Ancient Nuclear Receptor VDR With New Functions: Microbiome and Inflammation

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The biological functions of 1α,25-dihydroxyvitamin D_3 are regulated by nuclear receptor vitamin D receptor (VDR). The expression level of VDR is high in intestine. VDR is an essential regulator of intestinal cell proliferation, barrier function, and immunity. Vitamin D/VDR plays a protective role in inflammatory bowel diseases (IBDs), both ulcerative colitis and Crohn's disease. Emerging evidence demonstrates low VDR expression and dysfunction of vitamin D/VDR signaling in patients with IBD. Here, we summarize the progress made in vitamin D/VDR signaling in genetic regulation, immunity, and the microbiome in IBD. We cover the mechanisms of intestinal VDR in regulating inflammation through inhibiting the NF-κB pathway and activating autophagy. Recent studies suggest that the association of VDR single nucleotide polymorphisms with immune and intestinal pathology may be sex dependent. We emphasize the tissue specificity of VDR and its sex- and time-dependent effects. Furthermore, we discuss potential clinical application and future direction of vitamin D/VDR in preventing and treating IBD.

Key Words: immunity, infection, myeloid, nuclear receptor, *Salmonella*

INTRODUCTION

Vitamin D receptor (VDR) is an ancient nuclear receptor that has been highly conserved in birds, fish, and mammals.[1] Based on epidemiological studies, we know that vitamin D (VD) deficiency is implicated in various diseases.[2] VD absorbed from the diet or produced in the skin after exposure to sunlight is then converted to 25(OH) VD by the liver and to the active vitamin D metabolite 1α,25-dihydroxyvitamin D3 (1,25$(OH)_2D_3$) by the kidney.[3] VDR regulates the major biological functions of 1,25$(OH)_2D_3$. VDR is highly expressed in the small intestine and colon (The Human Protein Atlas, https://www.proteinatlas.org/ENSG00000111424-VDR/tissue; 20 April 2018, date last accessed).[4,5] Intestinal VDR plays critical roles in immunity, proliferation, differentiation, permeability, and host-microbial interactions.[6]

It is acknowledged that genetic factors, environmental triggers, immune responses, and intestinal bacteria contribute to the pathogenesis of Crohn's disease (CD) and ulcerative colitis (UC).[7–9] Low vitamin D status has been observed in inflammatory bowel disease (IBD) patients.[10–12] Due to insufficient sunlight in regions farther from the equator, a north-south gradient in CD rate indicates that vitamin D deficiency may be an environmental trigger contributing to IBD pathogenesis.[11] Evidence strongly supports a protective effect of vitamin D and VDR in IBD. Low VDR expression and dysfunction of vitamin D/VDR signaling has been reported in both CD and UC patients.[13,14] We show that a low level of intestinal epithelial VDR is accompanied by a reduction of *Atg16l1*, an IBD risk gene and regulator of autophagy, which leads to dysbiosis and impaired autophagic responses.[13]

Here, we will summarize the recent research advancement in human *Vdr* gene variation, vitamin D/VDR signaling in transcriptional regulation, immunity, and the microbiome in IBD. The mechanisms of VDR in regulating inflammation are also discussed. The vitamin D/VDR pathway is critical in maintaining intestinal homeostasis and regulating microbiota-host interactions. Further, we will discuss the potential clinical applications of vitamin D/VDR in IBD.

We performed an electronic literature search of papers written in English in the MEDLINE database via PubMed. Searches included combinations of the following terms: vitamin D, vitamin D receptor, microbiome/microbiota, inflammatory bowel disease, inflammation, autophagy, nuclear factor kappa B. Papers without clear relevance to the role of VDR signaling in inflammation or microbiota regulation were excluded.

