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REVIEW

Vitamin D and Microbiome

Molecular Interaction in Inflammatory Bowel Disease Pathogenesis

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Studies of systemic autoimmune diseases point to characteristic microbial patterns in various diseases, including inflammatory bowel disease (IBD). Autoimmune diseases, and IBD in particular, show a predisposition to vitamin D deficiency, leading to alterations in the microbiome and disruption of intestinal epithelial barrier integrity. This review examines the role of the gut microbiome in IBD and discusses how vitamin D—vitamin D receptor (VDR)—associated molecular signaling pathways contribute to the development and progression of IBD through their effects on gut barrier function, the microbial community, and immune system function. The present data demonstrate that vitamin D promotes the proper function of the innate immune system by acting as an immunomodulator, exerting anti-inflammatory effects, and critically contributing to the maintenance of gut barrier integrity and modulation of the gut microbiota, mechanisms that may influence the IBD development and progression. VDR regulates the biological effects of vitamin D and is related to environmental, genetic, immunologic, and microbial aspects of IBD. Vitamin D influences the distribution of the fecal microbiota, with high vitamin D levels associated with increased levels of beneficial bacterial species and lower levels of pathogenic bacteria. Understanding the cellular functions of vitamin D—VDR signaling in intestinal epithelial cells may pave the way for the development of new treatment strategies for the therapeutic armamentarium of IBD in the near future. *(Am J Pathol 2023, 193: 656—668; https://doi.org/10.1016/j.ajpath.2023.02.004)*

In autoimmune reactions, the immune system reacts against the body's own antigens, causing cell and tissue damage.[1] Crohn disease (CD) and ulcerative colitis (UC) are two chronic diseases characterized by chronic inflammation in the gastrointestinal tract and are referred to as inflammatory bowel disease (IBD). Although the exact cause of IBD remains unclear, significant progress has been made in recent years in deciphering the pathophysiology of these diseases. Over the past decade, IBD has become a global public health challenge,[2] and its prevalence and incidence are increasing significantly, making it one of the most common gastrointestinal tract diseases.[3] The pathogenesis and development of IBD include impaired regulation of immune responses, environmental changes, and disease-related genetic alterations.[4,5] In the pathology of IBD, alterations in the gut microbiota are a common feature; however, it is not clear whether these alterations are the cause or consequence of gut inflammation and how these bacterial communities contribute to the pathogenesis of IBD.[6] Epigenetics may partially explain how the exposome may modulate gene expression and contribute to the development of gut inflammation.[7] Several features of the exposome, including the gut microbiota and vitamin D, may enhance epigenetic alterations associated with IBD.[7]

Patients with IBD are at high risk for osteopenia and osteoporosis and usually have calcium and/or vitamin D

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malabsorption and altered dietary intake.[8] Vitamin D malabsorption has been associated with inadequate sunlight exposure, deficient enzymatic activation, decreased bioavailability, increased catabolism or excretion, inadequate exercise, and smoking.[9] Vitamin D exerts a variety of effects on the immune system, including regulation of immune modulation, cell proliferation and differentiation, and maintenance of intestinal homeostasis.[10,11]

Recent evidence supports a critical role for vitamin D in IBD, as vitamin D deficiency is inversely related to disease activity.[12] In parallel, vitamin D–associated vitamin D receptor (VDR) signaling has been shown to regulate immunity to gut pathogens and maintain gut barrier integrity, demonstrating a potential role in IBD pathogenesis.[12,13] This has attracted considerable attention because of its potential pathogenic impact on IBD progression.[8]

The human microbiome in the gastrointestinal tract consists of a community of commensal, symbiotic, and pathogenic microorganisms that live in the human body.[14] This population directly or indirectly influences human physiology by contributing to metabolic functions, protecting against pathogens, and modulating the immune system.[15] The microbiome is of great importance in autoimmune responses under the concept of molecular mimicry, in which microbial peptides have a similar structure and sequence to self-antigens, resulting in immune cell autoreactivity.[16]

There is growing evidence that vitamin D/VDR-mediated signaling ameliorates both clinical[17,18] and experimental IBD.[19,20] Impaired VDR signaling has been associated with the mucosal inflammation and damage observed in patients with IBD; however, the question of whether impaired VDR signaling causes IBD or whether IBD is the consequence of this impairment has not yet been answered.[21]

This review presents advances in the molecular mechanisms linking the gut microbiome and IBD, as well as recent findings on the interaction between vitamin D and the gut microbiome and how this relationship influences IBD pathogenesis and progression. Emphasis will be placed on preclinical experimental IBD models and translational data on clinical IBD.

Biosynthesis, Physiology, and Metabolism of Vitamin D

Vitamin D was first referred to as a vitamin, but it is actually a fat-soluble steroid hormone that occurs in two different isoforms, vitamin D_2 (ergocalciferol, mainly derived from plants) and vitamin D_3 (cholecalciferol, mainly derived from animals) (Figure 1A). Both are synthesized endogenously in the skin by exposure to UVB and by conversion of 7-dehydrocholesterol to previtamin D_3 and subsequent isomerization, or they are absorbed in the small intestine.[22] These isoforms are considered biologically inactive until enzymatically hydroxylated.[10] Vitamin D binds to vitamin D–binding protein; this complex is transferred to the liver and converted to the circulating form 25-hydroxyvitamin D by the 25-hydroxylase cytochrome P450, family 2, subfamily R, polypeptide 1.[10,11] 25-Hydroxyvitamin D is the most abundant circulating vitamin D metabolite. It is then converted to the biologically active form of vitamin D, 1α,25-dihydroxyvitamin D3 [1,25$(OH)_2D_3$], by sequential hydroxylation by the mitochondrial cytochrome P450, family 27, subfamily B, polypeptide 1 (CYP27B1) in the kidney[10,11] Renal CYP27B1 activity is essential for the production and maintenance of normal concentrations of circulating 1,25$(OH)_2D_3$. CYP27B1 production and activity are tightly regulated by endocrine factors induced in response to changes in plasma calcium and/or phosphorus; the major factors associated with CYP27B1 activity are parathyroid hormone and fibroblast growth factor 23 (FGF23).[23] The release of parathyroid hormone is induced in response to hypocalcemia and is the major activator of the biologically active form of vitamin D.[24] The transmembrane receptor Klotho is critical for FGF23, another important modulator of vitamin D metabolism, to exert its effects in the kidney.[25] In the presence of Klotho, FGF23 induces downstream signaling pathways to modulate phosphate homeostasis.[25] The vitamin D endocrine circuit may be involved in a feedback mechanism by which 1,25$(OH)_2D_3$ inhibits the expression of CYP27B1 in the kidney, down-regulates the production of parathyroid hormone by the parathyroid gland, and up-regulates FGF23 via the skeletal system.[26] Similarly, FGF23 acts via a negative feedback mechanism in the kidney to down-regulate CYP27B1 and in the parathyroid gland to inhibit parathyroid hormone.[27]

