Vitamin D and gastrointestinal diseases: inflammatory bowel disease and colorectal cancer

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***Abstract*:** Over the past 5 years, there has been a rapid resurgence of interest in vitamin D outside of its traditional role in metabolic bone disease. Some nontraditional roles ascribed to vitamin D include anti-inflammatory and immune-modulating effects. These effects have led to possible implications in the pathophysiology of immune-mediated diseases including multiple sclerosis and inflammatory bowel disease (IBD). In addition, vitamin D insufficiency has been linked to higher rates of cancers including colon, prostate and breast cancers. Given these diverse associations of vitamin D and disease states, this review describes recent advances with regard to vitamin D and gastrointestinal diseases, in particular IBD and colorectal cancer.

***Keywords*:** vitamin D, inflammatory bowel disease, colorectal cancer, immunology

Introduction

Over the past 5 years, there has been a rapid resurgence of interest in vitamin D outside of its traditional role in metabolic bone disease. Vitamin D is a hormone precursor present in two forms, ergocalciferol and cholecalciferol. Ergocalciferol or vitamin D2 is present in plants, yeast and fungi. In contrast cholecalciferol, vitamin D3, is synthesized in the skin upon exposure to sunlight. Vitamin D requirements may be fulfilled either by adequate ingestion and absorption or sun exposure. Historically, interest in vitamin D revolved around its major role in metabolic bone disease. However, novel insights into additional roles for vitamin D are being established, and interest in this vitamin is gaining popularity for many other reasons apart from those associated with bone disease.

Some nontraditional roles ascribed to vitamin D include anti-inflammatory and immune-modulating effects. These effects have led to possible implications in the pathophysiology of immune-mediated diseases including multiple sclerosis (MS) and inflammatory bowel disease (IBD). In addition, vitamin D insufficiency has been linked to higher rates of cancers including colon, prostate and breast cancers. For example, one group found that vitamin D and calcium supplementation reduced all cancer risk [Lappe *et al.* 2007]. In this study patients were randomized to receive 1400–1500 mg of supplemental calcium daily, supplemental calcium plus vitamin D3 1100 IU daily or placebo. Follow up was for 4 years. Patients receiving both supplemental calcium and vitamin D had a significant reduction in development of all cancers including colorectal cancer (CRC). When the analysis was confined to cancers diagnosed after the first 12 months, the reduction in cancer risk was even greater.

Given these diverse associations of vitamin D and disease states, our purpose in this review is to describe recent advances with regard to vitamin D and gastrointestinal diseases, in particular IBD and CRC.

The various roles of vitamin D

Vitamin D plays a key endocrine role in calcium and phosphate homeostasis. Most human vitamin D is derived endogenously upon exposure of the skin to UV light, leading to photochemical conversion of 7-dehydrocholesterol to cholecalciferol (vitamin D3). The skin production of vitamin D in response to UV exposure is self-limited, and sunlight exposure cannot cause vitamin D toxicity in normal vitamin D physiology.

Although vitamin D can be absorbed from the intestine, most foods contain insignificant amounts

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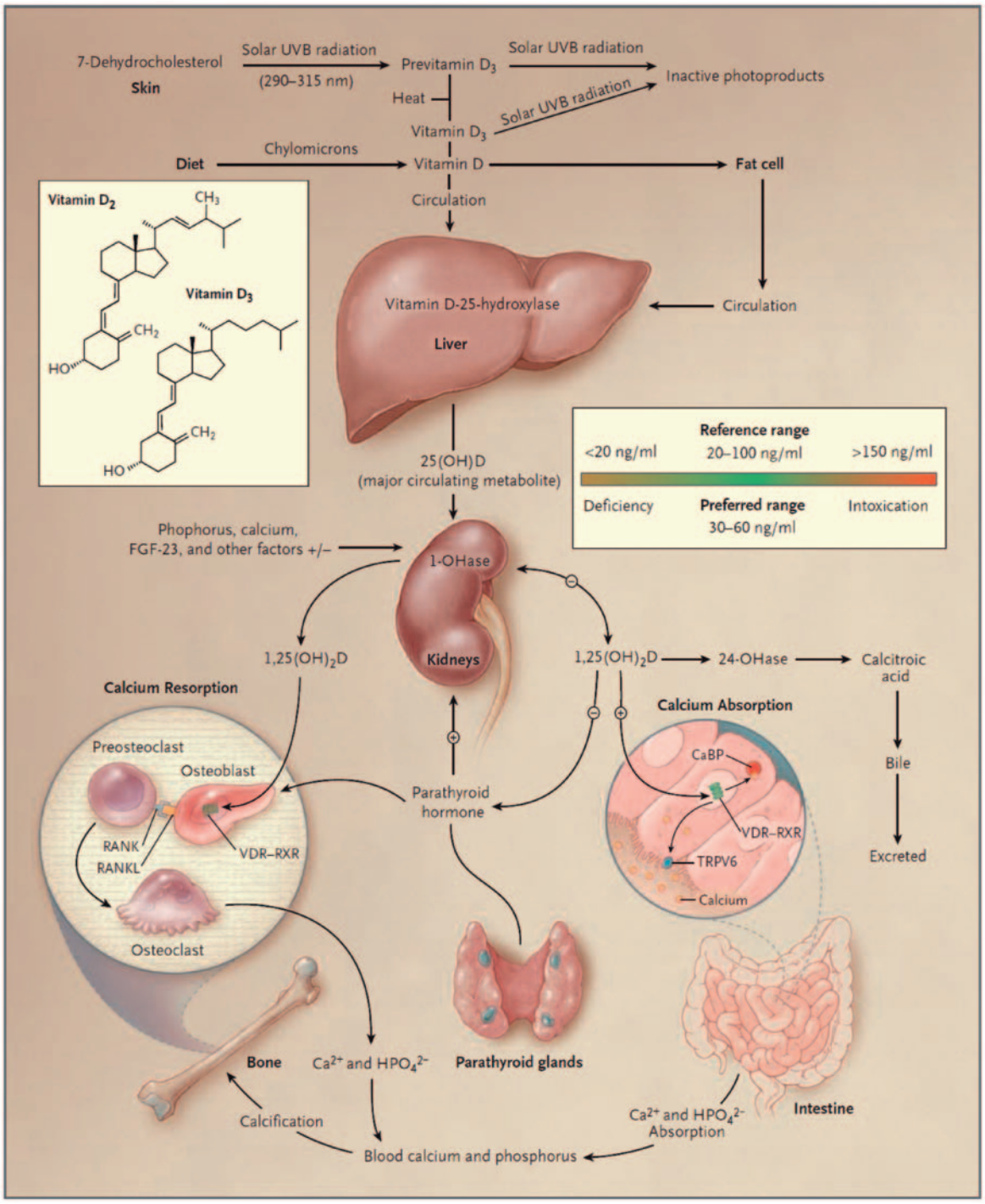

