JA, Martin TJ, MacIntyre I, et al. 1,25-Dihydroxyvitamin-D-receptor in breast cancer cells. Lancet 1979;2:1335.
2. Manolagas SC, Haussler MR, Deftos LJ. 1,25-Dihydroxyvitamin D3 receptors in cancer. Lancet 1980;1:828.
3. Abe E, Miyaura C, Sakagami H, et al. Differentiation of mouse myeloid leukemia cells induced by 1alpha,25-dihydroxyvitamin D3. Proc Natl Acad Sci U S A 1981;78:4990.
4. Colston K, Colston MJ, Feldman D. 1,25-Dihydroxyvitamin D3 and malignant melanoma: the presence of receptors and inhibition of cell growth in culture. Endocrinology 1981;108:1083.
5. Abe E, Miyaura C, Tanaka H, et al. 1 alpha,25-dihydroxyvitamin D3 promotes fusion of mouse alveolar macrophages both by a direct mechanism and by a spleen cell-mediated indirect mechanism. Proc Natl Acad Sci U S A 1983; 80:5583.
6. Bhalla AK, Amento EP, Serog B, et al. 1,25-Dihydroxyvitamin D3 inhibits antigen-induced T cell activation. J Immunol 1984;133:1748.

7. Bell NH, Stern PH, Pantzer E, et al. Evidence that increased circulating 1alpha, 25-dihydroxyvitamin D is the probable cause for abnormal calcium metabolism in sarcoidosis. J Clin Invest 1979;64:218.

8. Papapoulos SE, Clemens TL, Fraher LJ, et al. 1,25-Dihydroxycholecalciferol in the pathogenesis of the hypercalcaemia of sarcoidosis. Lancet 1979;1:627.

9. Adams JS, Gacad MA. Characterization of 1 alpha-hydroxylation of vitamin D3 sterols by cultured alveolar macrophages from patients with sarcoidosis. J Exp Med 1985;161:755.

10. Adams JS, Sharma OP, Gacad MA, et al. Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis. J Clin Invest 1983;72:1856.

11. Barbour GL, Coburn JW, Slatopolsky E, et al. Hypercalcemia in an anephric patient with sarcoidosis: evidence for extrarenal generation of 1,25-dihydroxyvitamin D. N Engl J Med 1981;305:440.

12. Hewison M. Vitamin D and the immune system. J Endocrinol 1992;132:173.

13. Hewison M, Burke F, Evans KN, et al. Extra-renal 25-hydroxyvitamin D3-1alpha-hydroxylase in human health and disease. J Steroid Biochem Mol Biol 2007; 103:316.

14. Griffin MD, Xing N, Kumar R. Vitamin D and its analogs as regulators of immune activation and antigen presentation. Annu Rev Nutr 2003;23:117.

15. Van Etten E, Decallonne B, Verlinden L, et al. Analogs of 1alpha,25-dihydroxyvitamin D3 as pluripotent immunomodulators. J Cell Biochem 2003;88:223.

16. Koeffler HP, Amatruda T, Ikekawa N, et al. Induction of macrophage differentiation of human normal and leukemic myeloid stem cells by 1,25-dihydroxyvitamin D3 and its fluorinated analogues. Cancer Res 1984;44:5624.

17. Tanaka H, Abe E, Miyaura C, et al. 1 alpha,25-Dihydroxyvitamin D3 induces differentiation of human promyelocytic leukemia cells (HL-60) into monocyte-macrophages, but not into granulocytes. Biochem Biophys Res Commun 1983;117:86.

18. Kreutz M, Andreesen R, Krause SW, et al. 1,25-Sihydroxyvitamin D3 production and vitamin D3 receptor expression are developmentally regulated during differentiation of human monocytes into macrophages. Blood 1993;82:1300.

19. Koeffler HP, Reichel H, Bishop JE, et al. Gamma-interferon stimulates production of 1,25-dihydroxyvitamin D3 by normal human macrophages. Biochem Biophys Res Commun 1985;127:596.

20. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006;311:1770.

21. Adams JS, Ren S, Liu PT, et al. Vitamin D-directed rheostatic regulation of monocyte antibacterial responses. J Immunol 2009;182:4289.

22. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J 2005;19:1067.

23. Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004; 173:2909.

24. Stoffels K, Overbergh L, Giulietti A, et al. Immune regulation of 25-hydroxyvitamin-d(3)-1alpha-hydroxylase in human monocytes. J Bone Miner Res 2006; 21:37.

25. Krutzik SR, Hewison M, Liu PT, et al. IL-15 links TLR2/1-induced macrophage differentiation to the vitamin D-dependent antimicrobial pathway. J Immunol 2008;181:7115.

26. Peric M, Koglin S, Kim SM, et al. IL-17A enhances vitamin D3-induced expression of cathelicidin antimicrobial peptide in human keratinocytes. J Immunol 2008;181:8504.

27. Ren S, Nguyen L, Wu S, et al. Alternative splicing of vitamin D-24-hydroxylase: a novel mechanism for the regulation of extrarenal 1,25-dihydroxyvitamin D synthesis. J Biol Chem 2005;280:20604.

28. Schauber J, Dorschner RA, Coda AB, et al. Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. J Clin Invest 2007;117:803.

29. Schauber J, Dorschner RA, Yamasaki K, et al. Control of the innate epithelial antimicrobial response is cell-type specific and dependent on relevant microenvironmental stimuli. Immunology 2006;118:509.

30. Yim S, Dhawan P, Ragunath C, et al. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3). J Cyst Fibros 2007;6:403.

31. Liu N, Kaplan AT, Low J, et al. Vitamin D induces innate antibacterial responses in human trophoblasts via an intracrine pathway. Biol Reprod 2009;80:398.

32. Evans KN, Bulmer JN, Kilby MD, et al. Vitamin D and placental-decidual function. J Soc Gynecol Investig 2004;11:263.

33. Makishima M, Lu TT, Xie W, et al. Vitamin D receptor as an intestinal bile acid sensor. Science 2002;296:1313.

34. D'Aldebert E, Biyeyeme Bi Mve MJ, Mergey M, et al. Bile salts control the antimicrobial peptide cathelicidin through nuclear receptors in the human biliary epithelium. Gastroenterology 2009;136:1435.

35. Matsunawa M, Amano Y, Endo K, et al. The aryl hydrocarbon receptor activator benzo[a]pyrene enhances vitamin D3 catabolism in macrophages. Toxicol Sci 2009;109:50–8.

36. Sadeghi K, Wessner B, Laggner U, et al. Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. Eur J Immunol 2006;36:361.

37. Brennan A, Katz DR, Nunn JD, et al. Dendritic cells from human tissues express receptors for the immunoregulatory vitamin D3 metabolite, dihydroxycholecalciferol. Immunology 1987;61:457.

38. Dam TN, Moller B, Hindkjaer J, et al. The vitamin D3 analog calcipotriol suppresses the number and antigen-presenting function of Langerhans cells in normal human skin. J Investig Dermatol Symp Proc 1996;1:72.

39. Penna G, Adorini L. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J Immunol 2000;164:2405.

40. Griffin MD, Lutz WH, Phan VA, et al. Potent inhibition of dendritic cell differentiation and maturation by vitamin D analogs. Biochem Biophys Res Commun 2000;270:701.

41. Gregori S, Casorati M, Amuchastegui S, et al. Regulatory T cells induced by 1 alpha,25-dihydroxyvitamin D3 and mycophenolate mofetil treatment mediate transplantation tolerance. J Immunol 1945;167:2001.

42. O'Garra A, Barrat FJ. In vitro generation of IL-10-producing regulatory CD4+ T cells is induced by immunosuppressive drugs and inhibited by Th1- and Th2-inducing cytokines. Immunol Lett 2003;85:135.

