Trends in Endocrinology & Metabolism

CellPress
REVIEWS

Review

Aligning the Paradoxical Role of Vitamin D in Gastrointestinal Immunity

Margherita T. Cantorna,[1,2,*] Connie J. Rogers,[2,3] and Juhi Arora[1,2]

Inflammatory bowel disease (IBD) is a chronic disorder characterized by inflammation of the gastrointestinal tract and an immune-mediated attack against the commensal microbiota. Vitamin D is an essential vitamin that not only promotes calcium and phosphate absorption but also regulates immune function. The active form of vitamin D [1,25(OH)$_2$D] has been shown to suppress symptoms of IBD by inhibiting T cell responses. Host protection from gastrointestinal infection depends on T cells. Paradoxically, vitamin D deficiency increases susceptibility to IBD and gastrointestinal infection. Here we review the roles of vitamin D in immune cells using a kinetic model of the vitamin D-mediated effects on infection to explain the sometimes paradoxical effects of vitamin D on gastrointestinal immunity.

Highlights

In the gastrointestinal tract, vitamin D and 1,25D increase IL-10 production from macrophages, dendritic cells, and T cells.

Vitamin D-regulated IL-22 production in the gastrointestinal tract protects against infection and inflammation.

The effects of vitamin D in the immune system following infection depend on the sequential activation of first innate cells (macrophages) and then T cells.

Locally produced 1,25D inhibits T cells, IFN-γ, and IL-17 to resolve inflammation following infection.

Introduction

In the early 1980s, it was discovered that immune cells and other cells outside those important in bone or mineral metabolism also express the vitamin D receptor (VDR) [1,2]. Since this discovery, the role of vitamin D and the VDR in immune response regulation has been an active area of study. Early experiments added the active form of vitamin D [1,25(OH)$_2$D or 1,25D] to human peripheral blood mononuclear cells *in vitro* and showed that 1,25D suppressed T cell proliferation, IL-2, and IFN-γ production [1,2]. Later experiments in animal models of T cell-mediated autoimmunity demonstrated that 1,25D suppressed the development of experimental models of type 1 diabetes, multiple sclerosis and IBD [3–5]. The limitation of using 1,25D clinically to treat autoimmunity was hypercalcemia that developed at the therapeutic doses of 1,25D [6]. Interestingly, limiting dietary calcium reduced the suppressive capacity of 1,25D on T cell function or autoimmunity, indicating that the effects of 1,25D on T cells are calcium dependent [6].

Immune Cells Express the VDR and Produce 1,25D

Vitamin D from the diet or following UV-light exposure of skin is 25-hydroxylated by several enzymes found in the liver to produce 25(OH)D [7]. The kidney is the exclusive source of the 1alpha hydroxylase Cyp27B1 for endocrine production of 1,25D [8]. Endocrine regulation of 1,25D production is through several P450 enzymes (Box 1). The VDR is a nuclear receptor, which is part of the steroid/thyroid superfamily of nuclear receptors that regulate transcription [9]. 1,25D is the-high affinity VDR-binding ligand [8].

In addition to the endocrine control of 1,25D, cells in the immune system also produce 1,25D and expresses the VDR. Local regulation of the production of 1,25D is controlled by the expression of Cyp27B1 in extrarenal tissues, including cells of the immune system [10,11]. Induction of Cyp27B1 in immune cells requires the activation via toll-like receptors or cytokines in macrophages and T cell receptor stimulation in T cells [10–13]. Macrophages, dendritic cells, B cells, and T cells are all vitamin D targets since they express the VDR [14–16]. In addition to the systemic control of 1,25D, the immune system can both produce 1,25D and respond to 1,25D by expressing the VDR.

[1]Department of Veterinary and Biomedical Science, The Pennsylvania State University, University Park, PA 16802, USA
[2]Center for Molecular Immunology and Infectious Disease, The Pennsylvania State University, University Park, PA 16802, USA
[3]Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA 16802, USA

*Correspondence:
mxc69@psu.edu (M.T. Cantorna).

Box 1. Vitamin D Regulation of 1,25D

Endocrine regulation of 1,25D production is a critical regulator of calcium homeostasis. When dietary calcium is adequate, 1,25D increases calcium absorption from the small intestine. Prolonged hypocalcemia results in the production of 1,25D to signal the bones to release calcium [61]. Control of 1,25D production is regulated by a feedback loop that includes inhibition of Cyp27B1 that produces 1,25D from 25(OH)D and induction of Cyp24A1 that inactivates 25(OH)D and 1,25D [61,62]. Other signals that induce Cyp27B1 production include parathyroid hormone and hypocalcemia [61,62]. The production of 1,25D suppresses Cyp27B1 and induces Cyp24A1 [62]. Mutations in Cyp27B1 result in secondary hyperparathyroidism and the development of rickets, and mutations in Cyp24A1 result in hypercalcemia [62]. The availability of 1,25D is carefully controlled by a feedback loop that includes regulation of Cyp27B1 and Cyp24A1 that maintain control of serum calcium.