Figure 1. Classical calcium and vitamin D homeostasis pathways. Vitamin D metabolism and functions. Under ultraviolet B light exposure, 7-dehydroxycholesterol is converted to vitamin D_3 in the shin. Vitamin D_3 is transported to the liver where it is converted to 25-hydroxyvitamin D_3 (25-OHD) by 25-hydroxylase (25-OHase). 25-hydroxyvitamin D_3 (25-OHD) is further converted to 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the hormonal metabolite, by renal 1α-hydroxylase (1-OHase). 1α-hydroxylase. the rate-limiting enzyme, is stimulated by parathyroid hormone and feedback inhibited by $1,25(OH)_2D_3$. 25-OHD and $1,25(OH)_2D_3$ are further metabolized by 24-hydroxylase (24-OHase) to initiate their catabolism, which is stimulated by $1,25(OH)_2D_3$. $1,25(OH)_2D_3$ feedback inhibits parathyroid homione production. $1,25(OH)_2D_3$ targets the intestine, kidney and bone to regulate calcium and phosphate homeostasis. The hormone also has other noncalcemic physiologic functions. $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 25-OHD, 25-hydroxyvitamin D_3; 1-OHase, 1α-hydroxylase; 24-OHase, 24-hydroxylase; 25-OHase, 25-hydroxylase; PTH, parathyroid hormone; UVB, ultraviolet B light. [Holick, M.F. (2007) Vitamin D deficiency. *N Engl J Med* 357: 266-281] Copyright© [2007] *Massachusetts Medical Society. All rights reserved.*

(except oily fish). Cholecalciferol is subsequently converted by hepatic vitamin D 25-hydroxylase to 25-hydroxyvitamin D (25OHD), the major circulating vitamin D metabolite. 25OHD is largely inactive, but a long half-life makes it the best indicator of overall vitamin D status. Finally, renal conversion of 25OHD by 25-hydroxyvitamin D 1α-hydroxylase produces the active vitamin D metabolite, 1,25-dihydroxyvitamin D3 (calcitriol or $1,25(OH)_2D_3$). Serum calcium regulates 1α-hydroxylase, as do fibroblast growth factor 23 (FGF-23), phosphate and $1,25(OH)_2D_3$ itself.

$1,25(OH)_2D_3$ stimulates intestinal absorption of orally ingested calcium and phosphate, tubular reabsorption of calcium filtered in the kidney, and mobilization of calcium and phosphate stores from the skeleton by stimulating maturation of osteoclasts (Figure 1). Chronic vitamin D deficiency results in undermineralization of bones, leading to rickets and osteomalacia [Holick, 2007].

Active $1,25(OH)_2D_3$ is now known to exert its biological functions via the vitamin D receptor (VDR), a member of a superfamily of nuclear hormone receptors. This family includes the perioxisome proliferator-activated receptor and glucocorticoid receptor, implicated in the therapeutic action of the 5-aminosalicylates and glucocorticosteroids, respectively, in IBD [Probert *et al.* 1992]. Upon entering the target cell, $1,25(OH)_2D_3$ binds with the VDR inducing a conformational change and heterodimerization with the retinoid X receptor (RXR). This increases the affinity of the VDR/RXR complex for a specific promoter region, the vitamin D responsive element, in vitamin D responsive genes leading to transcription [Carlberg and Polly, 1998] (Figure 2).

