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Conflict of Interest
The authors declare no potential conflicts of
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Abbreviations
1,25D3, 1,25 dihydroxy vitamin D3; 25D3,
25-hydroxyvitamin D3; AMP, antimicrobial
protein; AMPK, adenosine monophosphate-
activated protein kinase; ATG5, autophagy
related 5; BECN1, beclin-1; CAMP, cathelicidin
AMP; CRAMP, cathelicidin-related

Vitamin D-Cathelicidin Axis: at the Crossroads between Protective Immunity and Pathological Inflammation during Infection

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ABSTRACT

Vitamin D signaling plays an essential role in innate defense against intracellular microorganisms via the generation of the antimicrobial protein cathelicidin. In addition to directly binding to and killing a range of pathogens, cathelicidin acts as a secondary messenger driving vitamin D-mediated inflammation during infection. Recent studies have elucidated the biological and clinical functions of cathelicidin in the context of vitamin D signaling. The vitamin D-cathelicidin axis is involved in the activation of autophagy, which enhances antimicrobial effects against diverse pathogens. Vitamin D studies have also revealed positive and negative regulatory effects of cathelicidin on inflammatory responses to pathogenic stimuli. Diverse innate and adaptive immune signals crosstalk with functional vitamin D receptor signals to enhance the role of cathelicidin action in cell-autonomous effector systems. In this review, we discuss recent findings that demonstrate how the vitamin D-cathelicidin pathway regulates autophagy machinery, protective immune defenses, and inflammation, and contributes to immune cooperation between innate and adaptive immunity. Understanding how the vitamin D-cathelicidin axis operates in the host response to infection will create opportunities for the development of new therapeutic approaches against a variety of infectious diseases.

Keywords: Vitamin D; Cathelicidin; Infections; Autophagy; Immunity; Inflammation

INTRODUCTION

Vitamin D signaling and metabolism are crucial in the regulation of bone and calcium homeostasis. However, various cells and tissues, regardless of calcium metabolism, express the vitamin D receptor (VDR) and can undergo functional VDR signaling, suggesting that

antimicrobial peptide; EP, E prostanoid; HBD2, human β-defensin 2; hCAP-18, human cationic AMP 18; IBD, inflammatory bowel disease; mCRAMP, murine CRAMP; MKP-1, MAPK phosphatase-1; Mtb, Mycobacterium tuberculosis; PBA, phenylbutyrate; pDC, plasmacytoid dendritic cell; PGE2, prostaglandin E2; ROS, reactive oxygen species; TB, tuberculosis; UV, ultraviolet light; VDR, vitamin D receptor

vitamin D plays a greater biological role than solely skeletal homeostasis (1,2). Accumulating data suggest that vitamin D and downstream receptor signaling play key roles in the ability of macrophages and other immune cells to enhance host antimicrobial defense and coordinate a variety of biological responses, including immune and inflammatory activity. The outcome of vitamin D-induced innate immune responses depends, at least partly, on the induction of gene sets encoding antimicrobial proteins (AMPs). Several AMPs are induced by vitamin D signaling, including cathelicidins, defensins, hepcidins, and neutrophil peptides, acting as principal intrinsic antibiotics given their direct antimicrobial activities against various pathogens (3). Since infections caused by drug-resistant pathogens are emerging worldwide, host defense peptides could be valuable candidates for the development of new therapeutic agents (4).

AMPs are usually cationic, binding to anionic regions (i.e., anionic lipids) of plasma membranes of various bacteria and viruses to form and eventually rupture pores (5). AMPs also function as key signaling molecules to fine-tune immune and inflammatory responses in diverse living organisms, including mammals, insects, and plants (6). Numerous previous studies have shown that vitamin D signaling leads to transcriptional activation of AMPs, including cathelicidins and defensins (7,8). Importantly, vitamin D-induced cathelicidin, the best-characterized AMP involved in VDR signaling, has multiple functions as an essential anti-infective agent against numerous pathogens, including intracellular *Mycobacterium tuberculosis* (Mtb), a major pathogen of human tuberculosis (TB) (7,8), and as a crucial immune modulator that impacts innate and adaptive immunity, chemotaxis, and angiogenesis (9).

In this review, we discuss the effects of the vitamin D-cathelicidin axis on antimicrobial host defense in a variety of infections caused by bacteria, viruses, and parasites, as examined in both *in vitro* and *in vivo* studies, and in clinical trials. Specifically, we review the current understanding of how VDR signaling is linked to the activation of AMP cathelicidin to modulate the autophagy machinery, cytokine/chemokine generation, and crosstalk with innate and adaptive immune responses during infection. We also speculate about the engagement of vitamin D signaling, cathelicidin, and host defense to eliminate invading pathogens without resulting in inflammatory-induced tissue injury.

OVERVIEW OF VITAMIN D METABOLISM AND SIGNALING

Vitamin D3 (hereafter mentioned as vitamin D) can be obtained from dietary intake or produced by ultraviolet light (UV)-mediated synthesis in the skin, in which UV rays promote the conversion of 7-dehydrocholesterol into vitamin D3 (10). The inactive vitamin D compound is then sequentially hydroxylated in the liver and kidney to form 25-hydroxyvitamin D3 (25D3) and active compound 1,25 dihydroxy vitamin D3 (1,25D3) by essential enzymes 25- and 1-α-hydroxylases, respectively (10). Circulating 1,25D3 levels are tightly regulated to prevent excessive VDR signaling and activity by a metabolic feedback loop involving the conversion by the inactivating enzyme 24-hydroxylase (CYP24A1) into inactive calcitroic acid, which is subsequently excreted in the bile. Immune cells also produce both activating (25- and 1α-hydroxylase) and metabolizing (24-hydroxylase) enzymes to regulate local levels of 1,25D3 in immune reaction sites (10). It is now clear that immune cells express 1-α-hydroxylase (CYP27B1), which allows them to activate functional VDR

signaling pathways through the conversion of inactive 25D3 into active 1,25D3 (10). Various innate stimuli including TLR2/1 (11-13), TLR8 (14), and a combination of IL-12 and IL-18 (15) trigger local activation of functional VDR signaling to enhance cell-autonomous host defense mechanisms, which are mediated through cathelicidin induction, the autophagy pathway, and the production of IFN-γ, in host cells against intracellular pathogens.

During infection, VDR signaling activation coordinates innate immune signals for the production of AMPs, including human cathelicidin AMP (CAMP) and β-defensin 2 (HBD2) by binding to vitamin D response elements in target genes (8,16). Although antimicrobial effects have been the major function ascribed to AMPs in VDR signaling, recent studies have established AMP as a central regulator of autophagy/xenophagy, generation of cytokines, chemokines and reactive oxygen species (ROS), as well as modulation of IFN signaling, thus serving as a signaling node to regulate immune pathways (7,8,17). A number of excellent reviews have covered the antibacterial and immune regulatory roles of AMPs; therefore, this review mainly focuses on recent studies that have identified the roles of endogenous AMPs induced by VDR signaling, and on the therapeutic implications of vitamin D-AMPs in host-directed therapy against infections. The effector pathways of vitamin D-driven innate immune signaling, which will be discussed in this review.

CATHELICIDINS AND VITAMIN D-INDUCED ANTIMICROBIAL RESPONSES

The antimicrobial activity of human cathelicidin LL-37/human cationic AMP 18 (hCAP-18) has been reported in a variety of gram-positive and gram-negative pathogenic bacteria, viruses, and fungi (18,19). Vitamin D and its active analog upregulate the expression of the cathelicidin LL-37 in a wide range of cell types, including keratinocytes, epithelial cells, and human monocytes/macrophages (11,20,21). Interestingly, murine cells reportedly not to induce CAMP mRNA expression (22), suggesting that vitamin D-mediated LL-37 induction is critically involved in the human defense system. Cathelicidin is an antimicrobial weapon that eliminates intracellular mycobacteria and also plays a regulatory role in various processes of the autophagy pathway to enhance the fusion of mycobacterial phagosomes with autophagosomes and autolysosomes (11,23). In addition to its antibacterial roles, LL-37 has a pleiotropic role in a variety of biological responses, as a key immunomodulator with both pro- and anti-inflammatory functions in different cells/tissues and microenvironments in a context-dependent manner (24). The generation and detailed functional significance of cathelicidins have been expertly reviewed (24-26); therefore, we will discuss recent findings of the roles and mechanisms of cathelicidins in the context of vitamin D signaling during infection.