In parallel, the role of vitamin D in modulating the immune system and maintaining the homeostasis of the intestinal barrier and the composition of the gut microbiota has received increasing attention.[28] In particular, vitamin D contributes directly to the modulation of the innate immune response by exerting its effect on the proper function of monocytes, macrophages, and dendritic cells and the resulting secretion of cytokines. In addition, vitamin D influences the adaptive immune response, including the development and progression of various autoimmune diseases, by regulating the activation, proliferation, and differentiation of T and B cells.[29]

Vitamin D Receptor

One of the most important actions of vitamin D is the regulation of gene expression in certain tissues; this action is mediated by VDR, a ligand-activated transcription factor that belongs to the nuclear receptor superfamily[30] and is expressed on almost all immune cells.[31] VDR interacts directly with regulatory sequences near target genes and recruits chromatin-active complexes that contribute to genetic and epigenetic modifications of transcriptional output.[23] The active form of vitamin D binds to VDR and induces its activation, directly modulating regulation of

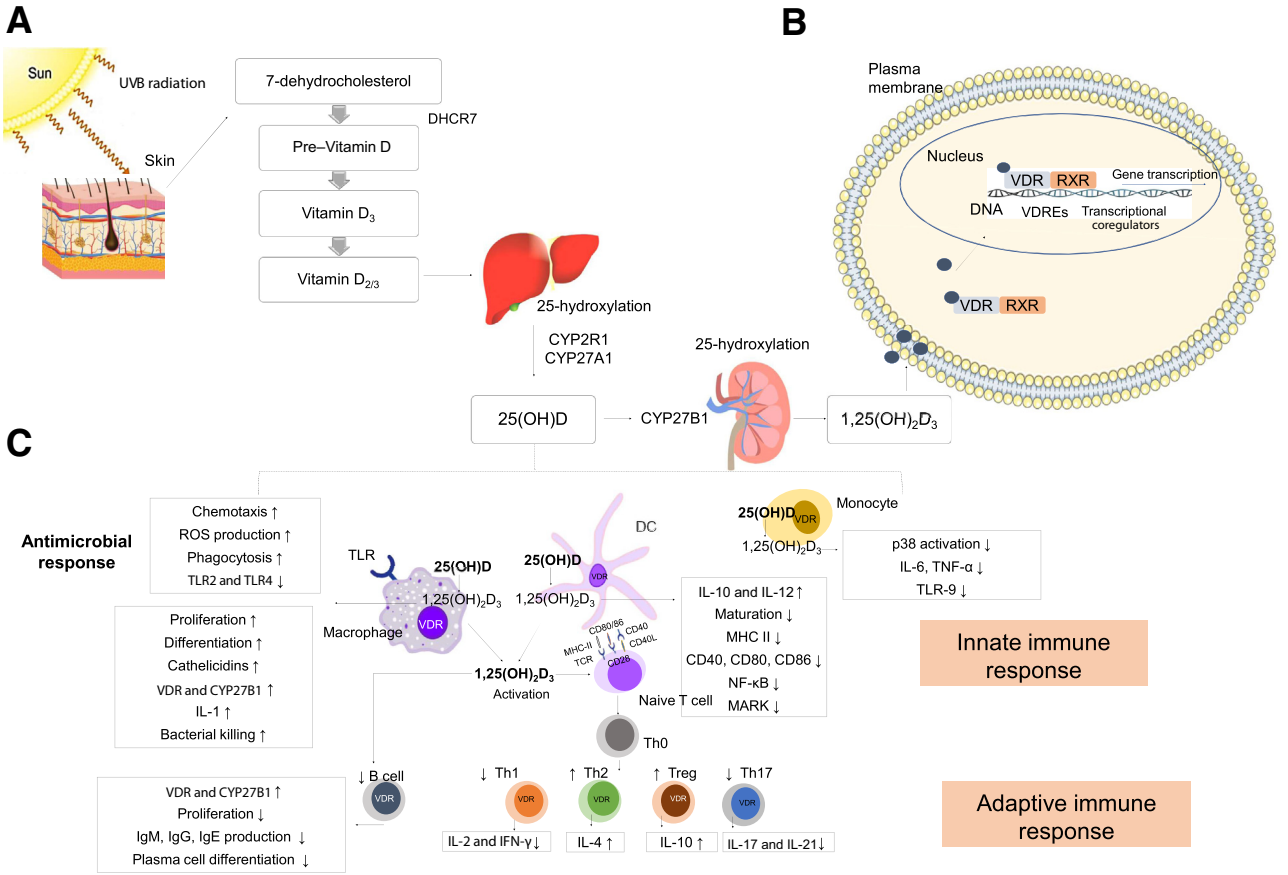

Figure 1 **A:** Vitamin D biosynthesis and metabolism. **B:** Vitamin D—vitamin D receptor (VDR) signaling axis. **C:** Vitamin D action in innate and adaptive immune responses. $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D3; 25(OH)D, 25-hydroxyvitamin D; CD40L, CD40 ligand; CYP27A1, cytochrome P450, family 27, subfamily A, polypeptide 1; CYP27B1, cytochrome P450, family 27, subfamily B, polypeptide 1; CYP2R1, cytochrome P450, family 2, subfamily R, polypeptide 1; DC, dendritic cell; DHCR7, 7-dehydrocholesterol reductase; IFN-γ, interferon-γ; MARK, microtubule affinity-regulating kinase; MHC, major histocompatibility complex; ROS, reactive oxygen species; RXR, retinoid X receptor; TCR, T-cell receptor; Th1, type 1 helper T cell; Th17, type 17 helper T cell; Th2, type 2 helper T cell; TLR, toll-like receptor; TNF-α, tumor necrosis factor-α; Treg, T regulatory cell; VDRE, vitamin D response element.