HUMAN *VDR* GENE VARIATION IN IBD

The human *Vdr* gene encodes 6 domains of VDR protein. The *Vdr* gene is on chromosome 12q, comprised of promoter and regulatory regions (1a–1f) and exons 2–9. When 1,25$(OH)_2D_3$, the active form of vitamin D, binds to VDR protein, VDR translocates to the nucleus and functions as a

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transcription factor.[15] The VDR target genes include the autophagy regulator ATG16L1,[13] barrier protein claudin 2,[16] scaffold protein Axin1,[17] and antimicrobial peptides cathelicidin[18] and defensin-β2.[19]

There are numerous single nucleotide polymorphisms (SNPs) in the *Vdr* gene. Susceptibility to IBD is associated with polymorphisms of *Vdr*.[20–22] Five are of particular interest in IBD research: the nonsynonymous *FokI* and synonymous *BsmI*, *ApaI*, *TaqI*, and *Tru9I*. These SNPs, each named for the restriction endonuclease site it disrupts, are associated with risk of colon cancer.[15, 23] Studies on the role of these SNPs in IBD susceptibility have been contradictory.[24] Recent studies suggest that the association of *Vdr* SNPs with immune and intestinal pathology may be sex dependent. The *Taq1* SNP is more frequent in male UC patients.[25] Another study found that the haplotype "baT," which contains the ancestral alleles for *BsmI*, *ApaI*, and *TaqI*, protects against adenomatous polyps only in females, whereas the *FokI* SNP increases risk particularly in males.[26] There is also sex disparity in the association of *FokI* with lumbar pathology. The Ff allele of *Fok1* is protective in females whereas ff is required for protection in males.[27] The sex-specific effects of *Vdr* SNPs are also observed in other immune-related diseases, including multiple sclerosis[28] and type I diabetes.[29]

RELEVANCE OF INTESTINAL VDR IN IBD

Although VDR is expressed in numerous tissues, intestinal epithelial expression is of particular importance in protecting against IBD. The expression level of VDR is critical in IBD pathology and treatment. Intestinal VDR expression decreases in both UC and CD patients,[14] which reduces the anti-inflammatory effect of the vitamin D analogue. Tumor necrosis factor–alpha (TNF-α), a pro-inflammatory cytokine associated with IBD, is known to reduce the expression level of intestinal VDR.[30] Constitutive RAS signaling, which is commonly observed in colon cancer, downregulates VDR expression and thus inhibits vitamin D signaling.[31] However, the heterogeneity of VDR and its variable expression in physiological and pathological conditions are overlooked when we assess the efficacy of vitamin D or vitamin D analogue for IBD treatment.

In experimental models of colitis, *Vdr* whole-body knockout mice are known to develop severe colitis.[32] This can be ameliorated by introducing transgenic expression of VDR in intestinal epithelial cells. Additionally, wild-type mice with transgenic overexpression VDR in the epithelium are less susceptible to chemical-induced models of colitis.[14] This protection is associated with decreased epithelial production of pro-inflammatory cytokines. Our study has demonstrated that the bacterial product butyrate or probiotics protect against colitis by increasing the expression of VDR at the mRNA and protein levels, thus activating VDR signaling in epithelial cells.[13, 33]

Conditional knockout of intestinal epithelial *Vdr* alters the microbiota, which promotes more severe inflammation in chemical-induced models of colitis. These conditional knockouts have impaired Paneth cell function marked by deficient autophagy and decreased production of antimicrobial peptides (AMPs).[13] Dietary vitamin D deficiency also impairs AMP production, resulting in dysbiosis and worsened high-fat diet–induced metabolic disease.[34] VDR signaling in Paneth cells may be critical in preventing dysbiosis and maintaining mucosal health.

The intestinal barrier is critical in maintaining intestinal homeostasis. Vitamin D treatment has been found to promote barrier function in in vitro and in vivo studies.[35–37] Loss of VDR expression in the intestine leads to decreased production of the barrier protein claudin 2[16] in cells without any stimulation. VDR also promotes barrier integrity by preventing intestinal epithelial apoptosis.[38] These studies suggest that VDR signaling may protect against IBD by promoting intestinal barrier function. Further study is warranted to determine if intestinal VDR influences the expression of additional barrier proteins.