Importantly, many other tissues and cells in the body, including the immune system, not involved in calcium homeostasis, have been found to express VDR and possess the enzymes necessary to produce local $1,25(OH)_2D_3$. This extrarenal enzyme activity is not subject to the same regulatory endocrine feedback mechanisms, but appears to be induced by other factors. These findings suggest vitamin D may have actions beyond simple endocrine activity, and may explain the far-reaching functions of vitamin D including its role in tuberculosis (TB)

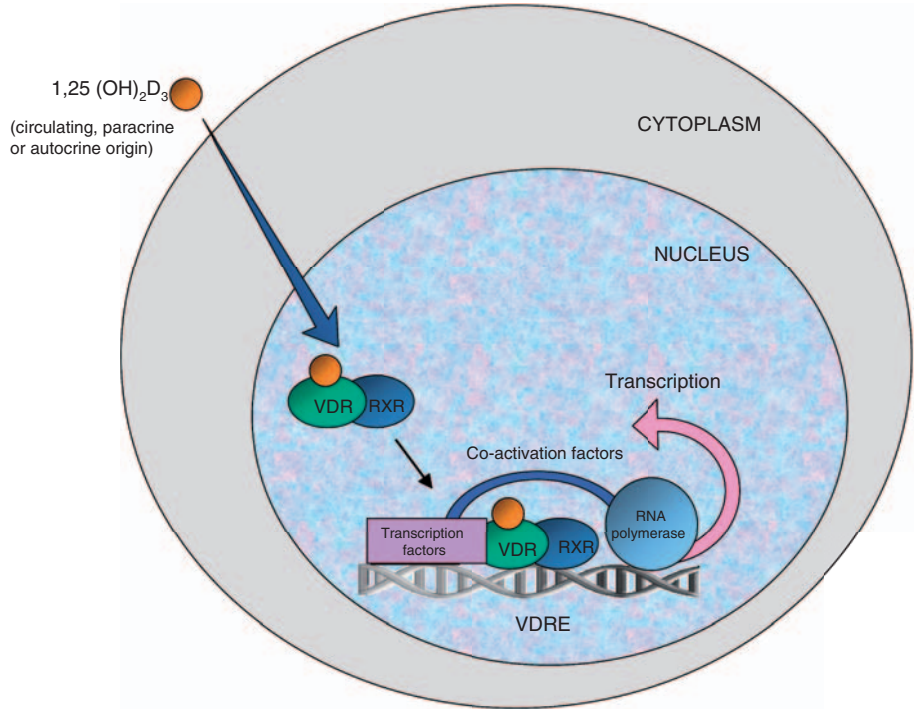

Figure 2. Ligand-dependent gene transcription by 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$). Lipid soluble $1,25(OH)_2D_3$ from serum, autocrine or paracrine sources enters the target cell and binds to the nuclear vitamin D receptor (VDR). This induces a conformational change and promotes heterodimerization with the retinoid X receptor (RXR). The VDR/RXR has an increased affinity for the vitamin D responsive element (VDRE). VRDE is a specific sequence of nucleotides in the promoter region of the vitamin D responsive gene. Binding of the VDR/RXR complex to the VDRE attracts a complex of co-activator proteins connecting VDRE with RNA polymerase II. Gene transcription then occurs, producing mRNA transcripts, which leave the nucleus for translation into the coded protein in the cytoplasm.

infection, diabetes mellitus, cardiovascular disease, congestive heart failure [Zittermann, 2006; Zittermann *et al.* 2003], and the regulation of blood pressure homeostasis through the renin–angiotensin system [Li *et al.* 2002]. In addition, vitamin D exhibits antiproliferative, cell differentiation and apoptotic effects in malignant cell lines, which may be protective against breast, colon and prostate cancer [Nagpal *et al.* 2010].

Vitamin D in colorectal cancer and inflammatory bowel disease

Colorectal cancer

The mechanisms by which vitamin D status may alter cancer development are still being delineated. Hundreds of genes contain vitamin D response elements [Carlberg, 2003], which encode for proteins important in the regulation of cell proliferation, differentiation and apoptosis [Diaz *et al.* 2000; Vanderwalle *et al.* 1994; Meggouh *et al.* 1991]. When vitamin D status is suboptimal, these activities are impaired. Similar to the prevalence of autoimmune diseases, it has long been noted that cancer mortality including mortality from CRC increases with geographical latitude [Garland and Garland, 1980]. In 1980, Garland and colleagues proposed that lower levels of vitamin D resulting from weaker UV-B radiation seen at higher latitudes may account for the geographical pattern of cancer mortality [Garland and Garland, 1980]. The latitudinal effect on serum 25OHD status, in combination with the potential biological effects of vitamin D deficiency on cancer incidence, resulted in numerous studies exploring the relationship between vitamin D and cancer risk; however until recently, the results had remained somewhat inconclusive. Recently, Yin and colleagues aimed to provide an updated review and meta-analysis of longitudinal epidemiological studies evaluating the association between 25OHD levels and CRC risk with a particular focus on potential variation by anatomic site [Yin *et al.* 2009]. Eight studies comprising 3556 patients were included in the analysis and support previous evidence that serum 25OHD levels are inversely associated with CRC risk (odds ratio (OR) 0.69; 95% confidence interval (CI) 0.55–0.86, $p < 0.001$) for patients with 25OHD levels in the highest compared with lowest quintiles. An increase of 25OHD by 20 ng/ml was associated with a risk reduction of 59% for rectal cancer and 22% for colon cancer. Analyses stratified by anatomical site suggest a stronger risk reduction for rectal cancers compared with colon cancers, however, this finding did not reach statistical significance.