Antimicrobial effects of cathelicidins in host cells

VDRs are found on monocytes/macrophages, which express and induce the 1α-hydroxylase isoenzyme (CYP27B1) to produce active 1,25D3, which promotes the adherence and differentiation of normal human monocytes (10). Innate immune signaling, e.g., TLR 2 activation, results in functional activation of vitamin D signaling to enhance CYP27B1-dependent synthesis of active vitamin D and enhancement of human cathelicidin (8,12). Cathelicidins are among the well-characterized classes of human AMPs and are usually produced as a prepropeptide to be processed for the generation of anti-microbial active fragments (27). The hCAP18 is the only member of human cathelicidins; LL-37 is the C-terminal, amphipathic, alpha-helical peptide generated by cleavage of the C-terminal end

of the hCAP18 protein by serine proteases and proteinase 3 (28). Due to its positive charge, LL-37 preferentially interacts with negatively charged bacterial membranes and forms pores via detergent-like effects (28). A recent study used super-resolution single-particle tracking tools to demonstrate LL-37 penetration and rigidification of bacterial cytoplasm through electrostatic linking of chromosomal DNA and a subset of ribosomes (29). Another recent study of LL-37 structure identified its core antimicrobial region for application in peptide designing of antimicrobial and antibiofilm agents (30).

Over the past 12 years, evidence has emerged for cathelicidin LL-37 as an integral regulator and effector of vitamin D-mediated anti-microbial responses in antibacterial immunity, particularly in mycobacterial infection (31). Synthetic LL-37 has shown a direct inhibitory effect on Mtb growth in broth and human macrophages (8,31). A study conducted by Liu et al. (8) showed that TLR2/1 triggering of vitamin D-mediated cathelicidin induction is crucial for anti-mycobacterial activity in human monocytes/macrophages. Numerous studies have demonstrated the beneficial effects of vitamin D-mediated cathelicidin in antimicrobial actions against Mtb infection (7,8,11). Importantly, the physiological levels of vitamin D3 contribute to the restriction of Mtb alone or Mtb/HIV replication in macrophages (11,32,33). In addition, a recent study showed that 25D3 treatment of human monocyte-derived macrophages, which are differentiated with IL-15, led to increased expression of both cathelicidin mRNA and protein, and upregulated the vitamin D-dependent antimicrobial response against intracellular *Mycobacterium leprae* (34). Besides mycobacterial infection, calcipotriol, a calcitriol-derivative, has been shown to inhibit the hepatitis C virus and robustly activate VDR target genes, including cathelicidin and hepcidin (35). Recent studies have shown that 1,25D3 directly activates the transcription of IL-1β, decreasing the mycobacterial burden in macrophages through IL-1β-driven epithelial production of the antimicrobial peptide DEFB4/HBD2 (36). This result suggests that, for a wide variety of infections, vitamin D-induced AMPs can serve as an antimicrobial immune effector to activate host defense.

LL-37 is internalized through an endocytotic process via the P2X7 receptor and traffics into lysosomes to enhance bactericidal activity against *Staphylococcus aureus* in human macrophages (37). Human cathelicidin is produced via vitamin D-mediated signaling and exhibits antimicrobial activity against intracellular pathogens through trafficking into endosomal/lysosomal compartments. Indeed, a previous study demonstrated that hCAP-18 is present in the phagolysosome with azurophil granule proteins, containing serine protease, which processes hCAP-18 to generate active AMP LL-37 (38).

Given the key roles of cathelicidin in the regulation of infection, it is not surprising that several pathogens have evolved strategies to inhibit LL-37 function (39). For example, human metapneumovirus, a major pathogen of respiratory tract infections in young children, markedly suppressed vitamin D-induced hCAP18 levels in human macrophages (40). A recent study also showed that the *Clostridium difficile* clnRAB operon binds directly to LL-37, thereby regulating the expression of a variety of gene sets involved in metabolism, cell signaling, and pathogenesis (41). These studies strongly suggest that microbial signals can modulate host responses through direct interaction with AMPs. These studies of vitamin D-cathelicidin during infection are summarized in **Table 1**. Further studies are required to clarify how pathogens modulate CAMP gene expression and/or degrade host cathelicidin proteins, to establish intracellular survival and replication during infection, and how they subvert vitamin D-mediated antimicrobial responses.

Table 1. *In vivo* and *in vitro* studies of vitamin D-cathelicidin during infection

Reagent	Disease/pathogen	Subject	Results	Reference
In vitro				
TLR2/1 ligand	Mtb	Human macrophage	TLR activation upregulates VDR and the vitaminD-1-hydroxylase genes Induction of the cathelicidin	(8)
Vitamin D	Mtb	Human monocyte and macrophage, THP-1 and RAW 264.7 cells	Upregulation of transcription of BECN1 and ATG5 through cathelicidin-dependent MAPK and C/EBPβ signaling Recruitment of cathelicidin to the autophagosomes through the Ca^{2+} and AMPK-dependent pathways	(11)
Vitamin D and its analog	Mtb, BCG	Human PBMC	Vitamin D inhibits the growth of mycobacteria through VDR signaling Up-regulated the cathelicidin hCAP-18gene	(31)
Vitamin D	Mtb, HIV	Human macrophage	Induction of autophagy and phagosomal maturation	(33)
Vitamin D, IL-15	*Mycobacterium leprae*	Human macrophage	Increased expression of cathelicidin	(34)
Vitamin D, calcipotriol	HCV	Human hepatoma cell lines, human macrophage	Induced local structure rearrangement of VDR	(35)
Vitamin D	Mtb	Murine macrophage, human macrophage, THP-1 cells	Enhanced IL-1β expression Epithelial IL1R1 signaling and DEFB4/HBD2	(36)
LL-37	*Staphylococcus aureus*	Human macrophage, THP-1 cells	Endocytotic process via P2X7 receptor Upregulation of ROS and lysosome formation	(37)
DrsG	*Streptococcus dysgalactiae* subsp. *equisimilis*	*In vitro* assay	Functions as a ligand of the cathelicidin LL-37 and inhibits the bactericidal activity of LL-37	(39)
Vitamin D	hMPV	Human macrophage	hMPV attenuates CAMP through C/EBPα	(40)
In vivo				
LL-37	*Clostridium difficile*	Syrian golden hamsters C57BL/6 mice	*C. difficile clnRAB* Operon is induced by LL-37, senses and binds to LL-37	(41)
-	Mtb	Patient sputum proteome	A shift of vitamin D binding protein-AMP axis in the lung of TB patients	(44)
-	Mtb	Ethiopian patient blood	Patients with extrapulmonary TB in local lymph nodes show higher 25(OH)D3 levels compared with pulmonary TB patients Plasma 25(OH)D3 levels correlate with local LL-37 expression in granulomatous lesions of lymph nodes from extrapulmonary TB	(45)
-	*Mycobacterium avium* complex	Patient serum	Serum hCAP18/LL-37 level and BMD are decreased in patients with MAC lung disease; No relation to serum vitamin D level.	(46)
-	Urinary tract infection	Infants and young children patient serum	Serum vitamin D levels negatively correlate with age and are significantly lower in girls; Vitamin D levels positively correlate with levels of cathelicidin but not with β-defensin-2	(47)
-	Cystic fibrosis	Children	No relationship between vitamin D in sera and HBD-2 or LL-37 in bronchoalveolar lavage No differences in infective or inflammatory markers between vitamin D-sufficient and deficient groups	(48)
Cathelicidin	*Plasmodium chabaudi* AS murine malaria	BALB/c mice	No curative effects by exogenous CAMP in infected mice	(49)
Vitamin D	*P. chabaudi* AS murine malaria	BALB/c mice	Vitamin D shows antimalarial activity in the acute phase of infection	(50)
Vitamin D	Influenza vaccine	Elderly person serum	No differences in cathelicidin level between vitamin D supplemented and untreated groups	(51)
Vitamin D	Nontypeable *Haemophilus influenzae*	C57BL/6JolaH mice	Vitamin D-deficient mice resolve infection and local lung inflammation faster than vitamin D-sufficient mice, possibly through a shift of protease/anti-protease balance and upregulation of CRAMP	(52)