Interleukin 10 (IL-10) knockout mice have lower intestinal VDR expression and spontaneously develop microbiota-dependent colitis, which is more severe in *Vdr*/*IL-10* double knockouts.[39] Transgenic expression of human VDR in the mouse epithelium ameliorates *IL-10* knockout colitis,[38] whereas $1,25(OH)_2D_3$ treatment does not.[40] VDR expression reduces epithelial pro-inflammatory cytokine production and immune cell infiltration into the mucosa,[38] indicating that epithelial VDR signaling is critical in modulating the immune response in IBD. Thus, VDR could be both a cause (eg, gene variation and conditional knockout) and a consequence (reduced intestinal VDR protein) of chronic inflammation.

VITAMIN D/VDR REGULATES IMMUNITY

VDR Regulates Both Innate and Adaptive Immune Cells

As described above, epithelial VDR expression alone is able to ameliorate genetic and chemical models of colitis. However, VDR expression in both innate and adaptive immune cells also affects the inflammatory response. Deletion of VDR in macrophages and granulocytes mildly affects colitis, but greatly enhances pro-inflammatory cytokine expression in the colon. These data indicate an essential role of VDR for innate immune cells in intestinal inflammation.[41] Stimulation of macrophages with $1,25(OH_2)D_3$ abrogates their pro-inflammatory and T-cell-recruiting cytokine production.[42]

VDR signaling promotes anti-inflammatory cytokine secretion and promotes tolerogenic dendritic cell and regulatory T-cell differentiation to reduce inflammation.[43, 44] The severe inflammation observed in *Vdr*/*IL-10* double knockout mice is attributed to an augmented pro-inflammatory T-cell response.[38] VDR signaling in T cells influences differentiation. Treatment with $1,25(OH)_2D_3$ prevents pathogenic Th17 differentiation,

along with decreased expression of the pro-inflammatory cytokines interferon γ (IFNγ) and IL-17.[45]

Mechanisms: VDR Signaling, Autophagy, and NF-κB Activation

The VDR target gene *Atg16l1*[13] is an IBD risk gene that regulates intestinal homeostasis. ATG16L1 is a key autophagy protein that mediates autophagosome formation. The *Atg16l1-T300A* mutation increases degradation of the ATG16L1 protein and is associated with increased risk of CD. In intestinal inflammation, the *T300A* risk allele results in impaired bacterial clearance and dysbiosis.[46] Our study has demonstrated that lack of VDR in the intestinal epithelium leads to dysbiosis and susceptibility to colitis via reducing ATG16L1 expression and dampening inflammatory responses.[13, 33] Furthermore, loss of intestinal VDR shifts the microbiota to a pro-inflammatory profile. These data are consistant with our findings in human IBD samples: Decreased VDR in the intestine is associated with reduced ATG16L1 expression.[13]

Impaired autophagy in myeloid cells caused by myeloid-specific knockout of *Atg16l1* contributes to intestinal inflammation.[47, 48] These mice exhibit increased susceptibility to dextran sulphate sodium (DSS)-induced colitis due to impaired autophagy. Both macrophages and dendritic cells deficient in ATG16L1 have impaired processing of MHC class II antigens, and thus impaired CD4+ T-cell activation.[47, 48] ATG16L1 is also critical for T-cell function. Targeted deletion of ATG16L1 in CD4+ T cells results in increased Th2 expansion and impaired Treg development, leading to spontaneous intestinal inflammation.[49] Overall, induction of *Atg16l1* expression by VDR[13] in intestinal epithelial cells may play a critical role in maintaining intestinal homeostasis.