target genes (Figure 1B).[32] Vitamin D–related target genes include genes closely associated with various cellular processes.[23] VDR is highly expressed in healthy intestinal epithelial cells (IECs)[33] with a surface-to-crypt gradient, and the highest VDR expression is found in the crypts.[33] Therefore, the biological functions of vitamin D are directly associated with $1,25(OH)_2D_3$-dependent changes in the transcriptome in VDR-expressing cells.[34] On activation by vitamin D, a conformational rearrangement of VDR occurs, allowing heterodimerization of VDR with the retinoid X receptor. The VDR–retinoid X receptor complex is translocated to the nucleus, binds to specific genomic sequences (vitamin D response elements) in the promoter of various genes, and modulates their transcription.[35]

There is growing evidence that vitamin D is an important modulator of the immune system that can directly influence both innate and adaptive immune responses. Considering that VDR is expressed in almost all immune cells,[36] it is hardly surprising that vitamin D is closely associated with immune modulation and the development of autoimmune diseases, including IBD.

Physiological Functions of the Gut Microbiome

The epithelium of the gut is in close contact with the gut microbial community, revealing a commensal and/or mutualistic dynamic interaction. The gut microbiome has important metabolic, immunologic, and protective functions. Regarding its role in metabolism, the microbiome can break down otherwise indigestible food components, degrade potentially toxic food components, and synthesize certain metabolites, including vitamins, amino acids, and short-chain fatty acids, such as butyrate, acetate, and propionate, which provide energy to the intestinal epithelium.[37] In parallel, the gut microbiome produces vitamins K and B, niacin, biotin, and folic acid, and it contributes to the enterohepatic cycling of bile acids.[38] It also regulates gut immunity by interacting with adaptive and innate immune responses through the production of microbial-associated molecular patterns.[39] Finally, the gut microbiome inhibits the development of potentially pathogenic bacteria by preventing their access to nutrients and receptors, synthesizing antimicrobial factors, and resisting colonization.[40]

Composition of the Gut Microbiome

The development of an inflammatory response in the gut is hindered by the mucosal layer and gut epithelium, which prevent the invasion of bacteria or their products into the interstitial space.[41] Impairments of the mucosal layer and gut epithelium are closely related to the pathogenesis of IBD.[42] The development of dysbiosis of the gut microbiota and the entry of immunogenic products into the interstitial space activate the immune system, potentially leading to the development and progression of IBD.[43]

The lumen of the human gut, particularly the colon, contains a complex ecosystem of microorganisms, including bacteria, fungi, parasites, viruses, and archaea that live in symbiosis and are referred to as the gut microbiota.[44,45] The amount and composition of the gut microbiota vary throughout the gastrointestinal tract.[45] The gastrointestinal microbiota is predominantly composed of bacteria from four different bacterial phyla (namely, Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria).[46] The gut microbiome encodes approximately 3 million genes and is estimated to harbor 100 times more genes than the host. The presence of a dysbiotic microbiota can lead to a loss of immunoregulatory action on the intestinal mucosa, resulting in a range of inflammatory and immune-mediated diseases.[45,47]

Composition of the Gut Microbiome in IBD

Over the past decade, a decline in the diversity of species composing the microbiome has been demonstrated in stool samples, associated with changes in inflamed mucosal tissues.[47] In addition, adherent-invasive bacteria, such as *Escherichia coli* (phylum: Proteobacteria) and *Fusobacterium* (phylum: Fusobacteria), appear to be more prevalent in North American, Italian, and Japanese patients with IBD.[48–50]

Influence of Vitamin D/VDR on the Gut Microbiome in IBD

The gut microbiota and its metabolites play a critical role in the integrity of the mucosal barrier[51,52]; however, the link between disturbed gut flora and the development of IBD remains unclear.[53] Vitamin D influences the distribution of the fecal microbiota, with increased vitamin D levels associated with higher levels of beneficial bacterial species and lower levels of pathogenic bacteria.[8,54,55] The *VDR* gene is not expressed in the bacterial microbiome, so the effect of vitamin D in IBD is likely mediated through VDR signaling in immune cells and IECs.[56]

Animal Studies

Studies in experimental animal models of IBD have shown how vitamin D affects IBD through its effects on the gut microbiota. Vitamin D–deficient mice exhibited dysbiosis and deficient antimicrobial activity and were susceptible to dextran sulfate sodium (DSS)–induced colitis.[57,58] In addition, vitamin D may influence the susceptibility of mice to DSS-induced colitis by modulating the gut microbiota and the number of retinoid orphan receptor gamma t (RORγt)/forkhead box P3 (FoxP3)$^+$ regulatory T cells in the colon.[59] These data highlight the potential role of vitamin D in alleviating intestinal inflammation and reducing disease activity in IBD by altering the gut microbiota, resulting in higher numbers of beneficial bacteria and lower numbers of pathogenic bacteria.