Vitamin D-cathelicidin during infection *in vivo*

To date, most studies of LL-37 function have been conducted using *in vitro* systems, which have allowed us to begin to understand the impact of LL-37 as a biomarker related to vitamin D levels in various infectious diseases, including TB and HIV infection (42,43). A study applied sputum proteomic analysis to show that vitamin D binding protein was abundant, but cathelicidin lacked in sputum samples from active pulmonary TB patients (44). In Ethiopian patients with lymph node TB, plasma 25D3 levels were significantly correlated with local LL-37 expression in granulomatous lesions in disease sites (45). Another recent study showed that serum hCAP18/LL-37 levels were significantly depressed in patients with *Mycobacterium avium* complex lung diseases, although they were not related to serum vitamin D levels (46);

serum cathelicidin levels, which were correlated with vitamin D levels, were significantly decreased in young children and associated with urinary tract infection (47).

However, no relationship has been detected between vitamin D levels and LL-37 production in bronchoalveolar lavage fluids in children with cystic fibrosis, a genetic disorder (48). Similarly, cathelicidin may not play an important role in antimalarial effects, although vitamin D and its analogs display potent antiplasmodial activity (49,50). Vitamin D supplementation in deficient elderly persons with influenza vaccination was not found to increase serum cathelicidin levels, although vitamin D treatment drove lymphocyte polarization to the tolerogenic type (51). A recent study using an acute infection model with nontypeable *Haemophilus influenzae* showed a faster resolution of infection and lung inflammation in vitamin D-deficient mice, presumably due to cathelicidin-related antimicrobial peptide (CRAMP) upregulation (52). Together, these data suggest that cathelicidin production *in vivo* during infection is regulated by vitamin D-dependent and independent pathways, depending on the bacterial strain, cell types, and host immune status (**Table 1**).

THE VITAMIN D-CATHELICIDIN AXIS AND AUTOPHAGY REGULATION

Autophagy is an intracellular homeostatic process that affects diverse biological responses in the human body through quality control of cell functions. Autophagy plays an essential role in the activation of cell-autonomous immune defense against infectious agents (53). Since autophagy is an essential housekeeping function, its dysregulation is often associated with pathological conditions such as inflammation (53). Therefore, autophagy and vitamin D signaling have overlapping functions in the maintenance of homeostasis during infection and inflammation (23). In this section, we will focus on vitamin D-induced autophagy and its regulatory mechanisms, which are partly mediated by cathelicidin, in the context of infection (**Fig. 1**). The precise mechanisms by which vitamin D signaling activates autophagy and cathelicidin and how they interact with other signaling pathways/molecules remain to be elucidated.

Vitamin D-mediated autophagy in the antimicrobial effect of host cells
Upon pathogen challenge, vitamin D treatment triggers the autophagy pathway to enhance host antimicrobial responses and control excessive inflammation (11,23,54). The best examples of vitamin D-cathelicidin function in autophagy have been studied in host responses to Mtb infection. One strategy by which Mtb subverts host innate defense is the inhibition of LL-37 and ATG (beclin-1 [BECN1] and autophagy related 5 [ATG5]) expression in human macrophages (55). Previous studies have demonstrated the role of the vitamin D pathway in overcoming intracellular Mtb infection through autophagy activation and cathelicidin induction (56). Early studies identified a link between vitamin D autophagy function and Mtb clearance via LL-37 in human macrophages (11,57). The combined effects of retinoic acid and vitamin D3 have been reported to activate autophagy, to increase phagocytosis of Mtb and restrict intracellular Mtb growth in human monocytic tetrahydropalmatine (THP)-1 cells (58). It has also been reported that 4-phenylbutyrate (4-PBA) and vitamin D, separately or in combination, function as efficient inducers of autophagy through LL-37 expression and promote the co-localization of LL-37 with autophagosomes, thereby potentiating intracellular killing effects against Mtb (55). Importantly, either the cell-autonomous induction of LL-37 or exogenous synthetic LL-37 participates in vitamin
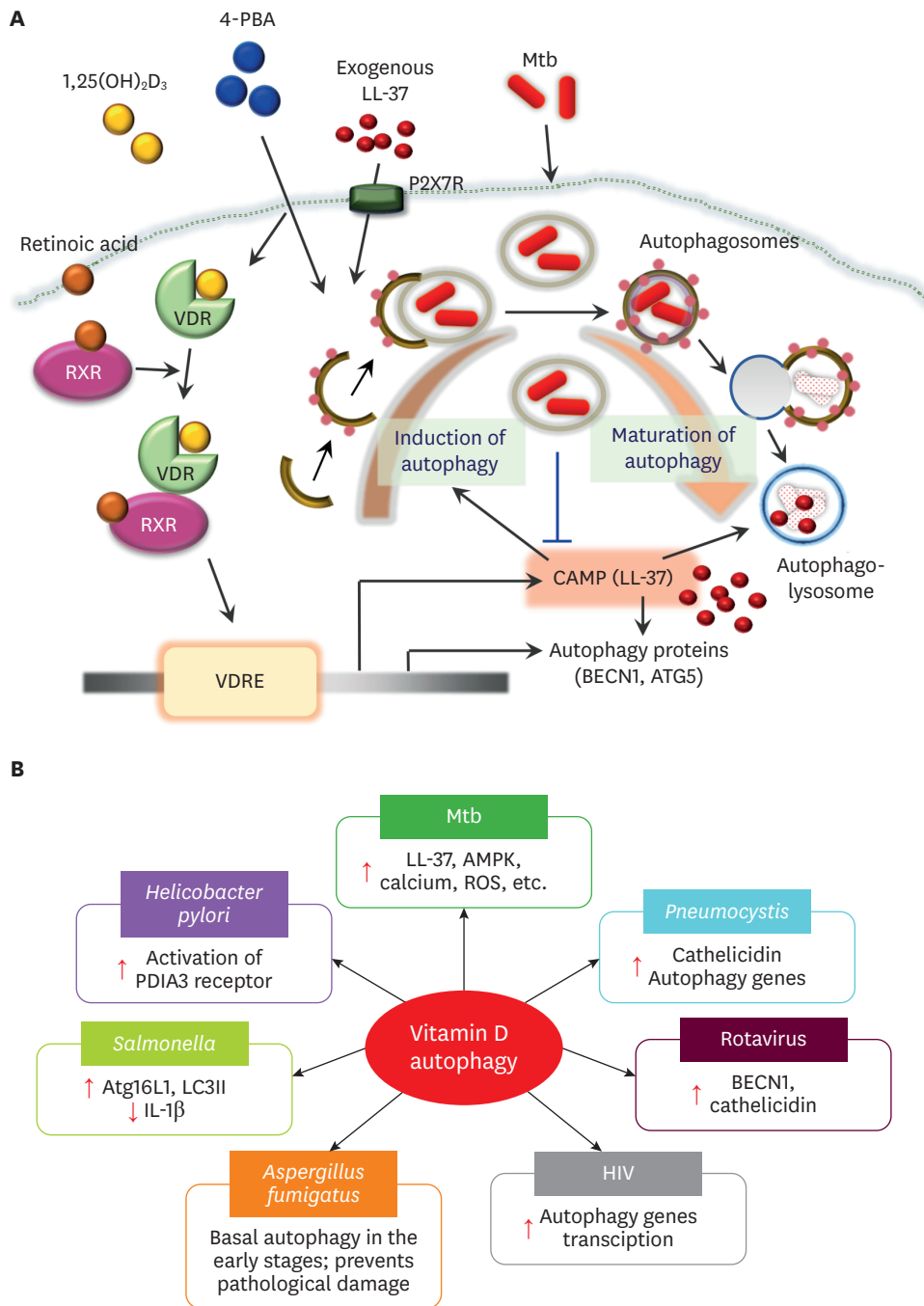
Figure 1. Vitamin D-mediated autophagy in antimicrobial host defense during infection. (A) Vitamin D-LL-37 functions in Mtb clearance through autophagy activation. Vitamin D3 treatment alone, or combined with retinoic acid or 4-PBA, or exogenous LL-37 via P2X7R, results in the activation of functional VDR signaling to trigger expression of cathelicidin, a secondary messenger for autophagy activation through transcriptional activation of ATGs and enhancement of autophagic flux, in human monocytes/macrophages. The activation of VDR-cathelicidin-mediated autophagy is beneficial for combatting intracellular Mtb infection. (B) Vitamin D-induced autophagy in various infections. Vitamin D signaling has been found to enhance autophagy in host cells during infection by Mtb, *Salmonella*, *Helicobacter*, *Aspergillus*, hepatitis C virus, HIV, or *Pneumocystis murina*. The known mechanisms involved in vitamin D-mediated autophagy are shown in the box of each pathogen.
VDRE, vitamin D response elements.