Previous work suggested a 51% (OR 0.49; 95% CI 0.35–0.68) lower risk of CRC associated with the highest serum 25OHD quintile compared with the lowest quintile [Gorham *et al.* 2007]. An inverse association between plasma 25OHD and risk for colon cancer was seen in the Nurses Health Study (NHS), a predominantly female cohort, between levels in the highest quintile compared with those in the lowest quintile [Feskanich *et al.* 2004]. In the Third National Health and Nutrition Examination Survey, higher mortality from colon cancer was observed in patients with serum 25OHD levels lower than 50 nmol/l compared with those with levels greater than 80 nmol/l [Freedman *et al.* 2007].

Similarly, the relationship between serum 25OHD levels and colon cancer was investigated in a nested case–control study within the Health Professionals Follow-up Study (HPFS) [Wu *et al.* 2007]. As this was a male cohort, results were pooled with results from the NHS, to increase statistical power. The combined results from the HPFS and NHS studies showed that higher plasma 25OHD concentrations were statistically significantly associated with decreased risks of CRC, without any predilection for location of tumour (proximal or distal colon).

One of the largest studies to date and one of the first based on European populations showed that compared with a mid-range concentration of 50–75 nmol/l, 25OHD levels lower than 50 nmol/l was associated with an increased risk of CRC [Jenab *et al.* 2010]. Patients with 25OHD levels greater than 100 nmol/l had a significant 40% lower risk of CRC than those patients with levels lower than 25 nmol/l.

Previous studies have also shown a significant 30% reduction in formation of colorectal adenomas among patients with higher *versus* lower 25OHD levels, further supporting biological plausibility for a potential role of vitamin D in colorectal carcinogenesis.

The data regarding decreased cancer mortality observed in randomized clinical trials with vitamin D supplementation is somewhat conflicting. The first study exploring the relationship between vitamin D supplementation and CRC risk was conducted by Wactawski-Wende and colleagues [Wactawski-Wende *et al.* 2006]. Patients were randomized to receive 1000 mg of elemental calcium carbonate and 400 IU of vitamin D3 or

placebo for 7 years. Results of this study did not show a significant reduction in the incidence of CRC in the 7-year follow-up period. During the time period that this study was conducted, doses of 400 IU/day were considered adequate and perhaps high. However, studies published since then have led to some recommendations of higher vitamin D daily intakes [Gorham *et al.* 2005]. In addition, a follow-up period of 7 years may not have been of sufficient duration to identify cancer development.

Inflammatory bowel disease

The pathogenesis of IBD such as Crohn's disease (CD) and ulcerative colitis (UC) is complex and incompletely understood. It is thought to involve a complex interplay between genetic, environmental and microbial environments in the context of an inappropriate and abnormal activation of the mucosal immune system. Particularly, a dysregulated intestinal mucosal T-cell-mediated immune response, specifically CD4+ T helper type-1 (Th1) lymphocytes, leads to production of Th1-associated pro-inflammatory cytokines such as interferon-γ (IFN-γ) and tumour necrosis factor-alpha (TNF-α). Another potential pathogenic factor in CD is impaired mucosal barrier function and intestinal hyperpermeability [Gibson, 2004]. A relatively high number of first-degree relatives of patients with CD have increased intestinal permeability in the absence of clinical symptoms suggesting that permeability issues may precede clinical symptoms [Peeters *et al.* 1997]. The genetics of CD demonstrate that nucleotide-binding oligomerization domain containing 2 (NOD2) insufficiency contributes to development of the disease. Recently, one group observed that 1,25D signalling is a direct inducer of NOD2 expression arguing strongly that vitamin D insufficiency/deficiency does play a causative role in the prevalence of CD [Wang *et al.* 2010].

Epidemiological evidence for vitamin D in the pathogenesis of inflammatory bowel disease

The association of temperate climates (e.g. northern latitudes with lower sunlight exposures) with higher incidences and prevalence of autoimmune diseases has led to the implication of vitamin D in their pathogenesis. The classical example of such a geographical association is the higher incidence of MS in temperate climates, interestingly with the acquisition of indigenous risk by young immigrants [Warrell, 1996]. A similar observation has been demonstrated in the higher incidence and prevalence of IBD in northern European countries (e.g. UK and Scandinavia) compared with their sunnier southern counterparts (e.g. Croatia) [Loftus and Sandborn, 2002]. In addition, people living near the equator are at low risk of developing IBD, however, upon migration to developed countries in temperate climates, the risk of IBD increases [Carr and Mayberry, 1999; Probert *et al.* 1990]. Furthermore, a seasonal variation in onset and exacerbations of IBD has been noted. For example, symptomatic onset of UC seems to peak in December [Moum *et al.* 1996; Sellu, 1986], whereas higher CD relapse rates have been noted in autumn and winter [Zeng and Anderson, 1996]. In keeping with these findings, a seasonal variation in vitamin D levels has been demonstrated in patients with CD [Vogelsang *et al.* 1989] and a high prevalence of vitamin D deficiency exists in patients with established CD [Vogelsang *et al.* 1989; Harries *et al.* 1985; Driscoll *et al.* 1982], but also remains common even when the disease is in remission [Andreassen *et al.* 1998, 1997]. In addition, IBD patients are also prone to vitamin D intestinal malabsorption, especially following small-bowel resection or the use of cholestyramine for postresectional diarrhoea, both of which deplete bile acids essential for vitamin D absorption.

