Vitamin D Deficiency Promotes Epithelial Barrier Dysfunction and Intestinal Inflammation

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Background. Vitamin D, an important modulator of the immune system, has been shown to protect mucosal barrier homeostasis. This study investigates the effects of vitamin D deficiency on infection-induced changes in intestinal epithelial barrier function in vitro and on Citrobacter rodentium–induced colitis in mice.

Methods. Polarized epithelial Caco2-bbe cells were grown in medium with or without vitamin D and challenged with enterohemorrhagic Escherichia coli O157:H7. Barrier function and tight junction protein expression were assessed. Weaned C57BL/6 mice were fed either a vitamin D–sufficient or vitamin D–deficient diet and then infected with C. rodentium. Disease severity was assessed by histological analysis, intestinal permeability assay, measurement of inflammatory cytokine levels, and microbiome analysis.

Results. 1,25(OH)2D3 altered E. coli O157:H7–induced reductions in transepithelial electrical resistance (P < .01), decreased permeability (P < .05), and preserved barrier integrity. Vitamin D–deficient mice challenged with C. rodentium demonstrated increased colonic hyperplasia and epithelial barrier dysfunction (P < .0001 and P < .05, respectively). Vitamin D deficiency resulted in an altered composition of the fecal microbiome both in the absence and presence of C. rodentium infection.

Conclusions. This study demonstrates that vitamin D is an important mediator of intestinal epithelial defenses against infectious agents. Vitamin D deficiency predisposes to more-severe intestinal injury in an infectious model of colitis.

Keywords. colitis; barrier function; dysbiosis; microbiome; vitamin D.

The incidence of chronic inflammatory bowel diseases (IBD) has increased over the past few decades, particularly in developed countries [1]. This change cannot be explained solely by genetic factors but, rather, is likely attributed to environmental insults, including changes in the composition of the intestinal microbial flora. 1,25-Dihydroxyvitamin D3 (1,25(OH)2D3), the active form of vitamin D, is a key modulator of the immune system [2]. Importantly, decreased serum 1,25(OH)2D3 levels, or vitamin D deficiency (<50 nmol/L or 20 ng/mL of 25(OH)D3), has been linked to autoimmune diseases, including type 1 diabetes [3] and multiple sclerosis [4]. While vitamin D deficiency is associated with an increase in prevalence of IBD [5], its precise role in the development of intestinal inflammation remains unclear.

Recent studies have identified an association between vitamin D receptor (VDR) polymorphisms and IBD susceptibility [6]. Moreover, colonic epithelial VDR expression is reduced in IBD patients [7]. Indeed, VDR gene–knockout mice develop chronic low-grade intestinal inflammation [8] and a more severe episode of colitis in response to challenge with dextran sodium sulfate (DSS) [9, 10]. VDR gene–knockout mice also exhibit increased bacterial burden and mortality after Salmonella infection, possibly via a direct downregulation of colonic epithelial VDR expression [11]. Similarly, 1,25(OH)2D3 deficiency predisposes to more severe experimental colitis [12, 13] through dysregulation of colonic antimicrobial activity [13, 14]. In vitro, vitamin D protects against DSS-induced disruption of the integrity of intercellular tight junctions [12], highlighting a
potential mechanism through which protective effects are mediated.

The intestinal epithelial barrier serves as the first line of defense against external insults [15]. These cells are bound together by apical junctional complexes including tight junctions (TJ), which regulate paracellular permeability and form a crucial barrier against toxins and enteric pathogens [16].

Altered permeability and dysfunctional TJ are described in the noninflamed distal ileum of patients with Crohn disease [17]. Increased epithelial permeability and impaired barrier function, owing to an alteration in TJ structure, is also reported in patients with ulcerative colitis [18]. However, it remains unclear whether the increased permeability observed in patients with IBD is causal in the development of disease or simply a consequence of the inflammatory processes.

The role of 1,25(OH)2D3 in pathogen-induced experimental colitis and infection-associated dysregulation of intestinal epithelial barrier function has not yet been established. Thus, the purpose of this study was to investigate the effects of 1,25 (OH)2D3 deficiency on Escherichia coli O157:H7–induced disruption of intestinal epithelial barrier in vitro and Citrobacter rodentium–induced experimental colitis in vivo.