43. Fritsche J, Mondal K, Ehrnsperger A, et al. Regulation of 25-hydroxyvitamin D3-1 alpha-hydroxylase and production of 1 alpha,25-dihydroxyvitamin D3 by human dendritic cells. Blood 2003;102:3314.

44. Hewison M, Freeman L, Hughes SV, et al. Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells. J Immunol 2003;170:5382.
45. Hewison M, Zehnder D, Chakraverty R, et al. Vitamin D and barrier function: a novel role for extra-renal 1 alpha-hydroxylase. Mol Cell Endocrinol 2004;215:31.
46. Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol 2005;23:275.
47. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. Annu Rev Immunol 2003;21:685.
48. Karmali R, Hewison M, Rayment N, et al. 1,25(OH)2D3 regulates c-myc mRNA levels in tonsillar T lymphocytes. Immunology 1991;74:589.
49. Nunn JD, Katz DR, Barker S, et al. Regulation of human tonsillar T-cell proliferation by the active metabolite of vitamin D3. Immunology 1986;59:479.
50. Provvedini DM, Manolagas SC. 1 alpha,25-dihydroxyvitamin D3 receptor distribution and effects in subpopulations of normal human T lymphocytes. J Clin Endocrinol Metab 1989;68:774.
51. Lemire JM, Archer DC, Beck L, et al. Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions. J Nutr 1995;125:1704S.
52. Boonstra A, Barrat FJ, Crain C, et al. 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. J Immunol 2001;167:4974.
53. Overbergh L, Decallonne B, Waer M, et al. 1alpha,25-dihydroxyvitamin D3 induces an autoantigen-specific T-helper 1/T-helper 2 immune shift in NOD mice immunized with GAD65 (p524-543). Diabetes 2000;49:1301.
54. O'Kelly J, Hisatake J, Hisatake Y, et al. Normal myelopoiesis but abnormal T lymphocyte responses in vitamin D receptor knockout mice. J Clin Invest 2002;109:1091.
55. Harrington LE, Mangan PR, Weaver CT. Expanding the effector CD4 T-cell repertoire: the Th17 lineage. Curr Opin Immunol 2006;18:349.
56. Weaver CT, Hatton RD, Mangan PR, et al. IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu Rev Immunol 2007;25:821.
57. Bettelli E, Korn T, Kuchroo VK. Th17: the third member of the effector T cell trilogy. Curr Opin Immunol 2007;19:652.
58. Korn T, Oukka M, Kuchroo V, et al. Th17 cells: effector T cells with inflammatory properties. Semin Immunol 2007;19:362.
59. Daniel C, Sartory NA, Zahn N, et al. Immune modulatory treatment of TNBS colitis with calcitriol is associated with a change of a Th1/Th17 to a Th2 and regulatory T cell profile. J Pharmacol Exp Ther 2007;323:23–33.
60. Liu N, Nguyen L, Chun RF, et al. Altered endocrine and autocrine metabolism of vitamin D in a mouse model of gastrointestinal inflammation. Endocrinology 2008;149:4799.
61. Barrat FJ, Cua DJ, Boonstra A, et al. In vitro generation of interleukin 10-producing regulatory CD4(+) T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. J Exp Med 2002;195:603.
62. Gorman S, Kuritzky LA, Judge MA, et al. Topically applied 1,25-dihydroxyvitamin D3 enhances the suppressive activity of CD4+CD25+ cells in the draining lymph nodes. J Immunol 2007;179:6273.
63. Gregori S, Giarratana N, Smiroldo S, et al. A 1alpha,25-dihydroxyvitamin D(3) analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. Diabetes 2002;51:1367.