The Gastrointestinal Immune System

The immune system of the gastrointestinal tract serves as an important barrier between the host and the commensal microbes that colonize the gut. The intestine contains unique populations of immune cells with specialized functions that mount immune responses to pathogens but not to commensals. Epithelial cells form a single cell barrier between the microbiota and the lamina propria (LP) of the gut. Intraepithelial lymphocytes (IELs) are found interspersed between the epithelial cells. The IELs are mostly (80%) T cells, while the LP has T cells, B cells, and myeloid cells (Box 2). A T and B cell response is critical for host protection against gastrointestinal infection [17]. Infection of mice with an enteropathogenic *Escherichia coli*-like gastrointestinal infection (*Citrobacter rodentium*) revealed that IL-22 and IL-17 are critical for survival as well as clearance of the infection [18,19]. *C. rodentium* is a natural mouse pathogen, which models human gastrointestinal infections caused by the enteropathogenic *E. coli* [17]. Innate lymphoid cell (ILC) type 3 (ILC3) are able to produce IL-22 and IL-17 immediately following injury or infection [20]. Later (7–10 days) the acquired immune system is engaged and Th17 cells also produce IL-22 and IL-17 [19]. Conversely, in IBD (Box 3), IL-17 is associated with disease and the IL-17 comes from Th17 cells that are responding to the dysbiotic commensal microbiota [21]. IL-22 is a cytokine that results in mucosal healing and is therefore thought to be an important therapeutic target for IBD [22]. The production of IL-22 is protective against IBD and gastrointestinal

Box 2. The Gastrointestinal Immune System

The unique features of the gastrointestinal tract include a heterogeneous mix of cells that are either absent or functionally different than immune cells in peripheral tissues.

Epithelial Cells

- Provide a physical barrier between the host and the luminal contents [63].
- Produce tight junction proteins and secrete mucins and antimicrobial peptides [63].
- Express pattern recognition receptors (toll-like receptors) that identifies microbes [64].

IELs

- Most are T cells (80%) with very few B cells and myeloid cells [64,65].
- Most of the T cells express CD8αα, which identifies the T cells T regs. The T cells that express CD8αα can also express CD4 or CD8αβ [66].
- High frequency of γδ T cells and $CD8^+$ T cells.

Lamina Propria

- Dendritic cells and specialized $CD103^+$ dendritic cells. The expression of $CD103^+$ on dendritic cells reduces the ability of the dendritic cells to activate T cells [67].
- Conventional CD4 cells: Th1, Th2, and Th17 cells and conventional CD8 T cells.
- ILCs that produce IL-22 (ILC3) [20].
- T regs that recognize commensal microbes, $FoxP3^+$/$ROR\gamma t^+$ T reg [68].

Box 3. IBD: Crohn's Disease or Ulcerative Colitis (https://www.crohnscolitisfoundation.org/)

Crohn's Disease

- Affects the entire gastrointestinal tract from mouth to anus.
- Dysbiosis of the microbiota, diarrhea, and weight loss.
- Vitamin D deficiency is associated with more severe Crohn's disease and treatment escalation [69,70].
- Animal models of Crohn's disease, Th1 and Th17 cell-driven: IL-10 KO, T cell transfer to immunodeficient mice, acute dextran sodium-induced colitis, and trinitrobenzene sulfonic acid colitis [71].

Ulcerative Colitis

- Disease is limited to the colon.
- Dysbiosis of the microbiota, diarrhea, and weight loss.
- The role of vitamin D deficiency has not been well studied [69].
- Animal models of ulcerative colitis, mixed Th1 and Th2: chronic dextran sodium sulfate-induced colitis and oxazolone colitis [71].

infection, while Th17 cells are needed to clear infection but are pathogenic in IBD. Thus, constraining the IL-17 response following infection is necessary for the host to resolve inflammation and return to gastrointestinal homeostasis.