**MATERIALS AND METHODS**

**Bacterial Strains**

Enterohemorrhagic E. coli (EHEC), strain CL56 serotype O157: H7 [19] and the murine pathogen C. rodentium [20] strain DBS 100 (kindly provided by the late David Schauer, Massachusetts Institute of Technology) were cultured for 18 hours at 37°C in static nonaerated Luria-Bertani broth to yield a total concentration of approximately 1 × 109 colony-forming units (CFU)/mL.

**Epithelial Cell Culture and Bacterial Infection**

Polarized Caco2-bbe human colonic adenocarcinoma cells (ATCC CRL-2102) were used as previously described [21]. T84 human colonic epithelial cells (ATCC, Manassas, VA) were cultured as previously described [19]. For all experiments, cells were grown to confluence in 6.5-mm or 12-mm Transwells (pore size, 0.4 µm; Costar, Corning, NY). Culture medium was changed to antibiotic-free culture medium with or without 10 µM of 1,25(OH)2D3 (Sigma-Aldrich, Oakville, Canada) and incubated with either primary rabbit anti-ZO-1 (Invitrogen) or rabbit anti-claudin-1 (Invitrogen) and a secondary Cy2-conjugated goat anti-rabbit immunoglobulin G (Jackson Immunoresearch). Nuclei were counterstained with 300 nM 4′,6-diamidino-2-phenylindole dilactate (Molecular Probes, Eugene, OR) and imaged using a Leica DMI600B fluorescence microscope equipped with a DFC 360FX monochromatic camera (Leica Microsystems, Ontario, Canada). Leica Application Suite AF software (Leica Microsystems) was used for all analyses.

**In Vitro Dextran Permeability Assay**

Following EHEC challenge, epithelial cells in the absence or presence of 1,25(OH)2D3 (10 µM) were rinsed 4 times with Dulbecco’s phosphate-buffered saline (PBS; Invitrogen, Ontario, Canada) to remove nonadherent bacteria. A fluorescein isothiocyanate (FITC)–conjugated dextran probe (10 kDa; Sigma-Aldrich; diluted in Dulbecco’s modified Eagle’s medium) was added apically. After incubation for 5 hours at 37°C, supernatants from the basolateral compartment were recovered and fluorescence intensity was measured using fluorometry (PerkinElmer, Ontario, Canada). Data recorded from the raw integrated intensity values were expressed relative to the intensity of samples collected from noninfected monolayers.

**Immunofluorescence Visualization of TJ Protein Expression**

Immunofluorescence staining for zonula occludens-1 (ZO-1) and claudin-1 was adapted from Zareie et al [19]. Briefly, infected and noninfected Caco2-bbe monolayers, in the presence or absence of 1,25(OH)2D3 (10 µM, 16 hours), were rinsed in PBS and fixed with 100% methanol. Monolayers were blocked with 5% normal goat serum (Jackson Immunoresearch, West Grove, PA) and incubated with either primary rabbit anti-ZO-1 (Invitrogen) or rabbit anti-claudin-1 (Invitrogen) and a secondary Cy2-conjugated goat anti-rabbit immunoglobulin G (Jackson Immunoresearch). Nuclei were counterstained with 300 nM 4′,6-diamidino-2-phenylindole dilactate (Molecular Probes, Eugene, OR) and imaged using a Leica DMI600B fluorescence microscope equipped with a DFC 360FX monochromatic camera (Leica Microsystems, Ontario, Canada). Leica Application Suite AF software (Leica Microsystems) was used for all analyses.

**Quantitative Polymerase Chain Reaction (qPCR) of TJ Protein Expression**

After treatment with 1,25(OH)2D3 (10 µM) Caco2-bbe monolayers were challenged with EHEC O157:H7, rinsed twice with cold PBS and scraped into 300 µL Trizol reagent (Invitrogen) for RNA isolation. Briefly, approximately 1 µg of RNA was treated with a DNase I kit (Invitrogen) before conversion into complementary DNA (cDNA), using the iScript cDNA synthesis kit system (Bio-Rad, Hercules, CA). cDNA was subjected to qPCR with iQ SYBR Green Supermix and 500 nM of a primer pair. Primer sequences (5′–3′) were as follows:

ZO-1, CGAGTTGCAATGGTTAAGGGA (forward) and TCAGGATTCAGGACTTTACTGG (reverse); claudin-1, GCCGCAATATTTCTCTCAGGG (forward) and TTCGTA CCTGGAATGACTGG (reverse). A CFX1000 thermocycler and CFX96 qPCR detection system (Bio-Rad) was used with 60°C annealing temperature. Levels of ZO-1 and claudin-1 were measured 3 hours after EHEC challenge and the fold change in expression was calculated relative to uninfected control monolayers.
were calculated by the $2^{-\Delta\Delta Ct}$ analysis. $2^{-\Delta\Delta Ct}$ values are presented as the relative expression of gene expression.