64. Mathieu C, Badenhoop K. Vitamin D and type 1 diabetes mellitus: state of the art. Trends Endocrinol Metab 2005;16:261.
65. Spach KM, Nashold FE, Dittel BN, et al. IL-10 signaling is essential for 1,25-di-hydroxyvitamin D3-mediated inhibition of experimental autoimmune encephalomyelitis. J Immunol 2006;177:6030.
66. Adorini L, Penna G, Giarratana N, et al. Dendritic cells as key targets for immunomodulation by vitamin D receptor ligands. J Steroid Biochem Mol Biol 2004; 89-90:437.
67. Dong X, Bachman LA, Kumar R, et al. Generation of antigen-specific, interleukin-10-producing T-cells using dendritic cell stimulation and steroid hormone conditioning. Transpl Immunol 2003;11:323.
68. Urry Z, Xystrakis E, Richards DF, et al. Ligation of TLR9 induced on human IL-10-secreting Tregs by 1alpha,25-dihydroxyvitamin D3 abrogates regulatory function. J Clin Invest 2009;119:387.
69. Vanham G, Ceuppens JL, Bouillon R. T lymphocytes and their CD4 subset are direct targets for the inhibitory effect of calcitriol. Cell Immunol 1989; 124:320.
70. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. Arch Biochem Biophys 2000;374:334.
71. Willheim M, Thien R, Schrattbauer K, et al. Regulatory effects of 1alpha,25-dihydroxyvitamin D3 on the cytokine production of human peripheral blood lymphocytes. J Clin Endocrinol Metab 1999;84:3739.
72. Iho S, Iwamoto K, Kura F, et al. Mechanism in 1,25(OH)2D3-induced suppression of helper/suppressor function of CD4/CD8 cells to immunoglobulin production in B cells. Cell Immunol 1990;127:12.
73. Meehan TF, DeLuca HF. CD8(+) T cells are not necessary for 1 alpha,25-dihydroxyvitamin D(3) to suppress experimental autoimmune encephalomyelitis in mice. Proc Natl Acad Sci U S A 2002;99:5557.
74. Topilski I, Flaishon L, Naveh Y, et al. The anti-inflammatory effects of 1,25-dihydroxyvitamin D3 on Th2 cells in vivo are due in part to the control of integrin-mediated T lymphocyte homing. Eur J Immunol 2004;34:1068.
75. Sigmundsdottir H, Pan J, Debes GF, et al. DCs metabolize sunlight-induced vitamin D3 to 'program' T cell attraction to the epidermal chemokine CCL27. Nat Immunol 2007;8:285.
76. Yu S, Bruce D, Froicu M, et al. Failure of T cell homing, reduced CD4/CD8alphaalpha intraepithelial lymphocytes, and inflammation in the gut of vitamin D receptor KO mice. Proc Natl Acad Sci U S A 2008;105:20834.
77. Provvedini DM, Tsoukas CD, Deftos LJ, et al. 1 alpha,25-Dihydroxyvitamin D3-binding macromolecules in human B lymphocytes: effects on immunoglobulin production. J Immunol 1986;136:2734.
78. Shiozawa K, Shiozawa S, Shimizu S, et al. 1 alpha,25-dihydroxyvitamin D3 inhibits pokeweed mitogen-stimulated human B-cell activation: an analysis using serum-free culture conditions. Immunology 1985;56:161.
79. Lemire JM, Adams JS, Sakai R, et al. 1 alpha,25-dihydroxyvitamin D3 suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. J Clin Invest 1984;74:657.
80. Chen S, Sims GP, Chen XX, et al. Modulatory effects of 1,25-dihydroxyvitamin d3 on human B cell differentiation. J Immunol 2007;179:1634.
81. Chapuy MC, Preziosi P, Maamer M, et al. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 1997;7:439.
82. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266.

83. Ginde AA, Liu MC, Camargo CA Jr. Demographic differences and trends of vitamin D insufficiency in the US population, 1988–2004. Arch Intern Med 2009;169:626.

84. Martineau AR, Honecker FU, Wilkinson RJ, et al. Vitamin D in the treatment of pulmonary tuberculosis. J Steroid Biochem Mol Biol 2007;103:793.