D-induced autophagy activation (11,55). The detailed mechanisms by which LL-37 mediates autophagy in the context of Mtb infection is discussed in the following session.

Several studies have explored the role of vitamin D-induced autophagy in various pathogenic infections. A recent study of *Helicobacter pylori* infection showed that vitamin D treatment improved lysosomal acidification and degradation through activation of the protein disulfide-isomerase A3 receptor, promoting autolysosomal function and antibacterial responses, even against antibiotic-resistant bacteria (54). Vitamin D signaling also enhances the expression of Atg16L1 and LC3II, as well as the abundance of LC3 punctae in intestinal epithelial cells during *Salmonella* infection (59). In this context, vitamin D-mediated Atg16L1 expression counteracts IL-1β expression (59), suggesting a role for vitamin D in the activation of autophagic clearance of bacteria and prevention of excessive harmful inflammation (59). Vitamin D-mediated autophagy also contributes to antifungal immunity. In studies of *Aspergillus fumigatus* infection models, whose pathology is related to excessive autophagy, vitamin D deficiency has been shown to cause defective pulmonary resistance to *A. fumigatus*. Mechanistically, vitamin D functions in the maintenance of basal autophagy in the early stages of infection and prevents pathological damage induced by excessive autophagy (60). Together, the findings of these studies suggest that vitamin D plays a pivotal role in controlling optimal and efficient levels of autophagy to improve host defense against diverse pathogens.

In addition to bacterial and fungal infection, vitamin D-mediated autophagy plays an important role in the regulation of antiviral host defense. In human macrophages, vitamin D treatment, even at physiologically relevant concentrations, can enhance autophagy to suppress the replication of HIV infection through transcriptional activation of autophagy genes (61). In patients with hepatitis C virus infection only or combined with hepatocellular carcinoma, vitamin D and VDR levels are depressed, and correlated with serum concentrations of LC3 and caspase-3, suggesting disturbance of autophagy and apoptosis function in these patients (62). It remains unclear whether vitamin D supplementation would recover autophagy activation in such patients. In rotavirus infection, vitamin D supplementation has been reported to upregulate autophagy gene BECN1 expression and autophagic flux, and increase porcine cathelicidin levels in virus-infected porcine intestinal epithelial cells (63). Since numerous viruses subvert, block, and even hijack host xenophagy systems (64), whether vitamin D-induced autophagy restricts or favors viral replication/survival in host cells may depend on the individual virus and host cell types, as well as different pathogenic mechanisms.

Vitamin D supplementation increases the efficacy of the drug primaquine in a murine infection model with *Pneumocystis murina*. Treatment with vitamin D enhances the number of phagocytes, decreases inflammatory cytokine production, and enhances cathelicidin/autophagy gene expression (65). These results show the beneficial effects of vitamin D in the enhancement of host defense and amelioration of excessive inflammation during treatment of *Pneumocystis* infection (65), although the regulation of cathelicidin in autophagy and inflammation was not addressed in this study. Given the low vitamin D levels observed in TB, HIV, and other infectious diseases (42,43), these findings provide new insights into potential approaches to developing vitamin D-based autophagy adjunctive therapy against various infections. However, it remains largely unknown which autophagic biomarker(s) might be useful in monitoring clinical stages and/or disease outcomes. Future studies are needed to identify useful autophagic status markers that are linked to clinical parameters and vitamin D levels in individual patients during infection.

Regulatory mechanisms involved in vitamin D-induced autophagy
Vitamin D signaling regulates autophagy at different steps: initiation, elongation, maturation, and degradation (23). Mechanistically, vitamin D/VDR signaling activates

autophagy through multiple pathways, including triggering intracellular calcium release/calcium-dependent kinases and inhibition of the mTOR, a negative regulator of autophagy (23,56). Notably, the lipoprotein LpqH, a mycobacterial TLR2/1 agonist, activates autophagy linked to functional VDR signaling and mediated by the adenosine monophosphate-activated protein kinase (AMPK) pathway (12). AMPK is an autophagy-activating kinase essential to various stages of the autophagy pathway and activation of lysosomal function through induction of transcription factor EB (66). The treatment of human macrophages with 4-PBA alone, or combined with vitamin D, has been shown to induce LL-37-mediated autophagy via the AMPK pathway to enhance the intracellular killing of Mtb (55). Since AMPK is a master regulator of anti-mycobacterial innate immune defense through multiple signaling pathways (67), the interplay between cathelicidin and AMPK signaling is likely to involve synergistic interaction through activation of several innate effector systems within phagocytic cells.

Redox status is crucial for the pathophysiology of numerous diseases, and strongly related to vitamin D-mediated signaling. For example, recent *ex vivo* analysis of redox potential in critically ill children showed that plasma LL-37 levels did not significantly differ from vitamin D levels; however, vitamin D sufficiency was closely associated with reduced oxidative stress in pediatric critical illness (68). Moreover, another epidemiological study found the relationship between serum 25D3 levels and plasma thiol/disulphide redox systems (69). The relevance of ROS functioning, as signaling molecules in autophagy regulation, has been suggested in the context of the vitamin D-cathelicidin axis during infection and inflammation; 1,25D3 leads to significant ROS production, which promotes autophagy (70) and antimicrobial activity (71). Indeed, TLR2-induced cathelicidin expression is mediated by ROS signaling in human monocytes/macrophages (72). Given the critical role played by ROS signals in autophagy and vice versa (73), future studies are needed to examine the function of the vitamin D-cathelicidin axis in redox homeostasis regulation and its ultimate link to autophagy activity.