Intestinal inflammation is largely mediated by activation of nuclear factor-kappa B (NF-κB). VDR is known to downregulate NF-κB signaling and ameliorate intestinal inflammation through its interaction with NF-κB and its inhibitor inhibitory kappa B kinase beta (IKKβ).[50] Interaction of VDR with NF-κB p65 blocks nuclear translocation to lessen the inflammatory response. Loss of VDR in intestinal epithelial cells and MEF cells leads to increased NF-κB signaling, which promotes pro-inflammatory cytokine production.[5] In macrophage cells, vitamin D activation of VDR promotes interaction of VDR with the NF-κB p50 subunit, which prevents LPS-induced proliferation by preventing colocalization of NF-κB p50 with KLF5.[51] Similarly, vitamin D treatment can prevent lipopolysaccharide (LPS)-induced placental inflammation by promoting dimerization of VDR with NF-κB p65.[52] Thus, suppression of NF-κB signaling by VDR ameliorates inflammation, which supports the use of vitamin D supplementation in inflammatory diseases like IBD.

The article by Singh et al.[53] has broadened our understanding of the interaction of VDR and NF-κB and further supports a critical role of VDR in regulating inflammatory disease. By integrating disease-associated SNPs identified by genome-wide association studies (GWAS) with VDR genomic binding sites identified by Chip-Seq, the authors identified 42 SNPs associated with immune phenotypes and VDR binding. Interestingly, most SNPs were not within canonical DR3-type vitamin D response elements (VDREs) that bind the VDR-retinoid X receptor (RXR) heterodimer. Rather, the transcription factor most frequently found to bind these 42 SNPs was NF-κB. Activated VDR and NF-κB share many genomic binding sites, including 22 of the 42 disease-associated SNPs. The data suggest that VDR likely influences transcription indirectly by interacting with NF-κB and other non-RXR transcription factors. The identification of non-VDRE SNPs bound by VDR opens new avenues of study in assessing the mechanism of VDR signaling in disease.

VDR SIGNALING REGULATES THE GUT MICROBIOTA

The human microbiota modulates host physiology and is a critical factor in the development of IBD.[54, 55] Vitamin D may benefit the intestinal microbiome[56] and improve glucose homeostasis in diabetes.[57] We are pioneers in studying how intestinal VDR expression influences the microbiome.[5, 13] From the results of our studies utilizing mouse models of *Salmonella* colitis, and mono-association of the commensal *Escherichia coli* F18 in originally germ-free mice models, we have begun to understand that enteric bacteria activate VDR signaling.[5] We have demonstrated that VDR expression protects the host from invasive pathogens and maintains intestinal homeostasis.[5, 58] Our recent study[59] demonstrated that *Vdr* gene variation in humans influences the intestinal microbiota. A meta-analysis in humans showed *Parabacteroides* to be the most significant taxon correlated with the *Vdr gene*. $Vdr^{-/-}$ mice showed changes in *Parabacteroides* abundance.[59] *Lactobacillus* was depleted, whereas *Clostridium* and *Bacteroides* were enriched in $Vdr^{-/-}$ mice. We have identified several important pathways (eg, cancer, detoxification, infections, signal transduction, and metabolism) affected by *Vdr* gene status.[60]

Intriguingly, lack of VDR in the intestine leads to dysbiosis.[13] We have reported profound alterations in the gut microbiome profile, with increased abundance of *Bacteroidaceae* in intestinal epithelial *Vdr* conditional knockout ($Vdr^{\Delta IEC}$) mice.[13] Likewise, IBD patients have altered mucosal colonization by the *Bacteroidaceae*.[61] $Vdr^{\Delta IEC}$ mice had dysbiosis and abnormal Paneth cells without any induction of colitis, suggesting the genetic role of intestinal epithelial *Vdr* in the development of chronic inflammation. The microbiota may play a role in the vulnerability of $Vdr^{\Delta IEC}$ mice to colitis. The absence of intestinal epithelial VDR confers a transmissible risk for DSS-induced colitis, based on the cohousing study. Thus, intestinal epithelial VDR contributes to microbial homeostasis and host protection against inflammation.