Vitamin D Regulation of Immunity

Vitamin D controls gastrointestinal homeostasis by regulating epithelial cells, innate immune cells, and acquired immune cells. Vitamin D status and expression of the VDR are important regulators of the epithelial barrier in the intestine [23]. VDR knockout (KO) mice exhibit increased permeability of the intestine, and expression of the VDR in the gastrointestinal epithelial cells only rescues epithelial integrity and protects the mice against experimental colitis [23–25]. The innate immune cells (macrophages, dendritic cells, and ILCs) are also important vitamin D targets. In dendritic cells and macrophages, 1,25D inhibits IL-12, toll-like receptor expression, and the ability of dendritic cells to activate T cells [26–28]. Conversely, 1,25D induces the production of IL-10 in dendritic cells and cathelicidin in macrophages [11,29,30]. Vitamin D-deficient mice have fewer ILC3 cells and produced significantly less IL-22 than vitamin D-sufficient mice [14]. In the acquired immune system and following activation, 1,25D inhibits B cells and T cells from proliferating [1,31]. In addition, 1,25D inhibits IL-2, IFN-γ, IL-17, and TNF-α production from T cells [31,32]. Conversely, 1,25D induces regulatory T cells that produce IL-10 and Th2 cells that produce IL-4 [31–33]. It was because of the inhibitory properties of 1,25D on T cells that the early research focused on the effects of vitamin D in diseases where T cells caused pathology [34,35]. In the gastrointestinal tract there is clear evidence of the immunosuppressive effects of 1,25D on experimentally induced IBD [36]. However, T cells and the production of IL-17 are also critical for an anti-infectious response to *C. rodentium* [37]. Paradoxically, however, vitamin D-deficient mice are more susceptible to experimental IBD and gastrointestinal infection with *C. rodentium* [14,36]. The recent data demonstrate that the mechanisms whereby vitamin D inhibits IBD are also critical for the effects of vitamin D in controlling gastrointestinal infection, and these ideas are the focus of this review.

Vitamin D and 1,25D Suppress T Cell-Mediated Disease

The inhibitory effects of 1,25D on T cells suggested that immune-mediated diseases such as multiple sclerosis and IBD could be affected by vitamin D status. 1,25D treatments suppress the development of experimental models of multiple sclerosis [experimental autoimmune encephalomyelitis (EAE)] and IBD [3–5,38]. In addition, vitamin D-deficient and VDR KO mice develop severe experimental IBD [39–41]. The transfer of VDR KO T cells into immunodeficient mice resulted in more severe IBD than the transfer of WT T cells [39]. T cell-specific VDR KO

exacerbates EAE and eliminates the capacity of 1,25D to suppress EAE [42]. Furthermore, 1,25D did not suppress EAE in mice without invariant natural killer T (iNKT) cells, suggesting that vitamin D can also directly regulate iNKT cells [43]. T cells are also indirectly regulated by 1,25D. Macrophages and dendritic cells have reduced abilities to stimulate T cells in the presence of 1,25D [27]. Furthermore, treating dendritic cells with 1,25D prior to transfer induced regulatory T cells (T regs) that suppressed allograft rejection in mice [27]. Vitamin D-deficient or VDR KO mice have fewer CD8αα-expressing T regs in the gastrointestinal mucosa, suggesting that vitamin D is important in the development or function of CD8αα T cells [44]. The mechanisms by which vitamin D regulates IBD include both the direct inhibition of T cells that produce IL-17 and IFN-γ and the induction of regulatory cells (T regs, CD8αα T cells, and iNKT cells) that produce IL-10 [36].

Vitamin D and Infection

The ability of vitamin D and 1,25D to inhibit Th1/Th17 cells and induce T regs should compromise the hosts' ability to fight infection. This hypothesis was tested in mice following a systemic fungal or viral infection [45]. Mice were treated with 1,25D or the T cell suppressor cyclosporin A and then infected with either a herpes virus or *Candida albicans* [45]. The cyclosporin A-treated mice exhibited reduced survival, while mice treated with 1,25D had no reduction in survival [45]. Vitamin D deficiency had no effect on *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, or *Pseudomonas aeruginosa* infections in mice [46,47]. However, *Listeria monocytogenes* and *Salmonella* infections were cleared more slowly in mice that could not respond to vitamin D (VDR KO) [48,49]. Some infections, like *L. monocytogenes* and *M. tuberculosis*, replicate inside macrophages and other infections, such as *Salmonella*, *S. pneumoniae*, and *P. aeruginosa*, are extracellular infections. The other possible difference between the effects of vitamin D on infection is the tissues being infected, with *L. monocytogenes* and *Salmonella* infecting the gastrointestinal tract and *M. tuberculosis* infecting the lung. The final issue that might affect the effect of vitamin D on infection is the suitability of the model and how well the model mirrors the human effects of 1,25D. The induction of cathelicidin by 1,25D that is important for inhibiting *M. tuberculosis* growth in human cultured macrophages is not regulated by 1,25D in the mouse [50]. Thus the effect of vitamin D on infection may be tissue, pathogen, or model specific.

Immune Cells Express the VDR and Produce 1,25D

Expression of the VDR depends on the tissue and cell type. In the kidney of vitamin D-sufficient hosts the VDR is expressed constitutively [51]. Calcium and 1,25D are *in vivo* positive regulators of the VDR in the kidney but not the intestine [51]. Tissue-specific regulation of the VDR by 1,25D has also been demonstrated by ChIP-seq analysis of VDR expression in the kidney, intestine, and bone [52]. All cells of the immune system that have been evaluated express the VDR. The signals that regulate VDR expression in immune cells are different than those in kidney, intestine, and bone. Cytokines, toll-like receptor ligands, T cell receptor stimulation, and nonspecific activation using PMA and ionomycin all increase VDR expression in immune cells [12,15,16]. Unlike the kidney that constitutively expresses high amounts of the VDR, the immune system maximally expresses the VDR 2–3 days after activation. T cell activation for 48–72 h upregulates the VDR in mouse and human $CD8^+$ and $CD4^+$ T cells [12,15,16]. Other immune cells including macrophages also require activation for maximal expression of the VDR [16]. The VDR is constitutively expressed in the kidney and inducible in immune cells.