85. Martineau AR, Wilkinson RJ, Wilkinson KA, et al. A single dose of vitamin D enhances immunity to mycobacteria. Am J Respir Crit Care Med 2007;176:208.

86. Nursyam EW, Amin Z, Rumende CM. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. Acta Med Indones 2006;38:3.

87. Wejse C, Gomes VF, Rabna P, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. Am J Respir Crit Care Med 2009;179:843.

88. Raghuwanshi A, Joshi SS, Christakos S. Vitamin D and multiple sclerosis. J Cell Biochem 2008;105:338.

89. Spach KM, Hayes CE. Vitamin D3 confers protection from autoimmune enceph-alomyelitis only in female mice. J Immunol 2005;175:4119.

90. Pedersen LB, Nashold FE, Spach KM, et al. 1,25-Dihydroxyvitamin D3 reverses experimental autoimmune encephalomyelitis by inhibiting chemokine synthesis and monocyte trafficking. J Neurosci Res 2007;85:2480.

91. Spach KM, Pedersen LB, Nashold FE, et al. Gene expression analysis suggests that 1,25-dihydroxyvitamin D3 reverses experimental autoimmune encephalo-myelitis by stimulating inflammatory cell apoptosis. Physiol Genomics 2004; 18:141.

92. Mathieu C, Gysemans C, Giulietti A, et al. Vitamin D and diabetes. Diabetologia 2005;48:1247.

93. Littorin B, Blom P, Scholin A, et al. Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). Diabetologia 2006;49:2847.

94. Harris SS. Vitamin D in type 1 diabetes prevention. J Nutr 2005;135:323.

95. Ramos-Lopez E, Jansen T, Ivaskevicius V, et al. Protection from type 1 diabetes by vitamin D receptor haplotypes. Ann N Y Acad Sci 2006;1079:327.

96. Bailey R, Cooper JD, Zeitels L, et al. Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. Diabetes 2007;56(10):2616.

97. Giulietti A, Gysemans C, Stoffels K, et al. Vitamin D deficiency in early life accel-erates type 1 diabetes in non-obese diabetic mice. Diabetologia 2004;47:451.

98. Pappa HM, Gordon CM, Saslowsky TM, et al. Vitamin D status in children and young adults with inflammatory bowel disease. Pediatrics 1950;118:2006.

99. Pappa HM, Grand RJ, Gordon CM. Report on the vitamin D status of adult and pediatric patients with inflammatory bowel disease and its significance for bone health and disease. Inflamm Bowel Dis 2006;12:1162.

100. Vagianos K, Bector S, McConnell J, et al. Nutrition assessment of patients with inflammatory bowel disease. JPEN J Parenter Enteral Nutr 2007;31:311.

101. Froicu M, Cantorna MT. Vitamin D and the vitamin D receptor are critical for control of the innate immune response to colonic injury. BMC Immunol 2007;8:5.

102. Froicu M, Weaver V, Wynn TA, et al. A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. Mol Endocrinol 2003;17:2386.

103. Kong J, Zhang Z, Musch MW, et al. Novel role of the vitamin D receptor in main-taining the integrity of the intestinal mucosal barrier. Am J Physiol Gastrointest Liver Physiol 2007;294:G208–16.

104. Zehnder D, Bland R, Williams MC, et al. Extrarenal expression of 25-hydroxyvi-
      tamin d(3)-1 alpha-hydroxylase. J Clin Endocrinol Metab 2001;86:888.
105. Abreu MT, Kantorovich V, Vasiliauskas EA, et al. Measurement of vitamin D
      levels in inflammatory bowel disease patients reveals a subset of Crohn's
      disease patients with elevated 1,25-dihydroxyvitamin D and low bone mineral
      density. Gut 2004;53:1129.
106. Packey CD, Sartor RB. Commensal bacteria, traditional and opportunistic path-
      ogens, dysbiosis and bacterial killing in inflammatory bowel diseases. Curr Opin
      Infect Dis 2009;22:292.