**Animals**

Weaned female C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME), aged 3 weeks, were raised in the containment unit of the Laboratory Animal Service facility at the Hospital for Sick Children (Toronto, Canada) with access to water and either standard chow or vitamin D–deficient chow (both from Ren’s Pets Depot, Ontario, Canada) for 5 weeks ad libitum, according to the protocol of Lagishetty et al [13].

Mice were infected with the murine-specific enteric pathogen *C. rodentium* ($10^8$ CFU administered by orogastric gavage) [24] or administered an equal volume of Luria-Bertani broth (both from Ren’s Pets Depot, Ontario, Canada) for 5 weeks ad libitum, according to the protocol of Lagishetty et al [13].

Assessment of Colonic Injury

To assess histological changes, distal colonic segments were fixed in 10% neutral-buffered formalin and paraffin embedded. Samples were sectioned, stained with hematoxylin and eosin, and visualized using a Leica DMI 6000B microscope equipped with a Leica DFC420 camera (Leica). Crypt lengths were measured on coded sections. Results represent the average of 5–10 crypt lengths per section of tissue from at least 2 colonic sections per animal.

**Determination of Serum Vitamin D and Calcium Levels**

Whole blood was obtained by cardiac puncture. Samples were centrifuged at 2300×g, (at 4°C for 20 minutes), and serum was isolated for storage at −20°C. 25-OH vitamin D levels were measured by immunoassay (25-OH vitamin D enzyme-linked immunosorbent assay, Eagle Bioscience, Nashua, NH). Calcium
levels were measured by colorimetry (MaxDiscovery Calcium Assay Manual, Austin, TX).

**FITC-Dextran In Vivo Permeability Assay**

Alterations in epithelial barrier function were assessed by measuring the recovery of a 4-kDa FITC-conjugated dextran probe (Sigma-Aldrich) in serum [25]. Four hours after administration, mice were euthanized and whole blood specimens were collected, as described above. Serum levels of the probe were determined using fluorometry (PerkinElmer).

**qPCR Analysis of Proinflammatory and Antiinflammatory Markers**

Full-thickness specimens from the distal colon were homogenized, and RNA was harvested by Trizol extraction (Invitrogen). After treatment with DNase A (Invitrogen), samples were converted into cDNA, using an iSCRIPT cDNA synthesis kit (Bio-Rad). DNA was amplified by qPCR, using SsoFast EvaGreen Supermix and a CFX96 C1000 Thermal Cycler (Bio-Rad). Primers against mouse β-actin (housekeeping gene), interleukin 10, interleukin 17a (IL-17a), tumor necrosis factor α (TNF-α), interferon γ, and transforming growth factor β were used [26].

**Gut Microbiome Composition**

The colonic microbiota was analyzed using denaturing gradient gel electrophoresis (DGGE) and 16S ribosomal RNA (rRNA) qPCR. Briefly, fecal pellets were collected and stored at −80°C. Bacterial DNA was extracted using the QIAamp DNA stool kit (Qiagen, Toronto, Canada).

DGGE was used to generate general bacterial 16S rRNA gene profiles, as previously described [27], using the primers
357F-GC clamp and 518R. Electrophoresis consisted of running a 30%–70% denaturing gradient in a 10% polyacrylamide gel for 14 hours at 85 V in a DGGEK-2001 system (CBS Scientific, Del Mar, CA). Gels were stained using SYBR Green I (Invitrogen) and visualized using a Typhoon FLA 9500 Molecular imager (GE Healthcare, Quebec, Canada). Community fingerprints were subjected to the unweighted pair group method of arithmetic mean cluster analysis based on the Pearson correlation of the fingerprint densitometric curves, using GelCompar II (Applied Maths).

qPCR was performed using SsoFast EvaGreen Supermix and a CFX96 C1000 Thermal Cycler (Bio-Rad). 16S rRNA primer sequences targeting Bacteroidetes, Firmicutes, Actinobacteria, Beta-proteobacteria, and Gammaproteobacteria were obtained from a previous study [28]. Colonization patterns were presented as the relative quantity of total bacteria in the infected group, compared with the uninfected and vitamin D–sufficient control groups.