One recent group sought to describe the clinical disease characteristics based on serum 25OHD levels in patients with CD [Joseph *et al.* 2009]. They found that disease activity as assessed by the Harvey Bradshaw score correlated negatively with the serum 25OHD levels. Interestingly, lower vitamin D levels were seen in patients with jejunal involvement. The only predictors of 25OHD levels in this study not surprisingly were disease severity and sunlight exposure. One needs to ask however whether pre-existing vitamin D deficiency was the initiating event leading to disease severity, or whether vitamin D deficiency was the consequence of severe underlying illness. It is likely that the truth is somewhere in between as patients with more active disease are likely to receive less sunlight and not absorb adequate amounts of vitamin D received in the diet. Although, this was one of the first studies describing the correlation between serum 25OHD levels and disease severity, conclusions regarding the role of vitamin D deficiency as a predictor of disease severity cannot be generalized from these limited data.

Vitamin D and the immune system

The observation that VDR is expressed significantly in the immune system, including peripheral blood monocytes, leucocytes, antigen-presenting cells and activated $CD4^+$ T cells has raised the possibility that VDR agonists may have immunomodulatory activity [Veldman *et al.* 2000; Brennan *et al.* 1987; Manolagas *et al.* 1986; Bhalla *et al.* 1983; Provvedini *et al.* 1983]. In addition, the demonstration that dendritic cells (DCs) and, to a lesser extent, activated T lymphocytes have the capacity to synthesize $1,25(OH)_2D_3$ from sunlight-derived precursors suggests immune autocrine/paracrine activity [Sigmundsdottir *et al.* 2007]. Although vitamin D has no direct antimicrobial activity, there is evidence that $1,25(OH)_2$ can modulate host response while deficiency increases susceptibility and severity in *Mycobacterium tuberculosis* infection. Novel experiments have shown that monocytes and macrophages exposed to TB upregulate both the VDR and 25(OH)D-1α-hydroxylase. In addition, $1,25(OH)_2D_3$, enhances the ability of mononuclear phagocytes to suppress the intracellular growth of TB *in vitro*, particularly in American-African individuals known to have an increased susceptibility to both TB and vitamin D deficiency [Liu *et al.* 2006]. Furthermore, a recent study has also shown that a single high oral dose of vitamin D3 (100,000 IU) significantly enhances the antimycobacterial immunity of tuberculosis contacts by restricting recombinant *M. bovis in vitro* [Martineau *et al.* 2007]. Interestingly, these recent findings of modern science add credence to the historical TB treatment of sunlight and cod liver oil (rich in vitamin D) back in the 1800s [Liu *et al.* 2006].

Effect of vitamin D on T-cell-mediated immunity

Monocyte-derived DCs are highly specialized antigen-presenting cells (APCs), which play a critical and central gatekeeping role in the initiation of mucosal $CD4^+$ T-cell responses. Following the uptake of antigen, DCs mature into potent APCs and present antigen, in association with major histocompatibility complex (MHC) class II molecules, to the T-cell receptor (TCR) of naïve T cells. T-cell activation then occurs in the presence of required additional DC (CD80/CD86/CD40) and T-cell (CD28 and CD154) costimulatory signals [Banchereau *et al.* 2000]. Several studies have demonstrated inhibition of precursor monocyte differentiation into DCs by $1,25(OH)_2D_3$ or its analogues [van Halteren *et al.* 2002; Canning *et al.* 2001; Berer *et al.* 2000; Griffin *et al.* 2000; Penna and Adorini, 2000; Piemonti *et al.* 2000]. This has been confirmed in both *in vitro* studies performed on peripheral blood monocyte-derived DCs obtained from healthy individuals and IBD patients [Stio *et al.* 2005; Rigby *et al.* 1984]. There is also substantial evidence that $1,25(OH)_2D_3$ directly inhibits T-cell activation and proliferation [Bhalla *et al.* 1984; Rigby *et al.* 1984]. The expression of the essential costimulatory markers is also inhibited [van Halteren *et al.* 2002; Canning *et al.* 2001; Berer *et al.* 2000; Griffin *et al.* 2000; Penna and Adorini, 2000; Piemonti *et al.* 2000]. Furthermore, it is now recognized that vitamin-D-cultured DCs have tolerogenic properties (characterized by decreased costimulatory expression of CD40, 80, 86 and class II MHC molecules), which in the mixed lymphocyte reaction demonstrated a reduced ability to activate allogenic T cells, which themselves were hyporesponsive with limited IFN-γ production. These tolerogenic DCs also induce T cells with suppressive activity [Canning *et al.* 2001; Berer *et al.* 2000; Griffin *et al.* 2000; Jonuleit *et al.* 2000; Penna and Adorini, 2000; Piemonti *et al.* 2000]. Importantly, vitamin-D-treated DCs retained an immature phenotype despite the withdrawal of vitamin D, a feature not seen in the response to corticosteroids, which also impair DC maturity and proliferation [Griffin *et al.* 2001]. Also peripheral blood monocyte cells cultured *in vitro* with dexamethasone and/or $1,25(OH)_2D_3$ demonstrated reduced lymphocyte proliferation with $1,25(OH)_2D_3$ alone, but with an additive antiproliferation effect in combination with steroids. These tolerogenic effects have also been demonstrated *in vivo* during treatment with $1,25(OH)_2D_3$ and mycophenolate mofetil, which induced tolerance to fully mismatched islet allografts in mice. This finding is associated with an increased percentage of $CD4^+CD25^+$ regulatory T cells which, when transferred, induced transplant tolerance in the recipient [Gregori *et al.* 2001].