Human Studies

Studies have shown that oral vitamin D supplementation is associated, at least in part, with a change in gut microbial composition in patients with IBD[60–62] (Table 1). Vitamin D supplementation results in a positive outcome, with an increase in *Enterobacteriaceae* and a decrease in overall gut inflammation in both CD and UC.[60–62] Administration of vitamin D (40,000 IU, once weekly) for a period of 8 weeks does not alter α diversity, except for a small decrease in *Ruminococcus gnavus* in patients with UC.[60] Despite a significant increase in the abundance of *Enterobacteriaceae* in patients with UC, no changes in total fecal bacterial diversity were observed.[60] Vitamin D supplementation (300,000 IU in 4 weeks) causes a change in the composition of the gut microbiota in patients with CD in remission, with an increase in favorable bacteria, such as *Roseburia*, *Alistipes*, *Parabacteroides*, and *Faecalibacterium*.[61] The effect of vitamin D supplementation is transient, as the microbial profile recovered within 4 weeks, although vitamin D levels continued to increase.[61] In addition, vitamin D has a positive effect on the treatment of IBD by modulating the gut microbiome. Administration of vitamin D resulted in an increase in beneficial bacteria and a decrease in pathogenic bacteria in stool samples from healthy individuals.[54] In addition, vitamin D supplementation resulted in a dose-dependent increase in bacteria (*Bacteroides* and *Parabacteroides*) associated with decreased IBD activity,[54] whereas these bacterial populations were reduced in patients with active IBD.[63,64] Recently, vitamin D was shown to have a specific effect on bacterial communities in CD. In patients with CD, vitamin D intake modulated gut bacterial composition by increasing the abundance of potentially beneficial bacterial species.[61] A recent study investigated the possible association between seasonal vitamin D levels and microbial changes.[62] *Pediococcus*, *Clostridium*, and *Escherichia/Shigella* species were enriched in summer/fall, whereas *Eggerthella lenta*, *Helicobacter* species, *Fusobacterium* species, and *Faecalibacterium prausnitzii* were relatively rare.[62]

Regulation of Vitamin D/VDR in IBD by Probiotics

Probiotics, which produce vitamins, have attracted increasing interest because of their effectiveness in reducing

the adverse effects of medications. Lactic acid bacteria are most commonly used because they block the inflammatory process through multiple mechanisms, including protecting the intestinal barrier and mucosal function, regulating immune response, and modulating gut flora in patients with IBD.[65] Probiotics, including *Lactobacillus rhamnosus* GG and *Lactobacillus plantarum*, have beneficial effects on vitamin D and VDR activity. In parallel, the role of probiotics in modulating VDR signaling *in vivo* was investigated using a *Salmonella* colitis model in VDR knockout (KO). The results showed that probiotics provided physiological and histologic protection in VDR-positive mice, whereas they had no effect in VDR KO mice.[66] In addition, it was shown that bile salt hydrolase−active *Lactobacillus reuteri* NCIMB 30242 can regulate plasma vitamin D concentrations. The combination of *L. reuteri*, vitamin D, and krill oil significantly decreased the pathologic score and secretion of inflammatory markers and induced mucosal healing.[67] Pretreatment with VSL#3 significantly increased VDR levels, protected intestinal mucosa, and prevented intestinal injury.[68] Gut microbes synthesize lithocholic acid, which acts as a bridge between VDR and microbiota and increases vitamin D levels.[69] Treatment of HCT116 cells or intestinal organoids with probiotic lactic acid bacteria resulted in the release of P40 and P75 proteins, which contribute to the anti-inflammatory function by increasing VDR and promoting autophagy.[70]

Cosupplementation of Vitamin D and Probiotics in IBD

There is increasing evidence of the synergistic effect of combined supplementation with vitamin D and probiotic bacteria in regulating the gut microbiota and metabolome.[71] Vitamin D absorption in the gut and VDR expression at both the protein and transcriptional levels are increased by the use of probiotics.[68] At the same time, VDR plays an important role in modulating the mechanisms underlying the action of probiotics and regulating their immunomodulatory and anti-inflammatory effects.[72] In a recent systematic review, combined supplementation with vitamin D and probiotics was recommended to be superior to vitamin D or probiotics alone or placebo.[71] A hypothetical model for the interaction of probiotics and vitamin D in terms of their beneficial role in patients with IBD has been proposed.[73] In this model, specific probiotic bacteria can increase circulating vitamin D levels and induce VDR expression and activity in the mucosa, resulting in modulation of mucosal immunity, enhancement of innate immunity and antibacterial defenses, reduction of type 1 helper T-cell (Th1) cytokine expression, and increase of anti-inflammatory effects in the mucosa.[73] Activation of innate immunity promotes modulation of the gut microbiota and elimination or prevention of dysbiosis.[73] This process leads to transient colonization with supplemental probiotics and proliferation of butyrate-producing bacteria that activate vitamin D−VDR pathway in a loop-like manner.[73]

Vitamin D/VDR Signaling Pathway Improves Intestinal Mucosal Barrier Integrity in IBD

Epithelial Junction Complexes

Paracellular permeability occurs via intercellular junctional complexes, such as adherens junctions, tight junctions (TJs), and desmosomes. Vitamin D contributes significantly to maintaining the integrity of the intestinal epithelium and modulates intestinal epithelial cell function by maintaining the expression of TJs in epithelial cells and preventing cytokine-triggered epithelial cell apoptosis.[74] Recent evidence has shown that vitamin D deficiency can attenuate the defective function of the intestinal epithelial barrier and increase susceptibility to DSS-induced colitis in experimental models.[75] Deficiency of vitamin D or VDR led to dysbiosis, which resulted in increased susceptibility to gut injury.[76] Deletion of VDR in the intestinal epithelium caused increased apoptosis of IECs and impaired permeability of the mucosal barrier.[76] Mechanisms involved in the development of dysbiosis in VDR KO and CYP27B1 KO mice include lower E-cadherin expression on the gut epithelium and immune cells, and lower numbers of tolerogenic dendritic cells, resulting in higher intestinal inflammation.[58] Clinical studies have shown that vitamin D deficiency resulted in decreased expression of VDR, occludin, E-cadherin, and zonula occluden-1 in patients with UC[77] and decreased expression of claudin-1, occludin, zonula occluden-1, and junctional adhesion molecule in patients with CD (Figure 2).[78]