Statistics
Results were expressed as means ± standard errors of the mean. Statistical differences between multiple groups were calculated using analysis of variance. To examine differences between 2 experimental groups, the unpaired Student t test was used. A P value of <.05 was considered statistically significant.

RESULTS

1,25(OH)2D3 Attenuates E. coli O157:H7–Induced Decrease in TER in a Dose- and Time-Dependent Manner

Vitamin D alone at concentrations of 10−6–10−9 M did not alter the TER of the 2 complimentary cell lines (P > .05 vs untreated monolayers [n = 3–5]; data not shown).

E. coli O157:H7 challenge resulted in reduced TER in both Caco2-bbe (8 hours; Figure 1A) and T84 (12 hours) cells (Figure 1B) after inoculation (66.2% ± 11.1% [n = 12] and 49.7% ± 2.5% [n = 3], respectively; P < .05, compared with uninfected, untreated cell lines at time 0). Pretreatment of the basolateral compartment with vitamin D (10−7) for 16 hours before infection attenuated EHEC-induced reduction in TER in Caco2-bbe (94% ± 2.5%; n = 8) and T84 (102.5% ± 2.3%; n = 3). The protective effect of vitamin D was maintained for...
concentrations of $10^{-6}$–$10^{-11}$ M ($P < .05$) and disappeared at a concentration of $10^{-12}$ M (74.8% ± 1.4% [n = 3]; $P < .05$). At 24 hours, both infected cell lines treated with vitamin D ($10^{-12}$ M) demonstrated a significant reduction in TER, compared with uninfected, untreated cell lines (n = 3–12; $P < .05$). Interestingly, an attenuation in TER reduction was not observed with apical exposure to vitamin D (35.8% ± 1.5% [n = 3]; $P < .05$). The protective effect of vitamin D on Caco2-bbe monolayers was diminished at 16 hours after infection (22.5% ± 6.5% vs 11.6% ± 1.5%; n = 8).

1,25(OH)$_2$D$_3$ Attenuates E. coli O157:H7–Induced Changes in Paracellular Permeability
As shown in Figure 1C, samples from the basolateral compartment of E. coli O157:H7–infected monolayers incubated with FITC-conjugated dextran (10 kDa) showed a greater fluorescent signal intensity relative to uninfected monolayers (fluorometry relative integrated intensity [RI] vs untreated monolayers, 3.3 ± 1.04 [n = 5]; $P < .05$). Pretreatment of the basolateral compartment of polarized monolayers with vitamin D ($10^{-7}$ M) for 16 hours before pathogen challenge attenuated EHEC-induced increases in paracellular permeability (RI vs untreated monolayers, 0.96 ± 0.16; [n = 5]; $P > .05$). Treatment with vitamin D in the absence of infection did not alter paracellular permeability (RI, 1.34 ± 0.24 [n = 5]; $P > .05$, compared with controls).

1,25(OH)$_2$D$_3$ Prevents Pathogen-Induced Redistribution of ZO-1 and Claudin-1 but Does Not Alter Protein Expression
Uninfected Caco2-bbe cells demonstrated intact tight junctions in the absence or presence of vitamin D, as evidenced by continuous circumferential ZO-1 and claudin-1 staining (Figure 2A and 2B). In contrast, E. coli O157:H7–challenged monolayers demonstrated diffuse fragmentation of ZO-1 and claudin-1 staining (Figure 2C). Pretreatment of the basolateral compartment of
compartment with vitamin D \( (10^{-7} \text{M}) \) for 16 hours before infection attenuated the observed changes in TJ integrity (Figure 2D). Complementary qPCR demonstrated no significant difference in either ZO-1 or claudin-1 messenger RNA (mRNA) expression in infected monolayers regardless of pre-treatment with vitamin D (Figure 2E and 2F).

**Vitamin D Deficiency Affects Mice Serum 25(OH)D3 Levels and Weight Gain**

Serum 25(OH)D3 levels were significantly lower in vitamin D–deficient animals \( (22 \pm 5.8 \text{ ng/mL} \text{ vs } 71 \pm 3.1 \text{ ng/mL} \text{ [n = 8]; } P < .0001; \text{ Figure 3A}) \). Calcium levels were also significantly different between groups \( (9.3 \pm 0.98 \text{ mg/dL} \text{ vs } 14 \pm 1.17 \text{ mg/dL}, \text{ respectively [n = 5]; } P < .05; \text{ Figure 3B}) \). At week 5, mice fed a vitamin D–deficient diet gained significantly less weight \( (7.5 \pm 0.21 \text{ g vs } 8.95 \pm 0.26 \text{ g [n = 30]; } P < .0001; \text{ Figure 3C}) \). Body weights of vitamin D–deficient mice infected with \textit{C. rodentium} decreased significantly at 10 days after infection, compared with infected, vitamin D–sufficient mice \( (1.03 \pm 0.36 \text{ g vs } 0.02 \pm 0.18 \text{ g [n = 20]; } P < .05; \text{ Figure 3D}) \).