The phenotype of T-cell-mediated response upon DC-modulated activation is also under the direction of specific DC-derived cytokines [Mannon *et al.* 2004]. Interleukin (IL)-12 is an important cytokine that plays a major role in driving pro-inflammatory Th1 differentiation implicated in the pathogenesis of IBD. IL-12 is also suspected of inhibiting T-cell apoptosis [Marth *et al.* 1999]. $1,25(OH)_2D_3$ has been convincingly demonstrated to inhibit IL-12 production [Penna and

Adorini, 2000; Lemire *et al.* 1995], probably by interfering with nuclear factor kappa B (NFκβ)-induced IL-12 transcription. Conversely, $1,25(OH)_2$ upregulates DC-derived IL-10 production, promoting the anti-inflammatory Th2 cell phenotype [Canning *et al.* 2001; Penna and Adorini, 2000], whilst inhibiting Th1 pathways by both downregulating IL-12 production and by blocking IFN-γ synthesis by differentiated Th1 T cells [Moore *et al.* 2001]. IL-10 also induces regulatory T cells and strongly inhibits production of other pro-inflammatory monokines, such as IL-1, IL-6 and TNF-α. Furthermore, when antigen-stimulated peripheral blood monocytes from CD patients were cultured *in vitro* with a vitamin D analogue, not only was there a significant reduction in cell proliferation, production of TNF-α and the associated inflammatory transcription factor NFκβ were also impaired [Stio *et al.* 2007]. T-cell production of IFN-γ is also directly inhibited by reduced $1,25(OH)_2D_3$-mediated IFN-γ gene transcription [Cippitelli and Santoni, 1998].

An important observation with potential therapeutic implications is the apparent synergistic effect of vitamin D when combined with other conventional treatments and immunomodulators. A combination of steroids with vitamin D was more effective in reducing the Th1 cytokine IFN-γ and increasing Th2 cytokines IL5/IL10/IL13 [Barrat *et al.* 2002; Jirapongsananuruk *et al.* 2000]. Pretreatment *in vivo* with anti-TNF-α treatment (infliximab) is synergistic with vitamin D in reducing TNF-α [Stio *et al.* 2005]. However, unlike other immunosuppressants, which also appear able to induce a tolerogenic DC phenotype (e.g. MMF, sirolimus and glucocorticosteroids), only vitamin D and its analogues appear able to specifically increase IL-10 [Penna and Adorini, 2000]. Interestingly, a vitamin D analogue also worked synergistically with the calcineurin inhibitor cyclosporin, often used in the treatment of UC, when used in a mouse model of experimental autoimmune encephalitis and with T cells from UC patients, by potent inhibition of APC antigen presentation and inhibition of TCR-mediated T-cell activation and proliferation, but possibly also by suppression of IL-2 transcription, an autocrine T-cell growth factor [Van Etten, 2007; Stio *et al.* 2002; Alroy *et al.* 1995].

Chemotactic cytokines (or chemokines) and their cell receptors are important in determining the tissue-specific homing and microenvironmental destination of immune cells [Kunkel and Butcher, 2002]. There is also recent evidence that vitamin D can influence DC-mediated homing marker expression on activated T cells, including integrin intestinal-homing receptor α4β7, chemokine receptor (CCR) type 9 (CCR9) (gut homing) and CCR10 (skin homing). Vitamin D has been shown to inhibit the spontaneous upregulation of α4β7 during T-cell activation and prevent upregulation of CCR (in response to retinoic acid. Vitamin D also upregulates CCR10. This modulation of homing marker expression has obvious implications for redirection of immune cells implicated in IBD [Sigmundsdottir *et al.* 2007].

Overall $1,25(OH)_2D_3$ appears to inhibit Th1 immune responses, promote desirable Th2 responses and influence immune cell homing marker expression. This *in vitro* laboratory data is supported by several novel *in vivo* animal models of IBD, which demonstrate powerful evidence of therapeutic immunomodulation by vitamin D.

Vitamin D and maintenance of the intestinal mucosal barrier

The integrity of the intestinal mucosal barrier is preserved by the enormous regenerating capacity of the mucosal epithelium. One potential pathogenic factor in the etiopathology of IBD is impaired mucosal barrier function [Gibson, 2004]. Recent animal data suggest that maintenance of the epithelial barrier integrity of the large intestine by vitamin D is critical in preventing IBD development [Kong *et al.* 2008]. In this study, it appeared that the VDR was required for mucosal repair in the mouse model of colitis, as supported by the observation that VDR expression was markedly induced in colonic mucosa during mucosal recovery in mice. *In vitro* studies have consistently demonstrated that vitamin D stimulates epithelial cell migration, suggesting that vitamin D is involved in the regulation of epithelial restitution in wound healing. These observations may explain at least in part the associations between vitamin D deficiency and IBD.

Animal models of inflammatory bowel disease

The IL-10 knockout (IL10-KO) mouse develops spontaneous severe panenterocolitis due to lack of IL-10 (a regulatory anti-inflammatory Th2 cytokine) [Kühn *et al.* 1993]. Vitamin-D-deficient IL10-KO mice develop an accelerated form of enterocolitis with early mortality,

while vitamin-D-deficient normal (wild-type) mice do not develop enterocolitis. Interestingly, when vitamin-D-deficient IL10-KO mice received dietary vitamin D or $1,25(OH)_2D_3$ enterocolitis did not develop. Furthermore, $1,25(OH)_2D_3$ supplementation ameliorated and blocked progression of IBD symptoms in IL10-KO mice with established IBD [Cantorna *et al.* 2000]. This is strong evidence for vitamin D as an anti-inflammatory immunomodulator in IBD.