Cathelicidin as a regulator of vitamin D-induced autophagy during infection

The role of the vitamin D-cathelicidin axis in antibacterial autophagy has been widely studied in mycobacterial infection. In human monocytes/macrophages, 1,25D3 treatment, through cathelicidin induction, robustly induces autophagosome formation, increases Mtb phagosomal maturation, and promotes antimicrobial responses against Mtb (11). Vitamin D treatment also enhances cathelicidin production and autophagic antimicrobial effects against *Mycobacterium marinum*, a pathogen causing skin lesions, in human THP-1 cells (74). Functional vitamin D signaling activation triggered by TLR2/1 leads to increased antimicrobial responses against Mtb infection through cathelicidin and autophagy activation (11-13,75). Vitamin D-mediated antimicrobial responses to coinfection by HIV and Mtb have been shown to be mediated by cathelicidin-dependent autophagy and enhancement of phagosomal maturation (32,33). Interestingly, TLR8 activation triggers gene expression for vitamin D signaling and human cathelicidin to the vitamin D-cathelicidin-mediated autophagy pathway, which is required for HIV restriction in human macrophages (14). A recent study showed that the combination of IL-12 and IL-18 enhances autophagy and anti-mycobacterial immune responses in human macrophages and pulmonary epithelial A549 cells. The protective host defense depends on the production of IFN-γ, VDR-derived antimicrobial peptide cathelicidin, and autophagy, but does not involve caspase-mediated apoptosis (15). Together, these findings suggest that numerous innate immune signals including TLR2/1, TLR8, and cytokines (IL-12+IL-18) are linked to a cell-autonomous defense mechanism, the CAMP/autophagy pathway, to promote antimicrobial action against Mtb in various human host cells.

Although it is not fully understood, the mechanisms by which cathelicidin regulates the induction of autophagy are mostly studied in mycobacterial infection, as summarized in **Fig. 2**. Vitamin D-induced autophagy activation is at least partly mediated by cathelicidin-mediated transcriptional activation of autophagy genes, including BECN1 and ATG5 (11). Similarly, a more recent study showed that the bioactive form of vitamin D enhanced the gene expression of ATG5, BECN1, and a mannose receptor in Mtb-infected monocytes/macrophages; these gene expression levels were lower in pulmonary TB patients (76). Importantly, a positive correlation has been reported between the expression of ATG5 and BECN1 and that of cathelicidin in monocytes/macrophages from healthy controls and TB patients (76). This result suggests that vitamin D-induced cathelicidin, as a secondary messenger, plays a role in transcriptional activation of autophagy genes, which are essential to all steps of the autophagy process (77). However, it remains an open question of how cathelicidin contributes to the transcriptional activation of autophagy genes in innate immune cells. LL-37 activates vitamin D and PBA-induced autophagy through an autocrine or paracrine pathway and that LL-37-mediated autophagy is associated with various intracellular signaling pathways, including the P2RX7 receptor, intracellular Ca^{2+} release, and the AMPK, and PI3K pathways (55). These findings suggest that cathelicidin-induced
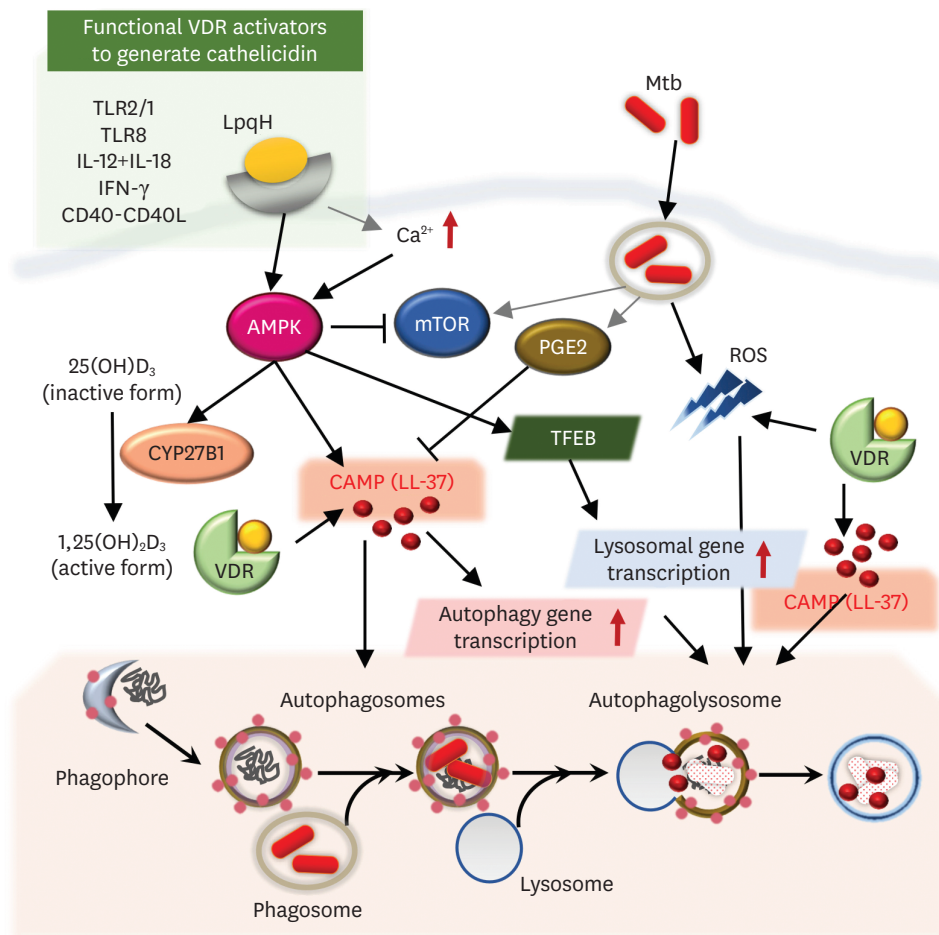

Figure 2. Regulatory mechanisms involved in vitamin D-induced autophagy. Vitamin D/VDR signaling activates autophagy through multiple pathways, including the triggering of intracellular calcium release/calcium-dependent kinases, the mTOR/AMPK pathway, ROS signaling, and cathelicidin. Cathelicidin, a key regulator of the vitamin D-autophagy pathway, is activated by functional VDR signaling downstream of TLR2/1, TLR8, CD40-CD40L, and cytokines, including IFN-γ and IL-12/IL-18. Mtb-derived PGE2 generation is known to inhibit LL-37 induction in human monocytes/macrophages. TFEB, transcription factor EB.

autophagy crosstalks with multiple intracellular signaling proteins and receptors, as well as other secondary messengers. Further studies are required to clarify the mechanism by which cathelicidin interacts with other transcription factors and/or regulates the signaling pathway, ultimately leading to the activation of autophagy at the transcriptional and post-translational levels.

To date, few mechanisms have been suggested for the regulation of vitamin D-cathelicidin signaling in host cells. A role for prostaglandin E_2 (PGE_2), a lipid mediator of inflammation and immunity, in vitamin D function has been identified (78). PGE_2 triggers the cyclic AMP/ protein kinase A pathway to induce inhibitory transcription factor signaling involving the E prostanoid (EP) 2 and EP4 receptors, thereby inhibiting cathelicidin expression and autophagy during Mtb infection. PGE_2-mediated impairment of cathelicidin expression and autophagy inhibition promotes intracellular Mtb growth in human macrophages (78). Most studies have indicated a link between cathelicidin in vitamin D-induced autophagy and antimicrobial responses. However, one study reported that the vitamin D-cathelicidin axis does not trigger autophagy against HIV infection, although vitamin D-mediated induction of autophagy was found to be beneficial in the inhibition of HIV-1 replication (61). Thus, the involvement of cathelicidin in vitamin D-mediated autophagy may depend on the type of pathogen; future studies should clarify this issue in relation to a more extended range of status, cell types, pathogens, and diseases.