Vitamin D deficiency in the diet induces intestinal dysbiosis; notable changes include increased *Helicobacter hepaticus* and decreased *Akkermansia municiphila* populations in the gut.[34] *H. hepaticus* is known to induce colon cancer

and colitis in mice with impaired immunity,[62] whereas low *A. muciniphila* is decreased in mouse models of colitis and a low population level is associated with ulcerative colitis.[63] *A. muciniphila* is also known to improve barrier function and metabolic health.[64, 65]

The *Vdr* gene transcribes for antimicrobial peptides and autophagy-related proteins (eg, ATG16l1).[13] Vitamin D deficiency reduces production of alpha defensin Defa1 and MMP7 in Paneth cells.[34] Further, Wang et al. reported that human *Vdr* gene variation is a key host factor influencing the gut microbiome.[59] Therefore, the *Vdr* gene may be critical in homeostasis and signaling between the microbiota and host in intestinal inflammation.

Gut bacteria can regulate the immune response in a VDR-dependent fashion. We have shown that the protective effect of probiotics against colitis is mediated by epithelial VDR signaling.[33] The secondary bile acid lithocholic acid, which is produced by *Clostridium* bacteria in the gut lumen, inhibits the Th1 immune response and suppresses IFNγ and IL-2 production by activating VDR in T cells.[66] *Vdr* knockout mice have lower *Clostridium* in the gut,[60] illustrating the influence of crosstalk between the microbiome and VDR signaling in immunity. Butyrate, a short-chain fatty acid produced by gut microbes, can increase epithelial VDR expression and thus decrease production of pro-inflammatory cytokines.[13]

Taken together, the current studies provide new insights into the mechanism of VDR regulation in bacterial-host interactions and inflammation.

VITAMIN D AND HUMAN INTESTINAL INFLAMMATION

A recent study has demonstrated that serum levels of 25-hydroxy vitamin D of 35 ng/mL or less in UC patients in clinical remission are associated with disease relapse. Patients in remission with serum levels <35 ng/mL had more basal inflammation, were more likely to relapse, and relapsed sooner than patients with levels >35 ng/mL.[67] Increasing levels of vitamin D might reduce their risk for UC relapse.

A small study of patients in remission from CD found that daily oral supplementation with 10,000 International Units (IU) of 1α,25-dihidroxy vitamin D_3 conferred greater protection against relapse than a smaller dose of 1000 IU/d over 12 months.[68] A similar study in UC patients with serum vitamin D levels under 30 ng/mL found that daily 4000 IU supplementation over 90 days improved patient quality of life but did not significantly improve clinical UC disease activity.[69]

To our knowledge, there is no report on the direct role of vitamin D supplementation on the microbiome in IBD patients. A recent study shows that high-dose vitamin D supplementation in humans markedly reduces opportunistic pathogens (eg, *Gammaproteobacteria*) and increases phylotype richness.[70] It is believed that vitamin D_3 supplementation reduces inflammation in the intestinal environment, thus diminishing the competitive advantage of opportunistic pathogens (eg, *Escherichia*/*Shigella* spp. or *Pseudomonas* spp.). Thus, the low inflammatory environment allows beneficial bacteria to outcompete pathogens for enhanced phylotype richness. These studies have shown that vitamin D may promote "healthy" microbiota that potentially confer protection against intestinal inflammation. However, we do not know the change of human VDR protein after using vitamin D supplementation and its role in regulating the gut microbiome in health and inflammation.

CONCLUSION AND FUTURE DIRECTION

In conclusion, vitamin D/VDR signaling contributes to the genetic, environmental, immune, and microbial aspects of IBD, making it a significant protein of interest in understanding IBD pathology and in developing treatments (Fig. 1). As a transcription factor, VDR regulates the expression and signaling of target genes contributing to intestinal inflammation and dysbiosis, including *Atg16l1*. Genetic variation of the *Vdr* gene and its targets is associated with inflammation. VDR also directly influences both the host immune system and microbiome through its expression in intestinal epithelial and immune cells. Using a vitamin D supplement confers protection against relapse of IBD. Thus, intestinal VDR expression and vitamin D/VDR signaling protect against intestinal inflammation and dysbiosis.