The importance of VDR in immunomodulation of the inflammatory response is highlighted in other models of IBD. In a T-cell transfer model of IBD, CD4+/$CD45RB_{high}$ cells are transferred into immunodeficient mice (recombinase-activated gene 2 (*Rag-2*) knockout mice that lack mature T and B cells) inducing enterocolitis. CD4+/$CD45RB_{high}$ T cells from VDR knockout (VDR-KO) mice induced a more severe form of IBD than CD4+/$CD45RB_{high}$ T cells from wild-type mice. In addition, in comparison to IL10-KO mice, VDR/IL-10 double knockout mice developed more severe colitis of more rapid onset with an increased 100% mortality [Froicu *et al.* 2003].

VDR-KO mice are extremely sensitive to chemically induced colitis and fail to recover spontaneously upon withdrawal of the chemical insult. Expression of several pro-inflammatory cytokines is also increased (e.g. TNF-α, IL-1α, IL-1β, IL-12, IFN-γ) and injection of lipopolysaccharide leads to a hyperactive inflammatory response. Dietary supplementation with $1,25(OH)_2D_3$ reduced severity of dextran sulphate sodium-induced colitis in wild-type mice but unsurprisingly not in VDR-KO mice, demonstrating a requirement for a functional VDR for vitamin D efficacy [Froicu and Cantorna, 2007]. The contribution of $1,25(OH)_2D_3$ in immune responses and maintenance of tolerance to self antigens is also suggested by the enlarged lymph nodes in VDR-deficient mice, which contain higher numbers of mature DCs, presumably as a result of the loss of vitamin-D-associated modulation of DC maturation and proliferation [Griffin *et al.* 2001].

Finally, an important study of 2,4,6-trinitrobenzenesulphonic acid-induced colitis (chemical model of Crohn's colitis in mice) compared the benefits of corticosteroids and $1,25(OH)_2D_3$ *in vivo* administered before and after induction of colitis. $1,25(OH)_2D_3$ alone reduced clinical severity significantly ($p < 0.05$), but combination treatment (dexamethasone plus $1,25(OH)_2D_3$) demonstrated the most effective reduction of IBD severity ($p < 0.001$). This was effective in both preventing severe colitis and ameliorating effects if given in established colitis. Both treatments independently downregulated Th1 (reduced Il-12, TNF-α, IFN-γ, IF-1β and T-bet expression) and upregulated Th2 responses (increased GATA3 and IL-4), as well as downregulating DCs responsible for pro-inflammatory differentiation of Th1 cells. In addition, Th17 responses (implicated in inflammation) were also downregulated. Interestingly, $1,25(OH)_2D_3$ alone, but reinforced by additional dexamethasone, also promoted a regulatory T-cell profile [Daniel *et al.* 2008]. This is important as it implies a potential therapeutic steroid-sparing clinical application of $1,25(OH)_2D_3$ derivatives in active IBD.

It should also be noted, that in addition to vitamin D, dietary calcium also appears to have an independent effect on IBD severity in animal models. Dietary calcium plus $1,25(OH)_2D_3$ resulted in maximal suppression in murine experimental IBD. This finding is also documented in other animal models of autoimmune disease [Zhu *et al.* 2005].

What 25-hydroxyvitamin D level is sufficient for immunomodulatory actions?

Serum 25OHD is the accepted biomarker of vitamin D status. It has been demonstrated that 25OHD concentrations greater than 20–25 nmol/l indicate severe vitamin D deficiency, which will lead to rickets and osteomalacia [Holick, 2007]. However, there is no formal consensus of optimal 25OHD status at present, although expert opinion ranges from 50 to 100 nmol/l. These target 25OHD levels are based on the rationale that only patients with 25OHD levels above 50 nmol/l show no significant change in circulating parathyroid hormone (PTH) levels subsequent to vitamin D therapy. Furthermore, if based on attaining 25OHD levels at which a functional effect is achieved, the inverse relationship between 25OHD and PTH levels, suggests PTH suppression beneficial to bones and based on fracture prevention studies occurs at 25OHD levels 75–100 nmol/l. Nevertheless, the circulating serum 25OHD level that is optimal for the immune system is not known, however, there is some suggestion that 25OHD levels of around 75 nmol/l may be optimal as evidenced in TB defence [Liu *et al.* 2006].

The daily vitamin D dosage required to achieve adequate 25OHD levels are also unclear. Vitamin D is potentially toxic and can lead to hypercalcaemia and hypercalciuria, however, the current recommended safe daily dose limits remain both controversial and conservative (1000 IU/day and 2000 IU/day in the UK and North America, respectively [Vieth, 2006; Food Standards Agency, 2003]. The optimum dose and 25OHD levels for bone protection have not yet been established.

Several recent expert reviews now suggest traditional doses of 800 IU–1000 IU/day used for bone protection are woefully inadequate and based on outdated and insufficient evidence [Holick, 2004; Lips, 2004; Vieth, 2004; Heaney *et al.* 2003]. Indeed, these guidance doses of 1000–2000 IU/day seem somewhat lacking when compared with the suggested physiological daily limit of 10,000 IU vitamin D/day generated by total-body sunlight exposure in the absence of toxicity [Vieth, 1999; Barger-Lux *et al.* 1996; Davie *et al.* 1982; Stamp, 1975].