Like the VDR, the level of 1,25D is tissue specific. In the kidney, the expression of 1alpha hydroxylase (Cyp27B1) is induced by hypocalcemia and the parathyroid hormone [13,53]. In a feedback loop, 1,25D inhibits Cyp27B1 expression in the kidney and induces Cyp24A1 to eliminate excess 1,25D (Box 1). The immune system can also be a source of locally produced 1,25D; however, the amount of Cyp27B1 made by immune cells is extremely low compared with the amount

produced in the kidney [11,13]. In addition, the signals that regulate the Cyp27B1 in immune cells are not the same as those that regulate renal Cyp27B1 [11,13]. Lipopolysaccharide (LPS) activation of human macrophages is required for induction of Cyp27B1 [11,54]. Conversely, LPS has no effect on renal production of Cyp27B1 [13]. *In vitro*, human macrophages produce 1,25D when activated with toll-like receptors (LPS) and cytokines [11,54]. Activated T cells are also a source of Cyp27B1 [13]. Immune cells produce 1,25D locally following 2–3 days of activation.

When the immune system is in homeostasis, the VDR and Cyp27B1 are expressed at low levels (i.e., absence of infection). In the first several days following activation, the immune system does not utilize or respond to vitamin D [31]. Early post-infection, the macrophages and innate cells that are activated immediately following infection are the first to produce 1,25D. Later the acquired T cells are activated 5–7 days post-infection and then 2–3 days after activation the VDR and Cyp27B1 gene are expressed in T cells. Therefore, it is not until 7–10 days post-infection that T cells express the VDR and are targets for 1,25D-mediated inhibition of proliferation, IL-17, and IFN-γ [36]. Within a few weeks of infection, the T and B cells clear the infection and antigen is eliminated. If the host is vitamin D deficient, they would still be able to eliminate the infection. Vitamin D-deficient mice cleared *C. rodentium* infection but with delayed kinetics compared with vitamin D-sufficient mice [14]. Conversely, in IBD the antigen cannot be eliminated and the T cells become chronically activated. In activated T cells, the availability of 1,25D would be essential to inhibit IL-17, IFN-γ, and T cell proliferation. In addition, 1,25D induces T regs that produce IL-10 [33,36]. These functions of 1,25D constrain the chronically activated Th1 and Th17 responses and therefore suppress IBD symptoms [55]. If the host is vitamin D deficient, the Th1 and Th17 cells remain chronically activated [36]. 1,25D is therefore critical for the induction of T regs that together with the direct effects of 1,25D on Th1/Th17 cells restrain the IFN-γ and IL-17 response and resolve inflammation.

Vitamin D and Host Resistance to Gastrointestinal Infection

Vitamin D has been shown to promote gastrointestinal homeostasis. Infections are often linked to autoimmunity and infections in the gastrointestinal tract contribute to the pathogenesis of IBD. At the peak of infection with *C. rodentium*, a strong Th17 response is observed, which is also characteristic of colitis in IBD [17]. 1,25D treatment of mice already infected with *C. rodentium* suppressed the IL-17 response in the colon, which increased the number of *C. rodentium* shed in the feces [56]. Paradoxically, vitamin D-deficient and VDR KO mice also developed more severe infections with *C. rodentium* [14,57]. Thus, it is unclear how vitamin D deficiency and 1,25D treatment both led to more severe infection with *C. rodentium*.

To date, we know that the innate immune system is required for early protection from *C. rodentium* and that mice without T and B cells use the innate immune system to control infection for 2 weeks but then develop a systemic infection that is fatal [17]. IL-22 KO mice develop an early and lethal infection within several days of *C. rodentium* infection and the IL-22 is produced by ILC3 [42]. Both T cells and B cells are important for protection from *C. rodentium* since mice without T cells (CD4 KO) or B cells (IgμM KO) also develop systemic infection [41]. IL-17 production by Th17 cells is required for clearance of *C. rodentium* [19]. ILC3 cells that produce IL-22, Th17 cells, and B cells are all critical for host defense against *C. rodentium*.