VITAMIN D-CATHELICIDIN SIGNALING IN THE REGULATION OF INFLAMMATION

The vitamin D-cathelicidin axis and inflammation activation

The balance between protective immunity and inflammation is vital to control intracellular bacterial growth while minimizing immune-mediated tissue damage. Numerous studies have reported anti-inflammatory effects of vitamin D in a variety of human diseases, including inflammatory bowel disease (IBD) (79), diabetes (80), atherosclerosis (81), and autoimmune diseases (82,83). However, the function of cathelicidin in the context of vitamin D signaling remains to be characterized in the regulation of inflammation, with some studies suggesting that cathelicidin promotes inflammation in other diseases (24,84). In this section, we focus on recent findings on the role of vitamin D-cathelicidin axis in the regulation of inflammatory responses during infection and inflammation (**Fig. 3**).

Several studies have identified the potential mechanisms by which cathelicidin promotes inflammatory responses. Human cathelicidin LL-37 efficiently senses and delivers the CpG motifs of bacterial DNA to immune cells, regardless of its bactericidal properties (85), suggesting a potential avenue for the development of vaccines and anti-cancer therapies based on the ability of LL-37 to deliver CpG/TLR9 agonists. However, other studies suggested a pathogenic role for LL-37 in autoimmune and inflammatory diseases. In atherosclerotic plasma and plaques, the mtDNA-LL-37 complex is enhanced, not degraded, by DNase II, resulting in the activation of TLR9-induced inflammatory responses (86). Systemic lupus erythematosus patients have a complex of AMPs and self-DNA in their sera; these complexes can activate innate plasmacytoid dendritic cells (pDCs) through TLR9 (87). Combined with the strong implication of links between vitamin D signaling and both autoimmunity and cardiovascular diseases (81,88), future studies must clarify whether and how the LL-37—self-DNA immune complex can be degraded and resolved through vitamin D treatment. Another
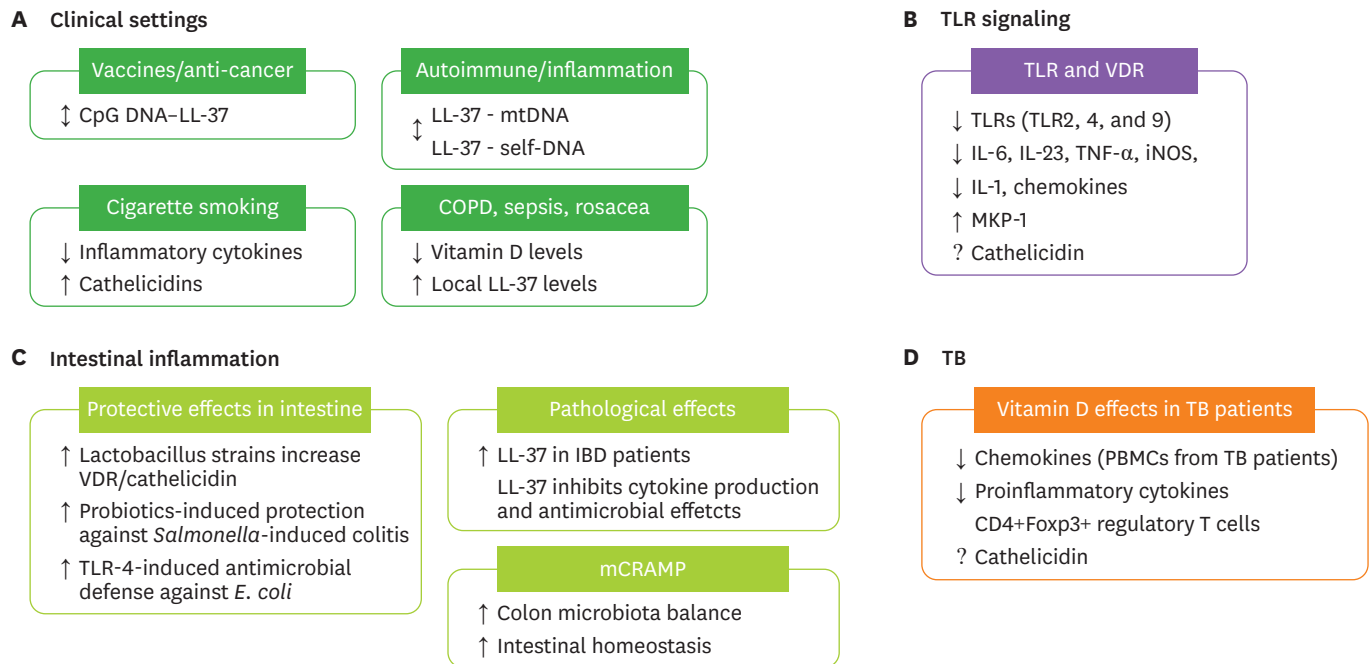
A Clinical settings

> **Vaccines/anti-cancer**
> ↕ CpG DNA–LL-37

> **Autoimmune/inflammation**
> ↕ LL-37 - mtDNA
> ↕ LL-37 - self-DNA

> **Cigarette smoking**
> ↓ Inflammatory cytokines
> ↑ Cathelicidins

> **COPD, sepsis, rosacea**
> ↓ Vitamin D levels
> ↑ Local LL-37 levels

B TLR signaling

> **TLR and VDR**
> ↓ TLRs (TLR2, 4, and 9)
> ↓ IL-6, IL-23, TNF-α, iNOS,
> ↓ IL-1, chemokines
> ↑ MKP-1
> ? Cathelicidin

C Intestinal inflammation

> **Protective effects in intestine**
> ↑ Lactobacillus strains increase VDR/cathelicidin
> ↑ Probiotics-induced protection against *Salmonella*-induced colitis
> ↑ TLR-4-induced antimicrobial defense against *E. coli*

> **Pathological effects**
> ↑ LL-37 in IBD patients
> LL-37 inhibits cytokine production and antimicrobial effetcts

> **mCRAMP**
> ↑ Colon microbiota balance
> ↑ Intestinal homeostasis

D TB

> **Vitamin D effects in TB patients**
> ↓ Chemokines (PBMCs from TB patients)
> ↓ Proinflammatory cytokines
> CD4+Foxp3+ regulatory T cells
> ? Cathelicidin

Figure 3. Vitamin D-cathelicidin signaling in the regulation of inflammation. (A) In clinical settings, LL-37 and the DNA complex play dual roles as potential candidates as vaccines/anti-cancer agents and in pathologic inflammation and pathogenesis of various inflammatory diseases, including chronic obstructive pulmonary disease, sepsis, and rosacea (see text, section IV). In cigarette smoking models, vitamin D treatment inhibits inflammatory cytokine production and increases cathelicidin levels. (B) VDR signaling inhibits the expression of TLRs and proinflammatory cytokines/chemokines in monocytes/macrophages and can activate MKP-1 signaling to suppress inflammatory responses. (C) VDR-cathelicidin maintains intestinal homeostasis. Lactobacillus strains increase VDR protein and cathelicidin expression. Probiotic treatment confers protective effects against *Salmonella*-induced colitis. Cathelicidin also elevates TLR4 response and protein expression and promotes antimicrobial defenses against *E. coli*. Murine cathelicidin is involved in colon microbiota balance and protective effects during experimental colitis. (D) In TB patients, vitamin D inhibits chemokines and inflammatory cytokines and increases Treg abundance. Cathelicidin levels remain to be characterized in a clinical setting (↕: dual role; ↑: increase/induce; ↓: decrease/inhibit).
mtDNA, mitochondrial DNA.

issue that may regulate pathogenesis is cell type-specific expression of pattern recognition receptors such as TLR9 and downstream immune functions for different cell types in the local environment, i.e., pDCs vs. inflammatory macrophages.