Mucosal barrier homeostasis is protected by vitamin D by maintaining the integrity of TJs through the induction of TJ-related mRNA and proteins in mice.[79] These findings were confirmed by *in vitro* studies showing that vitamin D rescued epithelial barrier function in rat IECs by improving permeability and restoring TJs (zonula occluden-1 and claudin-2), resulting in a reduction in inflammation.[80] In mouse models of colitis, vitamin D administration reduced TJ barrier impairment.[81−83] In an *in vitro* model of the intestinal epithelial barrier, $1,25(OH)_2D_3$ increased the expression of E-cadherin and TJ components, such as occludin and claudins (Figure 2).[84] In inflamed tissues of patients with UC, treatment with vitamin D up-regulated claudin-1 and claudin-2 and down-regulated claudin-4 and claudin-7.[83] Overexpression of VDR protected mice from bacterial- and chemical-induced colitis by promoting claudin-15 expression, whereas claudin-15 expression was suppressed in VDR KO mice.[85] Claudin-2 promoter activity was enhanced by VDR in a caudal-related homeobox 1 binding site.[86] VDR deletion resulted in decreased claudin-2 expression by abolishing VDR/promoter binding, whereas VDR deletion in IECs resulted in significantly lower claudin-2 levels in VDR KO and VDR-conditional KO mice

Table 1 Human Studies Evaluating Vitamin D_3 Effect on Microbiota Communities in IBD

Study	Patients, *n*	IBD type	Treatment	Study duration	Outcomes			
					Vitamin D levels	Microbial alterations	Disease course	Other
Garg et al[60]	8	Active UC	Vitamin D_3 40,000 IU weekly	8 Weeks	Increase	↑ *Enterobacteriaceae* Marginal ↓ *Ruminococcus gnavus*	↓ Clinical disease activity	↓ Fecal calprotectin and PLTs ↑ Albumin
	9	UC in remission			Increase		↓ Clinical disease activity	No difference in calprotectin, PLTs, and albumin
	8	HC			Increase	Overall Microbiota diversity unchanged	NA	
Schäffler et al[61]	7	CD in clinical remission	Vitamin D_3 day 1–3: 20,000 IU; day 4–28 (every other day): 20,000 IU	4 Weeks	Increase	Week 0–1 ↑ *Alistipes* ↑ *Barnesiella* ↑ *Roseburia* ↑ *Porphyromonadaceae* ↑ *Anaerotruncus* ↑ *Subdoligranulum* ↑ *Ruminococcaceae* Week 2 ↓ Bacteroidetes ↑ *Faecalibacterium*, ↑ *Veillonella* ↑ *Blautia* ↑ *Fusicatenibacter* ↑ *Intestinibacter* Week 3 ↑ *Parabacteroides* Week 4 ↑ *Lactobacillus* ↑ *Megasphera* ↓ Bacterial diversity	No difference in CDAI	No difference in calprotectin
	10	HC			Increase	No difference	NA	NA
Soltys et al[62]	39	CD	Supplemented and nonsupplemented	Winter/spring	Decrease	In mucosa: ↓ *Clostridium* species ↑ Clostridia (Firmicutes) In stool: ↑ Bacteroidetes Overall ↑ Firmicutes ↑ Bacteroidetes ↑ Proteobacteria ↑ Actinobacteria	NR	NR
				Summer/fall	Increase	In stool: ↑ Firmicutes ↓ *Bacteroides* species In sigma inflamed and noninflamed: ↓ Actinobacteria In noninflamed sigma: ↓ Fusobacteria ↑ *Pediococcus* species In inflamed sigmoid tissue: ↑ *Clostridium* species In noninflamed terminal ileum: ↓ *Collinsella aerofaciens*		
	35	UC	Supplemented and nonsupplemented	Winter/spring	Decrease	In inflamed sigma: ↑ Proteobacteria ↑ *Campylobacteralles* ↑ *Helicobacteraceae* In noninflamed sigma: ↑ Actinobacteria Overall ↑ Proteobacteria ↑ Firmicutes ↑ Bacteroidetes ↑ Actinobacteria	NR	NR
				Summer/fall	Increase	In stool: ↑ γ-proteobacteria		

↑, Increase; ↓, decrease; CD, Crohn disease; CDAI, Crohn Disease Activity Index; HC, healthy control; IBD, inflammatory bowel disease; NA, not applicable; NR, not reported; PLT, platelet; UC, ulcerative colitis.

in vivo.[86] These results were consistent with the findings of another study showing decreased claudin-2 mRNA and protein expression in VDR KO mice.[87]

In parallel, the vitamin D−VDR pathway may be involved in the control of gut barrier integrity through modulation of myosin light chain kinase (Figure 2).[88] Myosin light chain kinase promotes actomyosin ring contraction through phosphorylation of related proteins and has been proposed as a regulator of TJ permeability.[88] Long myosin light chain kinase isoform expression is up-regulated in the intestine during inflammation, and vitamin D/VDR signaling controls epithelial permeability in cultured intestinal cells and in experimental models by regulating the myosin light chain kinase pathway.[89] These data highlight that vitamin D/VDR signaling maintains the integrity of the intestinal mucosal barrier by regulating IECs that have a major impact on Paneth cells, autophagy, and the gut microbiome.

Paneth Cell Dysfunction

Mice with intestinal epithelium VDR KO showed impaired Paneth cell function, impaired autophagy, and dysbiosis accompanied by decreased expression of autophagy-related 16-like 1, a protein closely associated with IBD risk and an important regulator of autophagy (Figure 2).[90] VDR-deficient mice showed impaired ileal Paneth cell secretion of α-defensins and the matrix metalloprotease 7 in the ileum, which may lead to dysbiosis (Figure 2).[91] A recent study showed that Paneth cells derived from VDR KO mice exhibited decreased inhibition of pathogenic bacterial growth and autophagic responses (Figure 2).[92] These mice had significantly higher rates of inflammation and were highly susceptible to small intestinal injury after *Salmonella* infection, suggesting that loss of VDR in Paneth cells leads to deficient antibacterial activity and increased rates of inflammation.[92] VDR deficiency in the intestinal epithelium caused increased apoptosis of epithelial cells and abnormal autophagy due to decreased autophagy-related 16-like 1 and Beclin-1 expression (Figure 2).[93,94] Production of lysozyme and other antimicrobial peptides (AMPs), such as defensins, was lower in VDR KO mice.[92,93]