The effects of vitamin D status on the mucosal immune response to *C. rodentium* were recently examined. Vitamin D-deficient mice develop a severe infection with *C. rodentium* that resulted in the rapid mortality of the vitamin D-deficient mice [14]. IL-22 production was lower in vitamin D-deficient than vitamin D-sufficient mice [14]. Treating vitamin D-deficient mice with IL-22 completely protected them against infection, replacing the need for vitamin D early post-*C. rodentium* infection [14]. IL-17 mRNA expression was higher in the gastrointestinal tract of

C. rodentium-infected vitamin D-deficient mice and 1,25D suppressed IL-17 at the peak of *C. rodentium* infection [14,56]. However, vitamin D-deficient mice failed to expand the numbers of Th17 cells following *C. rodentium* infection [14]. Tissue-specific KO of the VDR in T cells (T-VDR KO) or in B cells (B-VDR KO) had no effect on the kinetics of *C. rodentium* clearance, which is consistent with a model where vitamin D in T and B cells is not required for the ability to eliminate an acute infection [14]. Vitamin D is required for early IL-22 production and the expansion of infection-induced Th17 cells that protect the gastrointestinal tract from *C. rodentium*.

Downregulating Immunity to Increase Resistance to Infection

The immune system is designed to eliminate invading pathogens without causing collateral damage to healthy tissue. Therefore, dampening immune responses post-infection is equally important as initiating the immune response to infection. The benefits of vitamin D in experimental models of *S. pneumoniae*, *P. aeruginosa*, *M. tuberculosis*, and malaria were shown to be via the reduction of the immunopathology from the infection and not more rapid elimination of the pathogen [46,47,58]. Vitamin D interventions enhanced the resolution of inflammation in patients infected with *M. tuberculosis* and 1,25D treatment of peripheral blood mononuclear cells from patients with pulmonary tuberculosis downregulated IFN-γ and cytotoxic cell mediators (perforin, granzyme-B, and granulysin) [59,60]. Based on the available data, we have developed a model to describe the effects of vitamin D in host resistance to gastrointestinal infection (Figure 1). The host mounts a robust innate immune response to infection (Figure 1). The macrophages and other

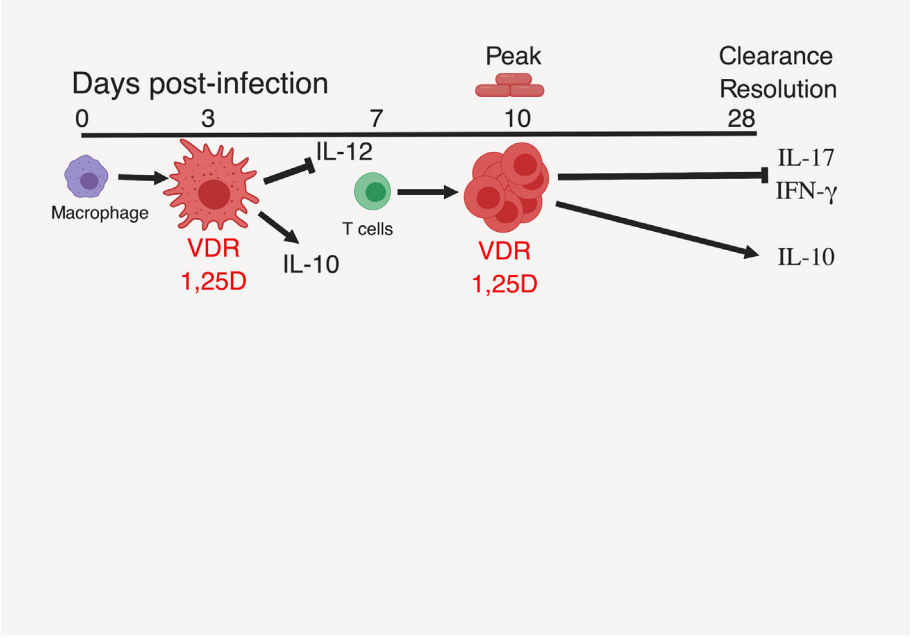

Figure 1. A Model for the Kinetics of the Immune-Mediated Effects of 1,25D Following Infection. The innate immune system responds first to an infection. Macrophages and other innate immune cells become activated by sensing the presence of the pathogen. The vitamin D receptor (VDR) and 1,25D are produced within 3 days of infection in macrophages and innate immune cells. At day 3 post-infection, the 1,25D regulates the activated macrophages to modify the ability to present antigen to T cells and to reduce IL-12 and induce IL-10. At day 7 post-infection, T cells begin to be activated and Th1 and Th17 cells proliferate rapidly. After 10 days of infection the activated T cells will begin to clear the infection and will express the VDR and produce 1,25D. As the infection is cleared, the 1,25D will decrease IL-17 and IFN-γ and induce regulatory cells to produce IL-10. The 1,25D-mediated inhibition of IL-17 and IFN-γ and the induction of IL-10 help to resolve the inflammation and prevent further damage.

innate immune cells express the VDR and produce 1,25D at 3 days post-infection (Figure 1). The 1,25D regulates the innate cells that present antigen to the T cells that expand following infection (Figure 1). At the peak of the infection, the T cells now express the VDR and can produce 1,25D (Figure 1). The ability of the immune system to both produce 1,25D and express the VDR requires activation and time (Figure 1). Since the innate immune system is activated prior to the T cells, macrophages and other innate cells are early targets of vitamin D following infection, while Th1 and Th17 cells are later targets of vitamin D following infection (Figure 1). In the absence of vitamin D, the damage from poorly controlled immune activation causes immune pathology while infections are cleared. The same mechanisms whereby vitamin D and 1,25D regulate Th1/Th17 cells in IBD are used to control the resolution of immunity following infection and to protect against immunopathology.