An immune alarm function for cathelicidin in the upregulation of proapoptosis and inflammasome activation during bacterial infection to enhance host protective immunity has been suggested (89,90). During Mtb infection, vitamin D functions in the general boosting of cytokine and chemokine levels, particularly via the induction of IL-1β, which controls the intracellular Mtb burden in macrophages through DEFB4/HBD2 production (36). The protective role of IL-1 in anti-mycobacterial responses has been reported in TB mouse models, suggesting a function for IL-1/IL-1R as a critical regulator of host defense (91). In patients with chronic obstructive pulmonary disease (COPD; n=215), high sputum hCAP18/LL-37 levels were associated with the risk of acute COPD exacerbation, suggesting that the local generation of hCAP18 is associated with airway inflammation (92). Sepsis patients have been reported to have lower vitamin D levels (93,94), but increased serum cathelicidin levels, which may be involved in the pathogenesis of systemic inflammation (93). Notably, vitamin D administration has been shown to reduce alveolar inflammation and cell damage in a sepsis model (94); however, this study did not address whether cathelicidin levels are modulated by vitamin D treatment. Several reports have indicated that high cathelicidin levels, but low vitamin D status, in chronic inflammatory diseases such as rosacea (95) represent the involvement of excessive cathelicidin in inflammatory disease pathogenesis. These reports

suggest the involvement of an altered vitamin D-cathelicidin axis in the pathogenesis of a variety of acute and chronic inflammatory diseases.

In contrast, several studies have reported a role for inflammatory cytokines in vitamin D-mediated antibacterial activity. For example, TNF-α/IL-1β treatment of primary bronchial epithelial cells inhibited vitamin D-induced hCAP18/LL-37 expression and killing effects against *H. influenzae*; interestingly, TNF-α/IL-1β treatment led to increased expression of CYP24A1 (a vitamin D-degrading enzyme), suggesting that chronic inflammation results in the impairment of vitamin D-mediated protective responses (96). Thus, in pathological settings with altered regulation of the vitamin D-cathelicidin axis, cathelicidin initiates cell-type-specific inflammatory damage that results in inappropriate inflammation, which may, in turn, affect vitamin D-mediated innate effector responses.

The inhibitory role of vitamin D in inflammation

Since vitamin D is a well-known immunomodulatory agent, the inhibitory role of 1,25D3 in inflammation has been widely studied in innate immune responses. In TLR signaling, vitamin D can decrease the expression of various TLRs (TLR2, 4, and 9), and inhibit the production of proinflammatory cytokines, including IL-6, IL-23, TNF-α, inducible nitric oxide synthase, IL-1, and various T cell-recruiting chemokines in monocytes/macrophages (97). It has been demonstrated that vitamin D inhibits LPS-induced cytokine production through the activation of MAPK phosphatase-1 (MKP-1) signaling in monocytes and macrophages (98). The expression of a variety of chemokines has been shown to be downregulated by 1,25D3 in PBMCs from pulmonary TB patients (99). Recent studies have shown that 1,25D3 treatment of PBMCs from TB patients and healthy controls has an anti-inflammatory effect through the inhibition of proinflammatory cytokines and chemokines (100,101). Vitamin D3 treatment of PBMCs has also been shown to significantly upregulate CD4+Foxp3+ Tregs (101). However, the role of cathelicidin in vitamin D-induced inhibitory function in inflammatory responses remains unclear. In a previous study using a cigarette smoke model, vitamin D treatment was found to reduce inflammatory cytokine levels, but enhance cathelicidin expression (102), suggesting that vitamin D plays dual roles in airways, attenuating airway inflammation, and the promotion antibacterial host defense.

Importantly, vitamin D administration in human TB patients resulted in a rapid drop in inflammatory cytokine and chemokine generation during antibiotic treatment, suggesting a vitamin D-mediated resolution effect on hazardous inflammation (103). A recent meta-analysis showed that adjunctive vitamin D accelerates sputum culture conversion in multidrug-resistant TB patients however; it did not influence drug-sensitive TB (104). This finding suggests that vitamin D-adjunctive therapy can improve clinical outcomes by controlling harmful inflammation and tissue damage during infection or treatment. However, there is little evidence that cathelicidin has a general function in vitamin D-mediated inhibition of inflammation, except in intestinal inflammation, which is discussed in the next section.

Vitamin D and cathelicidin in the regulation of intestinal inflammation

A large body of evidence shows that VDR function is connected to cathelicidin in intestinal homeostasis, barrier function, and gut inflammation regulation. Studies using mouse IBD models have found that AMP production via VDR signaling is responsible for modifying the intestinal microbiota and enhancing bacterial clearance at barrier sites in addition to immune cells (17). For example, the treatment of intestinal epithelial cells with probiotic

Lactobacillus rhamnosus strain GG and *Lactobacillus plantarum* resulted in increased VDR protein and cathelicidin expression. Probiotic treatment also confers a protective effect against *Salmonella*-induced colitis depending on VDR signaling and enhances the abundance of Paneth cells to increase AMPs (105). Regardless of VDR signaling, several studies have also shown beneficial roles for cathelicidin in the maintenance of intestinal homeostasis. In intestinal epithelial cells, cathelicidin amplifies TLR4 responses and protein expression and promotes epithelial antimicrobial defenses against *Escherichia coli* (106). Also, another recent study showed that LL-37 mRNA expression was significantly increased in mucosal tissues of subjects with ulcerative colitis and Crohn's disease and that LL-37 had antimicrobial effects interacting with LPS (107).

Murine CRAMP (mCRAMP) is induced by double-stranded RNA in intestinal epithelial cells, and intra-rectal administration of double-stranded RNA suppresses intestinal bacterial load and inflammation during *Shigella* infection through the induction of mCRAMP (108). Using CRAMP-deficient mouse models, it was recently reported that CRAMP plays an important role in the balance of colon microbiota and maintenance of intestinal mucosal homeostasis (109). However, it remains unknown whether and how vitamin D administration has any beneficial effect on cathelicidin-mediated protective responses against intestinal inflammation. Future studies are required to clarify the roles and mechanisms by which cathelicidin regulates host-pathogen interaction during intestinal homeostasis in the context of VDR signaling.

The vitamin D-cathelicidin axis: a key link between innate and adaptive immunity

Accumulating data suggest the function of vitamin D in adaptive immunity regulation in connection with innate immunity, in terms of mycobacterial infection. Fabri et al. (13) showed that IFN-γ-mediated antimicrobial responses in human macrophages were mediated by vitamin D, suggesting a role for vitamin D in acquired immunity against Mtb infection; notably, 25D3 treatment of human macrophages upregulated IFN-γ-induced antimicrobial responses, antimicrobial peptide expression, autophagy, and phagosome-lysosome fusion (13). Previous studies have also shown that pulmonary TB patients with vitamin D deficiency had depressed LL-37 levels in granulomatous lesions, but increased levels of IgG-secreting B cells and Tregs in TB lesions (110). Although these findings suggest that vitamin D-deficient TB patients have weak antibacterial responses as well as immunosuppression at disease sites (110), little is known about the molecular link between the vitamin D-cathelicidin pathway and Treg function in TB patients. A recent study showed that glucocorticoid-mediated cathelicidin induction is independent of the vitamin D signaling pathway, and failed to enhance antimicrobial responses against intracellular BCG mycobacteria. IFN-γ enhances, but glucocorticoid treatment decreases, lysosomal acidification of BCG via the expression of TCIRG1, a vacuolar H(+)-ATPase a3 subunit (111). Together, these findings indicate that cathelicidin production may be insufficient for host defense, requiring another key signaling of lysosomal acidification, presumably combined with IFN-γ, to augment anti-mycobacterial activity.

To support this hypothesis, a recent study showed that co-treatment with IFN-γ and IL-17A upregulated autophagy and autophagosome formation in primary monocytes from healthy controls and high-response TB patients; this effect was found to be related to anti-mycobacterial responses in human macrophages (112). However, a low-response group of TB patients exhibited a depressed autophagic response by monocytes in response to exogenous IL-17A (112). In contrast, cytokine IL-4 inhibited TLR2/1-induced, vitamin D-mediated

antimicrobial responses in human monocytes (113). IL-4 alone, or combined with the TLR2/1 ligand, induced catabolism of 25D3 through vitamin D-24-hydroxylase gene expression (113). Future studies should explore the reasons for intrinsic autophagy response defects among certain populations of TB patients.