One possible mechanism linking vitamin D deficiency to IBD pathogenesis is the dysregulation of autophagy by miR-142-3p (Figure 2); increased ileal expression of the autophagy-suppressing miR-142-3p was detected in vitamin D−deficient mice and in colon biopsies from vitamin D−deficient pediatric patients with IBD.[95] In parallel, Paneth cells from vitamin D−deficient mice exhibited impaired morphology and increased levels of the autophagy adaptor protein p62, a protein that was absent throughout the crypt epithelium,[95] suggesting that Paneth cells exhibit early autophagy dysregulation markers in response to vitamin D deficiency and increased miR-142-3p expression.[95] Although the effect of vitamin D level on mucus production has not been demonstrated, studies in experimental models suggest the absence of VDR on goblet cells.[96] However, degradation of the mucus layer was observed in CYP27B1 KO mice.[20] This finding suggests that vitamin D indirectly regulates mucus secretion by possibly promoting Ca^{2+} assimilation.[97] The positive role of Ca^{2+} and vitamin D on mucin 12, cell surface associated (MUC12) expression has also been suggested.[98]

Vitamin D/VDR Effects on the Intestinal Microbiome and Their Role on the Immunologic Barrier in IBD

Intestinal homeostasis is maintained by the interaction between the gut microbiome, IECs, and immune cells. A defect in any component of this complex interaction can lead to the development of an inflammatory response, as seen in IBD.[99]

Vitamin D contributes significantly to the regulation of immune responses through its binding to VDR, which is expressed in most immune cells, including activated or naive $CD4^+$ and $CD8^+$ T cells, B cells, neutrophils, APCs, monocytes, macrophages, and dendritic cells (Figure 1C).[100] A contribution of B cells/plasma cells in experimental or human IBD seems possible but has not been clearly demonstrated.[101] $CD4^+$ T cells differentiate into distinct proinflammatory and anti-inflammatory subpopulations.[102] The various T-cell subpopulations and the cytokines they secrete are critical to the physiological function of the intestinal mucosa and continuously modulate intestinal homeostasis and inflammation.[103] A growing body of evidence points to the role of T cells, particularly Th1/type 17 helper T cells, in experimental and human IBD; some of it is conflicting.[104] A useful tool to study the immunologic background of IBD is the experimental IBD model, which involves adoptive transfer of T cells to immunocompromised mice.[104] In this model, adoptive transfer of naive $CD4^+$ T cells to syngeneic severe combined immunodeficiency or recombination-activating gene (RAG) KO mice leads to the development of chronic progressive colitis that simulates IBD.

The vitamin D−VDR−retinoid X receptor complex induces chemotactic and phagocytic capabilities and concurrently stimulates the transcription of AMPs in various cells, including colon cells (Figure 2).[105] AMPs, including cathelicidin [human cationic antimicrobial protein (hCAP18)] and β-defensin 2/human beta-defensin-2 (DEFB4/HBD2), act as chemotactic factors for inflammatory immune cells[106] and exert antimicrobial effects.[100] In human macrophages, stimulation of the toll-like receptor promotes expression of the cathelicidin antimicrobial peptide via a vitamin D−dependent pathway.[107] Vitamin D−mediated up-regulation of cathelicidin antimicrobial peptide enhances antimicrobial activity against pathogens by down-regulating cathelicidin leucine−leucine-37 and up-regulating phagosome formation.[108] DEFB4/HBD2 and cathelicidin