A single high oral dose of vitamin D3 (100,000 IU) given to individuals with TB contacts, induced a 91% increase in mean serum 25OHD, correcting any pre-existing vitamin D deficiency (<20 nmol/l) for at least 6 weeks, without causing hypercalcaemia [Martineau *et al.* 2007]. Recent studies in healthy subjects have shown that intakes of vitamin D at 4000–11,000 IU/day over 5–6 months in healthy subjects are safe and do not result in hypercalciuria, which occurs when circulating levels of 25OHD3 are above with no reported toxicity up to 25OHD levels of 250 nmol/l [Hollis and Wagner, 2004; Heaney *et al.* 2003; Vieth *et al.* 2001; Vieth, 1999]. Indeed, Aloia and colleagues recently demonstrated doses of 3800 and 5000 IU/day are required over a 6-month period to raise baseline 25OHD levels of >55 nmol/l and <55 nmol/l, respectively, to over 75 nmol/l [Aloia *et al.* 2008].

Trials of vitamin D interventions for immune modulation in humans

There are now many documented animal studies of the immunomodulatory properties of vitamin D in IBD, however to date, there are no published clinical trials in human patients. Although fish oils, when naturally derived, are a good source of vitamin D3, studies of efficacy in IBD (UC) have used largely purified or manufactured forms likely to contain negligible amounts of vitamin D. Fish oils also contain polyunsaturated fatty acids, namely docosahexaenoic acid and eicosapentaenoic acid, which are also thought to exhibit anti-inflammatory activity. Nevertheless, despite trials of fish oils in UC suggesting some benefit, a recent Cochrane analysis of six papers of differing quality concluded there was insufficient evidence to recommend clinical use in IBD [De Ley *et al.* 2007].

It is important to highlight that basic physiological effects using interventions with vitamin D are designed specifically to target 25OHD levels, in contrast to treatments using the 'active' vitamin D hormone $1,25(OH)_2$ cholecalciferol or its analogues designed to achieve an immunomodulatory effect. In the first case, one is treating a deficiency, whereas in the second case, one is testing a potential therapy. The active form of vitamin D, $1,25(OH)_2D_3$ plays an important role in calcium homeostasis, cell differentiation and proliferation, immunity and cardiovascular function. Given its immunomodulatory properties, $1,25(OH)_2D_3$ or its analogues might be clinically useful for the treatment of inflammatory and autoimmune diseases. It is believed that the active vitamin D hormone exerts physiological and pharmacological effects by binding to the VDR and then sustains a conformational change. Although $1,25(OH)_2D_3$ could be a potentially useful immunomodulatory agent for clinical use, it can cause some serious adverse effects, in particular the induction of hypercalcaemia and bone resorption. Therefore, multiple drug development efforts are aimed at finding $1,25(OH)D_3$ analogues that exert immunomodulatory effects without causing these complications and are well under way.

In the appropriate context, physiological treatment of vitamin D deficiency does not necessarily guarantee the immunomodulatory benefits of higher pharmacological doses. In fact, it is more likely that the pharmacological benefits of supraphysiological doses of vitamin D are necessary to achieve immunomodulatory properties, in the face of vitamin D sufficiency.

There are a few limited clinical trials of vitamin D in autoimmune disease with Th1-mediated aetiology. Kimball and colleagues gave increasing oral doses of vitamin D (28,000–280,000 IU/week) over 4 weeks in 12 patients with an active phase of MS (together with 1.2 g calcium/day),

safely achieving mean serum 25OHD concentrations twice the upper physiological range [Kimball *et al.* 2007]. Although disease progression and activity did not appear to be affected, the number of gadolinium-enhanced lesions on magnetic resonance imaging significantly decreased [Kimball *et al.* 2007]. Other studies using lower doses of vitamin D (1000–5000 IU/day) have suggested significant increases in anti-inflammatory cytokine (transforming growth factor beta 1) [Mahon *et al.* 2003] and reported a reduction in exacerbations and clinical severity in MS [Nordvik *et al.* 2000; Goldberg *et al.* 1986]. In a small case–control study, rheumatoid arthritis patients supplemented with a synthetic $1,25(OH)_2D_3$ precursor (alphacalcidiol or 1α-hydroxyvitamin D3) also demonstrated a reduction in the severity of symptoms [Andjelkovic *et al.* 1999].

Summary

In summary, there is rapidly increasing epidemiological and strong experimental evidence, suggesting a role for vitamin D in IBD and CRC. Although data to date have been demonstrated in largely *in vitro* studies and murine models of IBD, it is clear that vitamin D potentially has potent immunomodulatory actions on the T-cell-mediated processes implicated in the pathogenesis of IBD, both at DC and T-cell level. In light of this evidence, well-conducted clinical trials of vitamin D or its analogues in human IBD patients are strongly indicated to assess further the potential therapeutic immunomodulatory properties of this much underestimated nutrient. Consideration to conducting randomized controlled clinical trials with vitamin D treatment using both physiological and pharmacological doses, in conjunction with standard of care therapy for CD should be provided. Of particular interest would be the effect of pre-existing serum 25OHD levels on the magnitude of response to therapy. Strong consideration for appropriate vitamin D supplementation should be provided to patients at risk for CRC.

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Conflicts of interest statement

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