**Vitamin D Deficiency Exacerbates \textit{C. rodentium}–Induced Paracellular Permeability**

\textit{C. rodentium} infection in vitamin D–sufficient animals resulted in an increase in intestinal permeability to a 4-kDa dextran probe (Figure 4C) 10 days after infectious challenge (fold increase, \( 1.68 \pm 0.13 \text{ [n = 4–8 per group]; } P < .05 \)). Vitamin D deficiency resulted in an increased permeability in noninfected animals (fold increase, \( 1.48 \pm 0.17 \text{ [n = 4–8 per group]; } P < .05 \)) and significantly increased permeability after infection (fold increase, \( 2.89 \pm 0.19 \text{ [n = 4–8 per group]; } P < .05 \)).

**Vitamin D–Deficient Mice Have Elevated Levels of Proinflammatory and Antiinflammatory Cytokines**

Uninfected vitamin D–deficient mice expressed higher mRNA transcripts for both pro- and anti-inflammatory cytokines in colonic homogenates (Figure 5). A similar pattern was also observed in pathogen-infected mice, where levels of all cytokines

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**Figure 5.** Vitamin D–deficient mice have elevated colonic mucosa proinflammatory and anti-inflammatory cytokine levels. Quantitative polymerase chain reaction analysis of interleukin 10 (IL-10), interleukin 17α (IL-17α), tumor necrosis factor α (TNF-α), transforming growth factor β (TGF-β), interferon γ (IFN-γ), and β-actin (housekeeping gene) showed significant changes between vitamin D–deficient and vitamin D–sufficient diets. Vitamin D deficiency resulted in elevated levels of both proinflammatory and anti-inflammatory cytokines, regardless of infection status (*\( P < .05 \), **\( P < .01 \), Student t test; n = 5–10).
except IFN-γ were significantly increased in the setting of vitamin D deficiency (Figure 5).

**Vitamin D Deficiency Alters the Composition of the Intestinal Microbiome in the Absence and Presence of C. rodentium–Induced Colitis**

DGGE analysis showed shifts in the gut microbiota of noninjected mice following 5 weeks of either vitamin D–deficient or sufficient diet (Figure 6A).

qPCR confirmed that a vitamin D–deficient diet for 5 weeks resulted in a significant increase in the relative quantities of Bacteroidetes (P = .0005), Firmicutes (P < .0001), Actinobacteria (P < .0001), and Gammaproteobacteria (P = .0088), compared with a vitamin D–sufficient diet, while there was no change in Betaproteobacteria (Figure 6B). Following 10 days of infection with *C. rodentium*, there was a significant increase in the relative quantity of Gammaproteobacteria (P < .0001) and Actinobacteria (P < .0001) in vitamin D–deficient groups, compared with controls. There were no significant differences in Bacteroidetes, Firmicutes, and Betaproteobacteria quantities from infected, vitamin D–deficient mice (Figure 6C).

**DISCUSSION**

This study is the first to demonstrate that vitamin D deficiency predisposes to more-severe infectious colitis. The murine noninvasive bacterial enteropathogen *C. rodentium* provides a robust and reproducible in vivo model of infectious colitis and colonic inflammation [29]. Two recent studies using a chemical (dextran sodium sulfate)–induced model of colitis confirm the detrimental effects of vitamin D deficiency on disease severity in experimental colitis [12, 13]. These findings are consistent with studies using VDR gene–knockout mice [7–10].
Several mechanisms by which vitamin D exerts anti-inflammatory effects have been suggested [13]. Vitamin D deficiency is associated with elevated levels of bacteria in mouse colonic tissue, with abrogation of bacterial translocation and increased expression of intercellular TJ proteins in vitamin D treated mice challenged with DSS [12]. The active form of vitamin D, 1,25(OH)$_2$D$_3$, is important for both innate and adaptive arms of the host immune system [2]. Vitamin D directly modulates the T cell antigen receptor (TCR) via induction of phospholipase Cy1 expression [30], and down-regulates pro-inflammatory cytokines, including interleukin 1, interleukin 6, IL-17, and TNF-α, and the chemokine CXCL8 (interleukin 8) [2, 31, 32]. Vitamin D also induces the expression of the FoxP3 transcription factor characteristic of regulatory T cells [33]. Expression of antimicrobial peptides, such as cathelicidin, is regulated by vitamin D [34], while the VDR mediates inhibition of NF-κB signaling via stimulation of the pattern recognition receptor NOD2 [35]. On the basis of these observations, it is postulated that susceptibility to infectious and inflammatory diseases could be increased in humans with suboptimal levels of vitamin D.