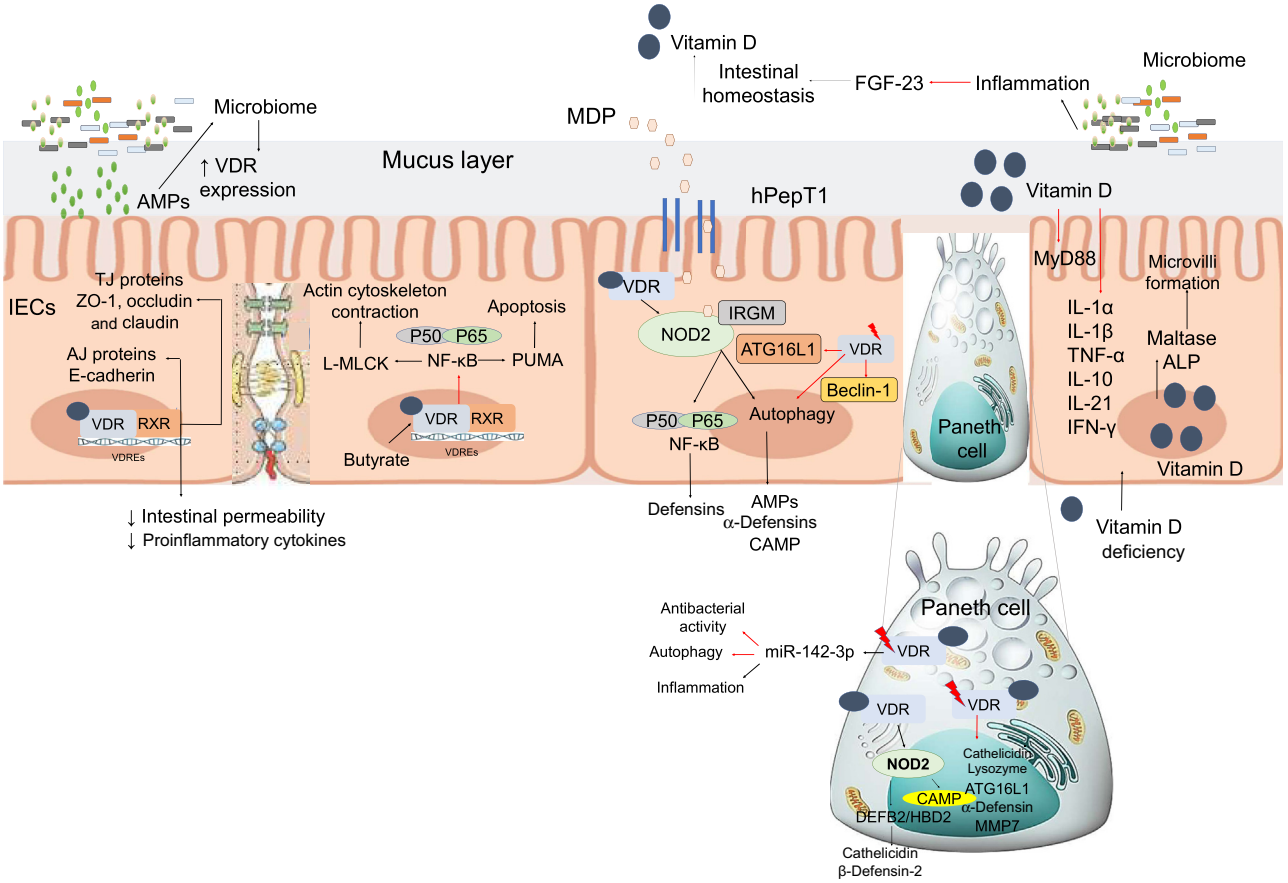

Figure 2 Schematic representation of the effect of vitamin D and the microbiota on the intestinal epithelial barrier. Vitamin D—vitamin D receptor (VDR) signaling is essential for maintaining intestinal barrier integrity by modulating apical junctional complexes through up-regulation of tight junctional proteins, zonula occluden-1 (ZO-1) and occludin, and adherent junctional proteins, such as E-cadherin, and down-regulation of myeloid differentiation primary response (MyD88) expression. Gut barrier permeability is also maintained by vitamin D—VDR signaling through the control of claudins. Vitamin D—VDR signaling protects against tumor necrosis factor (TNF)-α—induced intestinal barrier injury by contraction of the actin cytoskeleton via blockade of NF-κB—mediated activation of long myosin light chain kinase (L-MLCK). The vitamin D—VDR pathway is intimately involved in the autophagy process through its action on autophagy-related 16-like 1 (ATG16L1), nucleotide-binding oligomerization domain protein 2 (NOD2), and Beclin-1 and protects against apoptosis of intestinal epithelial cells (IECs) via down-regulation of the *PUMA* (P53 up-regulated modulator of apoptosis) gene, which occurs through suppression of NF-κB stimulation. The microbiome induces VDR expression in IECs, whereas it is regulated by vitamin D supplementation and by secretion of antimicrobial peptides (α-defensins from Paneth cells and β-defensin 2 and cathelicidin by IECs). Vitamin D deficiency induces secretion of IL-1α, IL-1β, IL-21, IL-10, TNF-α, and interferon (IFN)-γ. TNF-α promotes disruption of gastrointestinal barrier integrity and colon inflammation. Vitamin D increases the expression of alkaline phosphatase and maltase, which, in turn, promotes microvilli formation. AJ, adherent junction; ALP, alkaline phosphatase; AMP, antimicrobial peptide; CAMP, cathelicidin antimicrobial peptide; DEFB2/HBD2, antimicrobial peptide defensin β2; FGF-23, fibroblast growth factor 23; hPepT1, human PEPT1; IRGM, immunity-related GTPase M; MDP, muramyl dipeptide; MMP-7, matrix metalloproteinase 7; RXR, retinoid X receptor; TJ, tight junction; VDRE, vitamin D response element.

antimicrobial peptide were found to be induced both directly and indirectly by $1,25(OH)_2D_3$ stimulation in human monocytic and intestinal cell lines through activation of the intracellular pattern recognition receptor nucleotide-binding oligomerization domain protein 2.[109–111] Activation of nucleotide-binding oligomerization domain protein 2 by its ligand muramyl dipeptide triggers *HBD2* gene expression (Figure 2).[112] Vitamin D induces nucleotide-binding oligomerization domain protein 2 in primary human monocytic and epithelial cells.[110] These results suggest that the action of AMPs has a major impact on the composition of the gut microbiota. AMPs and IgAs synergistically protect the mucus layer outside epithelial cells.[113]

In addition, data have shown that activation of $1,25(OH)_2D_3$ in the intestinal epithelial cell line DLD1 promotes cathelicidin expression, inhibits *E. coli* growth *in vitro*, and protects against experimental colitis *in vivo*.[114] This effect was attenuated by blockade of cathelicidin, suggesting an antimicrobial role of vitamin D mediated by cathelicidin.[114]

In IECs, the expression of AMP is also regulated by the bioproducts of gut metabolism, which form a mucosal barrier and prevent the interaction of microbes and pathogens with the gut epithelium.[115] Short-chain fatty acids, such as sodium butyrate, are able to activate cathelicidin expression.[116] The vitamin D—VDR pathway is involved in this activation, as the regulation of cathelicidin expression in the colon by secondary bile acids is VDR regulated.[115] In addition, VDR signaling in mice is also controlled by bacterially produced metabolites, similar to butyrate, which

are associated with higher epithelial VDR levels.[93] Surprisingly, some bacterial enzymes hydroxylate steroids and stimulate vitamin D.[117] The intestinal microbiota also influences vitamin D metabolism by regulating FGF23 and CYP27B1 (Figure 2).[118]

Studies in experimental models have shown that the absence of VDR in the gut results in dysbiosis,[93] decreased secretion of proinflammatory cytokines from the epithelium, and attenuation of immune cell infiltration into the mucosa.[119] Deletion of VDR in the epithelium of a chemically induced colitis mouse model resulted in excessive apoptosis in colon epithelia due to overactivation of the gene *PUMA* (p53 up-regulated modulator of apoptosis), leading to epithelial barrier dysfunction (Figure 2). The disruption of the mucosal barrier led to increased invasion of antigens and luminal bacteria and triggered mucosal inflammatory responses.[76]

Vitamin D has been associated with the expression of intestinal alkaline phosphatase; alkaline phosphatase is a protein responsible for the hydrolysis of monophosphate esters and is a critical feature of the gut defense system, preventing the uptake of bacteria across the intestinal mucosal barrier and thereby maintaining gut homeostasis (Figure 2).[120] $1,25(OH)_2D_3$ increases the expression and activity of alkaline phosphatase and maltase and promotes microvilli formation (Figure 2).[121]