Microbiome studies and genetics analyses have brought new insights into an old topic on vitamin D/VDR in IBD. Future studies and clinical applications should consider the newly discovered functions of vitamin D/VDR in the intestine. The areas include:

- consideration of intestinal VDR expression as a clinical biomarker for identifying patients who might benefit from currently available interventions;
- development of strategies to restore the healthy host-microbiome interactions via vitamin D/VDR;
- exploration of interactions between the microbiota and vitamin D/VDR that may affect the outcome of therapies;
- consideration of VDR expression on the response to microbiome-targeted therapies, including fecal microbiota transplant;
- exploration of new roles of VDR in other digestive diseases.

VDR signaling in the intestine and immune system promotes mucosal homeostasis and protects against inflammatory disease. The tissue-specific roles of VDR may offer a diagnostic/prognostic indicator in IBD. The current research indicates that vitamin D/VDR will likely become a potential therapeutic target for IBD.

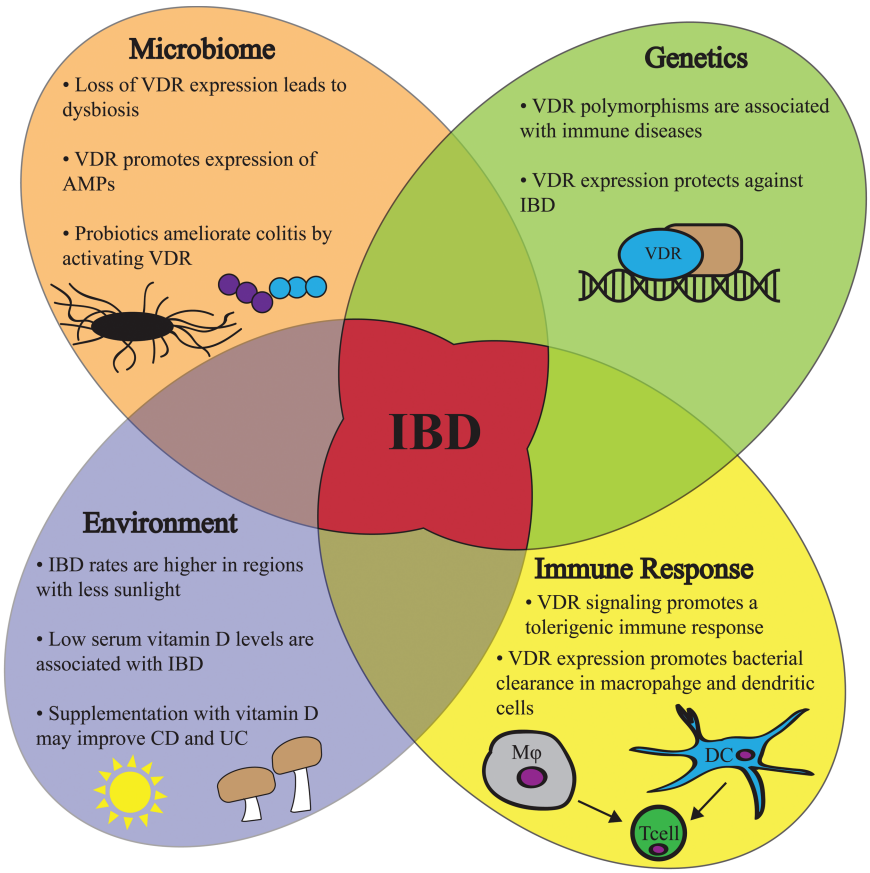

FIGURE 1. A working model of VDR signaling involved in the pathogenesis of IBD. Vitamin D/VDR regulates the genetic, environmental, immune, and microbial aspects in IBD, thus making VDR a significant host factor in IBD pathology and in developing treatments. Susceptibility to IBD is associated with polymorphisms in the *Vdr* gene. Moreover, VDR expression is significantly decreased in IBD patients. Vitamin D deficiency may be an environment trigger contributing to the pathogenesis of IBD. Vitamin D supplement may reduce the relapse of IBD. Vitamin D/VDR seems to be an important immunological regulator of IBD. Vitamin D/VDR signaling promotes microbial and mucosal homeostasis and protects against inflammatory diseases. DC, dendritic cell; Mφ, macrophage.

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