In the present study, mice deficient in vitamin D were more susceptible to colonic inflammation and exhibited enhanced C. rodentium–induced colonic injury, as determined by weight loss and elevated levels of colonic epithelial crypt hyperplasia. Interestingly, vitamin D deficiency alone resulted in low-grade colonic inflammation, indicating an altered immune status induced by low levels of vitamin D. These findings were supported by increased levels of proinflammatory cytokines in both vitamin D–deficient study groups (without and with infection), compared with vitamin D–sufficient animals.

C. rodentium infection causes tight junction instability and increased colonic permeability to macromolecules [36]. The present study showed that an increase in intestinal paracellular permeability was further enhanced by vitamin D deficiency, indicating that vitamin D plays a key role in maintaining mucosal epithelial barrier integrity. Analogous to previous studies [19], evidence for these protective effects were further supported by in vitro findings, in which 1,25(OH)$_2$D$_3$ protected polarized epithelial cells against E. coli O157:H7–induced disruption of epithelial tight junction structure, including ZO-1 and claudin-1 distribution, resulted in decreased TER and increased permeability to macromolecules. Taken together, these findings indicate that vitamin D can directly modulate epithelial barrier function in a variety of ways. These observations are supported by Kong et al [10], who demonstrated that vitamin D deficiency compromises the mucosal barrier, leading to increased susceptibility to mucosal damage.

We are the first to demonstrate that preincubation of polarized epithelial cells with vitamin D before challenge with E. coli O157:H7 attenuates pathogen-induced alterations in epithelial barrier function and redistribution of the TJ proteins ZO-1 and claudin-1. Gene expression analysis of ZO-1 and claudin-1 did not change in infected monolayers pretreated with vitamin D. These findings are contrary to those of Zhao et al [12], who reported that vitamin D induced an increase in gene expression, but are consistent with their finding of attenuated redistribution of TJ proteins in DSS treated monolayers. It is known that compromised intestinal barrier function caused by mucosal ulceration reduces the number of TJ strands and alters TJ protein expression and distribution. In addition, increases in apoptotic events result in increased leakiness, which exposes the host to luminal pathogens and dietary antigens that can lead to mucosal inflammation [37]. Colonic biopsy specimens obtained from patients with Crohn disease [38] and ulcerative colitis [39] revealed disrupted TJ complexes. Proinflammatory cytokines also play a key role in the induction of barrier defects in IBD [40]. In the current study, increased levels of proinflammatory cytokine mRNA were present in colonic homogenates isolated from vitamin D–deficient mice.

Epithelial barrier dysfunction is also caused by alterations in the composition of the intestinal microbiota [41]. An altered gut microbiome appears to be an essential factor driving dysregulated immune responses characteristic of recurring and relapsing mucosal inflammation in IBD [42]. qPCR analysis of colonic samples from patients with ulcerative colitis reveal less bacterial diversity, with increased populations of Actinobacteria and Proteobacteria and decreased populations of Bacteroidetes, compared with healthy twins [43]. Similarly, patients with Crohn disease exhibit reduced microbial diversity associated with temporal instability of dominant bacterial species [44]. Interestingly, in our study an altered composition of the gut microbiome was identified in vitamin D–deficient infected mice, including an increase in the abundance of Actinobacteria and Gammaproteobacteria. This finding indicates a direct effect of vitamin D on the intestinal microbiota, as recently demonstrated by Ooi et al [14]. These findings contribute to further understanding of the mechanisms by which vitamin D deficiency increases intestinal susceptibility to injury.

Several previous studies address the temporal association between vitamin D deficiency and IBD. In some publications, the prevalence of vitamin D deficiency was higher in patients with IBD than in age-matched community controls [45], and vitamin D deficiency was associated with increased disease activity [46]. Our experimental findings imply that vitamin D supplementation could prove beneficial in the management of a subset of patients with IBD.

Notes

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References