Bacteria can affect vitamin D metabolism and function, as germ-free mice had low vitamin D levels and hypocalcemia, a situation that was reversed when these mice were colonized with other commensal bacterial species.[118] In parallel, bacteria secrete substances that induce VDR signaling, such as butyrate, which increases VDR expression in the intestinal epithelium in mice,[122] and lithocholic acid (produced by *Clostridium* species in the gut), which suppresses Th1 immune responses and IL-2 production by stimulating VDR signaling in T cells.[123] Another study showed that commensal and pathogenic bacteria directly modulated VDR expression and localization in colonic epithelium, whereas VDR negatively regulated activation of bacterially induced intestinal NF-κB and attenuated the response to infection in the IECs of *Salmonella*-infected mice compared with VDR KO mice.[33]

Mouse models fed a vitamin D–deficient diet and models lacking expression of VDR in the intestinal epithelium exhibited higher susceptibility to experimental colitis, which may be due to different mechanisms of vitamin D action.[57,124,125] Vitamin D–deficient mice developed more severe DSS-induced colitis, had more bacteria in the colonic lumen (>50-fold), and had reduced antimicrobial protein angiogenin-4.[57] Diet-induced vitamin D deficiency increased intestinal permeability,[126] and deletion of VDR resulted in more severe disease in a DSS animal model.[93] VDR/IL-10 double-KO mice developed colitis after 8 weeks compared with single IL-10 or VDR-KO mice, which were relatively healthy at that time,[127] whereas epithelial VDR induction resulted in less disease activity.[124] However, the above data are at odds with studies showing that VDR-deficient mice had normal gut permeability with normal mucosal morphology in both the large[128] and small intestine.[87] This evidence for experimental colitis in animal models supports the critical role of vitamin D/VDR signaling in maintaining the integrity of the mucosal barrier. In contrast, data from human studies are unclear. VDR expression was found to be reduced in UC and CD colon biopsies and in UC activated inflamed colon, compared with high VDR expression in normal colon epithelial cells.[124,129] In contrast, other studies[130,131] showed no significant differences, although VDR expression was negatively associated with inflammatory activity. It was also shown that in patients with IBD, mucosal inflammation was related to tumor necrosis factor-α–mediated VDR down-regulation and induction of CYP27B1.[125,132]

Conclusions and Perspective

The immunomodulatory and anti-inflammatory effects of vitamin D provide plausible mechanisms for how vitamin D may influence the development, progression, and severity of IBD. Vitamin D contributes to the proper function of the innate and adaptive immune response, intestinal barrier integrity, and gut homeostasis. Specifically, the vitamin D/VDR pathway stimulates specialized epithelial cells (eg, Paneth cells) and lamina propria cells (eg, B cells/plasma cells) to limit the uptake of microbiota and their products into the interstitium. In the event of bacterial invasion of the lamina propria, immune cells, such as macrophages, dendritic cells, and Th1 and type 17 helper T cells, clear the affected tissue by direct or indirect action. After clearance, vitamin D exerts its immunoregulatory properties (ie, inhibits Th1 and type 17 helper T cells, activates T regulatory cells, and restores intestinal homeostasis). At the cellular level, vitamin D modifies the expression of TJ proteins, autophagy, and apoptosis, ensures proper epithelial barrier function, and induces the expression of AMPs. Many of these functions result from the complex ligand-receptor interaction between vitamin D and VDR, which has a major impact on the human microbiome.

Recently, the gut microbiome has attracted much interest because of rapid advances in sequence-based screening and the humanized gnotobiotic model to study the dynamic functions of the commensal microbiota. Vitamin D modulates the gut microbiota as vitamin D deficiency causes microbial imbalance in the gastrointestinal tract. The antibacterial role of vitamin D is closely linked to the expression of AMPs. The gut microbiota responds to vitamin D supplementation, and various fermentation products of the microbiota appear to promote VDR expression. More robust data on the immunologic, biochemical, functional, and genetic interplay between vitamin D and the human gut microbiota will pave the way to explore the complex function of the gut.

Although current understanding of the functional properties of the complex gut microbiome remains elusive, the results of recent studies promise to elucidate the etiology of IBD, which could lead to the development of new treatment strategies. However, the use of experimental models to study the mechanisms underlying IBD in humans appears to have limitations. Research on experimental animal models is usually conducted under specific conditions (eg, special diet and pathogen-free conditions). In contrast, patients with IBD often have severe comorbidities, including neuropsychological disorders, cardiovascular disease, and metabolic syndrome.[133] In parallel, although humans and mice are similar in some important features of the immune system, they also have crucial differences.[111,134] In particular, most gene expression motifs (estimated at 80%) are identical in mice and humans, which may be related to the presence of transcriptional regulators that control some of the similarities. On the other hand, the differences limit the translation of results from animal studies to humans, underlining the importance of interspecies inference. In parallel, the favorable results of vitamin D/VDR signaling in both experimental IBD models and human IBD have been associated with changes in the resident microbiota. However, most studies have been of insufficient duration to investigate the potential impact of microbiota resilience.[135] For example, after a disruption (harmful or therapeutic) of microbial composition, the altered state may persist or return to the pretreatment state. Exploring the resilience mechanisms that determine long-term community stability and understanding the perturbations are critical elements for a better understanding of the gut and important prerequisites for exploring microbiome-based precision medicine. Thus, the development of novel systems, such as culturing human primary epithelial cells or intestinal organoids derived from patients with IBD, may provide useful tools for the development of alternative therapies for this disease.

It is clear that the immune system and the microbiome are interconnected, and vitamin D is an essential mediator in this dynamic relationship. Therefore, a comprehensive understanding of the effects of vitamin D deficiency and supplementation on the gut microbiome in health and autoimmunity is needed.

Author Contributions

I.A. and C.T. designed the study; I.A. collected the data and wrote the manuscript; and M.M., S.F.A., A.M., K.T., and C.T. revised the manuscript. All authors approved the article for publication. I.A. is the guarantor of this work and, as such, had full access to all of the data in the study.

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