Concluding Remarks and Future Perspectives

Vitamin D is an important regulator of immunity and, in particular, critical for the downregulation of the immune system after infection. The immune response to infection requires rapid recognition of the infectious threat, elimination of the pathogen, and resolution of the immune response. There is strong evidence demonstrating that vitamin D and 1,25D inhibit Th1/Th17-mediated immunity directly, and indirectly by the induction of IL-10-producing T regs, which contribute to the resolution of an immune response. Vitamin D does not regulate innate immune responses before day 3 of infection and T cells before day 10 of infection, since immune cells do not express the VDR or produce 1,25D until activated. An important role of vitamin D in the anti-infectious immune response is to limit the inflammation and tissue injury. The same vitamin D-regulated mechanisms that are effective in the protection from IBD are in play and provide protection against gastrointestinal infection.

Outstanding Questions

What are the signals that regulate Cyp27B1 in immune cells *in vivo*?

What are the *in vivo* kinetics of the expression of the VDR in immune cells following infection?

Do the effects of vitamin D on host immunity depend on the tissue or the infectious agent causing the disease?

What are the effects of vitamin D and 1,25D on host resistance to chronic infection?

Acknowledgments

This work was supported by the National Institutes of Health (R01AT005378), the US Department of Agriculture (2914-38420-21822), and the US Department of Agriculture National Institute of Food and Agriculture/Hatch Appropriations (under Project #PEN04605, Accession #1018545).

References

1. Tsoukas, C.D. *et al.* (1984) 1,25-Dihydroxyvitamin D3: a novel immunoregulatory hormone. *Science* 224, 1438–1440
2. Rigby, W.F. *et al.* (1984) Inhibition of T lymphocyte mitogenesis by 1,25-dihydroxyvitamin D3 (calcitriol). *J. Clin. Invest.* 74, 1451–1455
3. Lemire, J.M. and Archer, D.C. (1991) 1,25-Dihydroxyvitamin D3 prevents the *in vivo* induction of murine experimental autoimmune encephalomyelitis. *J. Clin. Invest.* 87, 1103–1107
4. Cantorna, M.T. *et al.* (2000) 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. *J. Nutr.* 130, 2648–2652
5. Cantorna, M.T. *et al.* (1996) 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* 93, 7861–7864
6. Cantorna, M.T. *et al.* (1999) Dietary calcium is a major factor in 1,25-dihydroxycholecalciferol suppression of experimental autoimmune encephalomyelitis in mice. *J. Nutr.* 129, 1966–1971
7. Hoel, D.G. *et al.* (2016) The risks and benefits of sun exposure 2016. *Dermatoendocrinology* 8, e1248325
8. Prosser, D.E. and Jones, G. (2004) Enzymes involved in the activation and inactivation of vitamin D. *Trends Biochem. Sci.* 29, 664–673
9. Pike, J.W. (2011) Genome-wide principles of gene regulation by the vitamin D receptor and its activating ligand. *Mol. Cell. Endocrinol.* 347, 3–10
10. Hewison, M. *et al.* (2007) Extra-renal 25-hydroxyvitamin D3-1alpha-hydroxylase in human health and disease. *J. Steroid Biochem. Mol. Biol.* 103, 316–321
11. Liu, P.T. *et al.* (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 311, 1770–1773
12. Kongsbak, M. *et al.* (2014) Vitamin D up-regulates the vitamin D receptor by protecting it from proteasomal degradation in human $CD4^+$ T cells. *PLoS One* 9, e96695
13. Ooi, J.H. *et al.* (2014) Murine $CD8^+$ T cells but not macrophages express the vitamin D 1alpha-hydroxylase. *J. Nutr. Biochem.* 25, 58–65
14. Lin, Y.D. *et al.* (2019) Vitamin D is required for ILC3 derived IL-22 and protection from *Citrobacter rodentium* infection. *Front. Immunol.* 10, 1
15. Mahon, B.D. *et al.* (2003) The targets of vitamin D depend on the differentiation and activation status of CD4 positive T cells. *J. Cell. Biochem.* 89, 922–932
16. Veldman, C.M. *et al.* (2000) Expression of 1,25-dihydroxyvitamin D_3 receptor in the immune system. *Arch. Biochem. Biophys.* 374, 334–338
17. Mundy, R. *et al.* (2005) *Citrobacter rodentium* of mice and man. *Cell. Microbiol.* 7, 1697–1706
18. Zheng, Y. *et al.* (2008) Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat. Med.* 14, 282–289
19. Ivanov, I.I. *et al.* (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139, 485–498
20. Sonnenberg, G.F. and Artis, D. (2012) Innate lymphoid cell interactions with microbiota: implications for intestinal health and disease. *Immunity* 37, 601–610
21. Moschen, A.R. *et al.* (2019) IL-12, IL-23 and IL-17 in IBD: immunobiology and therapeutic targeting. *Nat. Rev. Gastroenterol. Hepatol.* 16, 185–196
22. Mizoguchi, A. *et al.* (2018) Clinical importance of IL-22 cascade in IBD. *J. Gastroenterol.* 53, 465–474
23. He, L. *et al.* (2018) Gut epithelial vitamin D receptor regulates microbiota-dependent mucosal inflammation by suppressing intestinal epithelial cell apoptosis. *Endocrinology* 159, 967–979

24. Ooi, J.H. *et al.* (2013) Vitamin D regulates the gut microbiome and protects mice from dextran sodium sulfate-induced colitis. *J. Nutr.* 143, 1679–1686
25. Liu, W. *et al.* (2013) Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. *J. Clin. Invest.* 123, 3983–3996
26. Sadeghi, K. *et al.* (2006) Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. *Eur. J. Immunol.* 36, 361–370
27. Griffin, M.D. *et al.* (2001) Dendritic cell modulation by 1α,25-dihydroxyvitamin D3 and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 98, 6800–6805
28. D'Ambrosio, D. *et al.* (1998) Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvement of NF-κB downregulation in transcriptional repression of the p40 gene. *J. Clin. Invest.* 101, 252–262
29. Verma, R. and Kim, J.Y. (2016) 1,25-Dihydroxyvitamin D3 facilitates M2 polarization and upregulates TLR10 expression on human microglial cells. *Neuroimmunomodulation* 23, 75–80
30. Korf, H. *et al.* (2012) 1,25-Dihydroxyvitamin D_3 curtails the inflammatory and T cell stimulatory capacity of macrophages through an IL-10-dependent mechanism. *Immunobiology* 217, 1292–1300
31. Cantorna, M.T. *et al.* (2015) Vitamin D and 1,25(OH)2D regulation of T cells. *Nutrients* 7, 3011–3021
32. Baeke, F. *et al.* (2010) Vitamin D: modulator of the immune system. *Curr. Opin. Pharmacol.* 10, 482–496
33. Mathieu, C. and Adorini, L. (2002) The coming of age of 1,25-dihydroxyvitamin D_3 analogs as immunomodulatory agents. *Trends Mol. Med.* 8, 174–179
34. Cantorna, M.T. (2000) Vitamin D and autoimmunity: is vitamin D status an environmental factor affecting autoimmune disease prevalence? *Proc. Soc. Exp. Biol. Med.* 223, 230–233
35. Cantorna, M.T. (2012) Vitamin D, multiple sclerosis and inflammatory bowel disease. *Arch. Biochem. Biophys.* 523, 103–106
36. Cantorna, M.T. *et al.* (2014) Vitamin D, immune regulation, the microbiota, and inflammatory bowel disease. *Exp. Biol. Med. (Maywood)* 239, 1524–1530
37. Valeri, M. and Raffatellu, M. (2016) Cytokines IL-17 and IL-22 in the host response to infection. *Pathog. Dis.* 74, ftw111
38. Zella, J.B. *et al.* (2003) Oral administration of 1,25-dihydroxyvitamin D3 completely protects NOD mice from insulin-dependent diabetes mellitus. *Arch. Biochem. Biophys.* 417, 77–80
39. Froicu, M. *et al.* (2003) A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. *Mol. Endocrinol.* 17, 2386–2392
40. Froicu, M. *et al.* (2006) Vitamin D receptor is required to control gastrointestinal immunity in IL-10 knockout mice. *Immunology* 117, 310–318
41. Froicu, M. and Cantorna, M.T. (2007) Vitamin D and the vitamin D receptor are critical for control of the innate immune response to colonic injury. *BMC Immunol.* 8, 5
42. Mayne, C.G. *et al.* (2011) 1,25-Dihydroxyvitamin D3 acts directly on the T lymphocyte vitamin D receptor to inhibit experimental autoimmune encephalomyelitis. *Eur. J. Immunol.* 41, 822–832
43. Waddell, A. *et al.* (2015) NKT cells can help mediate the protective effects of 1,25-dihydroxyvitamin D3 in experimental autoimmune encephalomyelitis in mice. *Int. Immunol.* 27, 237–244
44. Bruce, D. and Cantorna, M.T. (2011) Intrinsic requirement for the vitamin D receptor in the development of CD8αα-expressing T cells. *J. Immunol.* 186, 2819–2825
45. Cantorna, M.T. *et al.* (1998) 1,25-Dihydroxyvitamin D3 prolongs graft survival without compromising host resistance to infection or bone mineral density. *Transplantation* 66, 828–831
46. Reeme, A.E. and Robinson, R.T. (2016) Dietary vitamin D3 suppresses pulmonary immunopathology associated with late-stage tuberculosis in C3HeB/FeJ mice. *J. Immunol.* 196, 1293–1304
47. Niederstrasser, J. *et al.* (2016) Vitamin D deficiency does not result in a breach of host defense in murine models of pneumonia. *Infect. Immun.* 84, 3097–3104
48. Bruce, D. *et al.* (2009) Elevated non-specific immunity and normal *Listeria* clearance in young and old vitamin D receptor knockout mice. *Int. Immunol.* 21, 113–122
49. Wu, S. *et al.* (2010) Vitamin D receptor negatively regulates bacterial-stimulated NF-κB activity in intestine. *Am. J. Pathol.* 177, 686–697
50. Liu, P.T. and Modlin, R.L. (2008) Human macrophage host defense against *Mycobacterium tuberculosis*. *Curr. Opin. Immunol.* 20, 371–376
51. Healy, K.D. *et al.* (2003) Regulation of the murine renal vitamin D receptor by 1,25-dihydroxyvitamin D3 and calcium. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9733–9737
52. Lee, S.M. *et al.* (2018) The impact of VDR expression and regulation *in vivo*. *J. Steroid Biochem. Mol. Biol.* 177, 36–45
53. Brenza, H.L. and DeLuca, H.F. (2000) Regulation of 25-hydroxyvitamin D3 1alpha-hydroxylase gene expression by parathyroid hormone and 1,25-dihydroxyvitamin D3. *Arch. Biochem. Biophys.* 381, 143–152
54. Adams, J.S. *et al.* (2014) Regulation of the extrarenal CYP27B1-hydroxylase. *J. Steroid Biochem. Mol. Biol.* 144, 22–27
55. Cantorna, M.T. and Waddell, A. (2014) The vitamin D receptor turns off chronically activated T cells. *Ann. N. Y. Acad. Sci.* 1317, 70–75
56. Ryz, N.R. *et al.* (2012) Active vitamin D (1,25-dihydroxyvitamin D3) increases host susceptibility to *Citrobacter rodentium* by suppressing mucosal Th17 responses. *Am. J. Physiol. Gastrointest. Liver Physiol.* 303, G1299–G1311
57. Chen, J. *et al.* (2015) Dysbiosis caused by vitamin D receptor deficiency confers colonization resistance to *Citrobacter rodentium* through modulation of innate lymphoid cells. *Mucosal Immunol.* 8, 618–626
58. He, X. *et al.* (2014) Vitamin D inhibits the occurrence of experimental cerebral malaria in mice by suppressing the host inflammatory response. *J. Immunol.* 193, 1314–1323
59. Coussens, A.K. *et al.* (2012) Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. *Proc. Natl. Acad. Sci. U. S. A.* 109, 15449–15454
60. Afsal, K. *et al.* (2018) 1,25-Dihydroxyvitamin D3 downregulates cytotoxic effector response in pulmonary tuberculosis. *Int. Immunopharmacol.* 62, 251–260
61. Goltzman, D. *et al.* (2018) Physiology of the calcium–parathyroid hormone–vitamin D axis. *Front. Horm. Res.* 50, 1–13
62. Jones, G. *et al.* (2017) Genetic diseases of vitamin D metabolizing enzymes. *Endocrinol. Metab. Clin. N. Am.* 46, 1095–1117
63. Ahmad, R. *et al.* (2017) Gut permeability and mucosal inflammation: bad, good or context dependent. *Mucosal Immunol.* 10, 307–317
64. Eberl, G. (2005) Inducible lymphoid tissues in the adult gut: recapitulation of a fetal developmental pathway? *Nat. Rev. Immunol.* 5, 413–420
65. Mowat, A.M. (2003) Anatomical basis of tolerance and immunity to intestinal antigens. *Nat. Rev. Immunol.* 3, 331–341
66. Gangadharan, D. *et al.* (2006) Identification of pre- and postselection $TCR\alpha\beta^+$ intraepithelial lymphocyte precursors in the thymus. *Immunity* 25, 631–641
67. Johansson-Lindbom, B. and Agace, W.W. (2007) Generation of gut-homing T cells and their localization to the small intestinal mucosa. *Immunol. Rev.* 215, 226–242
68. Sefik, E. *et al.* (2015) Individual intestinal symbionts induce a distinct population of $ROR\gamma^+$ regulatory T cells. *Science* 349, 993–997
69. Cantorna, M.T. (2016) IBD: vitamin D and IBD: moving towards clinical trials. *Nat. Rev. Gastroenterol. Hepatol.* 13, 322–323
70. Kabbani, T.A. *et al.* (2016) Association of vitamin D Level with clinical status in inflammatory bowel disease: a 5-year longitudinal study. *Am. J. Gastroenterol.* 111, 712–719
71. Kiesler, P. *et al.* (2015) Experimental models of inflammatory bowel diseases. *Cell. Mol. Gastroenterol. Hepatol.* 1, 154–170