In addition to Mtb infection, IFN-γ treatment of human Langerhans cells, specialized dendritic cells in the skin, upregulates cathelicidin production and autophagy activation and promotes the fusion of *M. leprae* phagosomes with lysosomes. Importantly, the frequency of Langerhans cells that contain both cathelicidin and autophagic vesicles is higher in self-healing lesions than in progressive lesions. In addition, IFN-γ-induced autophagy promotes the ability of Langerhans cells to present Ags to T cells (114). A combination of CD40–CD40 ligand signaling and IFN-γ induced gene expression in CYP27b1-hydroxylase, which is essential for functional VDR signaling activation through the conversion of 25D3 to active 1,25D3, in human monocytes (115). The activation of functional VDR signaling upregulated cathelicidin and DEFB4 expression and activated autophagy in human monocytes (115). These findings suggest that vitamin D signaling contributes to a link between innate host defense and adaptive immune responses via T cell-mediated mechanisms involving CD40L and IFN-γ (13,115).

CLINICAL TRIALS OF VITAMIN D-CATHELICIDIN AXIS IN INFECTIOUS DISEASES

It is generally thought that inadequate levels of vitamin D are linked to an increased susceptibility to infectious diseases (2,116), yet clinical trials with to prevent or treat infectious diseases by vitamin D supplementation have yielded mixed results. So far, there have been many clinical studies of vitamin D supplements on human infectious diseases such as sepsis, viral infection, TB, pneumonia, peritonitis. Among the clinical studies with vitamin D treatment, several studies for patients with sepsis and HIV infection reported that the levels of cathelicidin correlate with the clinical responses and the cathelicidin levels after supplementing of vitamin D (117-119). In addition, several other studies on TB, bronchiectasis, and acute respiratory infection reported the serum cathelicidin levels for monitoring anti-infectious effects after supplementation of vitamin D (120-122). It was noted that vitamin D significantly increased the serum cathelicidin levels, which correlate with the improved clinical outcome of infectious diseases (121,122). These data provide strong evidence that vitamin D supplementation contributes to beneficial roles in human infectious diseases through the regulation of cathelicidin production. Studies showed that vitamin D supplementation in children with vitamin D deficiency markedly decreased the incidences of influenza A and acute respiratory tract infection (123,124). The most beneficial effect was observed in individuals with the lowest serum 25D3 levels, suggesting the need of new studies focused in this clinical group.

TB is the most widely studied infectious disease associated with vitamin D treatment; however, there are still debates in the effectiveness of vitamin D therapy. In patients with low baseline serum vitamin D levels, supplementation of high doses of vitamin D accelerated clinical and radiologic improvements as well as the increased immune activation (125,126). These studies indicate that the measurement of vitamin D and cathelicidin levels may be helpful for screening and selection of appropriate patients for vitamin D-based, host-directed therapy against TB. Decreased vitamin D levels are often associated with increased risk of

TB in HIV-positive patients (127), suggesting that vitamin D may play an important role in the case of immune-compromised state such as HIV co-infection. Vitamin D levels have been shown to be depressed in bronchoalveolar fluids from HIV-positive individuals (128). Combined with the finding that vitamin D treatment restores innate response in HIV-positive macrophages impaired by Mtb infection (128), vitamin D supplementation may help to restore defective innate effector functions at the physiological level. These findings support the potential application of vitamin D as adjunctive therapy for TB infection in HIV-positive patients through boosting antimicrobial peptide cathelicidin LL-37 production.

Table 2 summarizes the clinical trials of vitamin D supplementation with or without measuring cathelicidin levels in patients with several infectious diseases, including TB, sepsis, respiratory infections, etc. In some studies, detailed results/outcomes are still pending. Depending on the disease categories and patient criteria, vitamin D treatment can be either beneficial or non-efficacious. Nevertheless, the arsenal of vitamin D-based therapy could potentially offer favorable immunomodulation as host-directed therapy for various infectious diseases, particularly TB. Additionally, vitamin D response during infection remains to be monitored and interpreted in terms of the levels of antimicrobial peptide cathelicidin, which may predict the response of vitamin D treatment. Therefore, innovative clinical trials should be proposed or designed with regard to the subgroup selection of patients and functional monitoring of immune and autophagic systems based on cathelicidin levels.

CONCLUSION

In this review, we highlight the roles of vitamin D-cathelicidin signaling in cell-autonomous protection, autophagy regulation, and immune response modulation in host cells against various pathogen infections and inflammatory diseases. Functional VDR signaling activation, which is associated with cathelicidin induction, is clearly the best-studied area in the field of vitamin D-mediated antimicrobial responses to eradicate a broad spectrum of pathogens. Cathelicidin is involved in shaping our immune system to promote cell-autonomous defense mechanisms, simultaneously maintaining a balance with inflammation during infection. Recent studies have revealed key roles for vitamin D-induced cathelicidin in the maintenance of homeostasis as an autophagy process that is closely regulated by other intracellular signaling pathways, including calcium and AMPK. Indeed, vitamin D-cathelicidin interacts with other effector systems such as autophagy and lysosomal function, possibly converging into a dedicated protective role by preventing excessive immune pathology against a variety of infections. Nevertheless, several aspects in this field are only beginning to be understood; many open questions remain, including how the vitamin D-cathelicidin axis interconnects with key innate effector systems (e.g., autophagy and lysosomal acidification) and which factors are critically involved in the coordinated control of innate and adaptive immunity to promote protective immune responses during various infections. Clearly, further research is needed to manipulate vitamin D-cathelicidin signaling in different biological contexts. Topics for further study include the exact function of vitamin D-antimicrobial immunity in patients with various infectious diseases at different clinical stages, considering genetic variation in VDR genes and sera vitamin D levels.

Despite continuing efforts to develop new antibiotics, multidrug-resistant infections are emerging as a major health burden worldwide, with high associated morbidity and mortality. The activation of autophagy by VDR signaling is anticipated as a promising host-directed

Table 2. Clinical studies of vitamin D-cathelicidin in infectious disease

Study name	Study type	Outcomes measurement & Results	Interventions	Clinicaltrials.gov identifier (references)
Sepsis & infectious disease				
Vitamin D in ventilated ICU patients*	Interventional (phase 2)	Concentration of plasma 25(OH)D and LL-37 level	Dietary supplement: vitamin D	NCT01372995
Cholecalciferol supplementation for sepsis in the ICU	Interventional	Change in vitamin D Status and immunological profiles	Dietary supplement: vitamin D	NCT01896544
Effects of vitamin D and omega-3 fatty acids on infectious diseases and hCAP18*	Interventional (recruiting)	hCAP18 (LL-37) level	Vitamin D and omega-3 fatty acids	NCT01758081
Effect of high dose vitamin D3 in smokers and non-smokers with and without HIV*	Interventional (recruiting)	Difference in vitamin and peptide LL-37 levels	Dietary supplement: vitamin D	NCT03270709
Effects of smoking and vitamin D3 on the levels of human cathelicidin peptide LL-37*	Observational	Serum D3 vitamin and LL-37 levels	Cross-sectional observation	NCT03923218
Randomized trial of vitamin D supplement to prevent influenza A	Interventional	Vitamin D supplementation may reduce the incidence of influenza A	Dietary supplement: vitamin D	UMIN000001373 (124)
Randomized trial of vitamin D supplement and risk of acute respiratory infection	Interventional	Vitamin D supplementation reduced acute respiratory infection among children with vitamin D deficiency	Dietary supplement: vitamin D	NCT00886379 (123)
TB				
Clinical trial of PBA and vitamin D in TB*	Interventional (phase 2)	PBA and vitamin D promoted immunomodulation to improve TB treatment outcomes / PBA and vitamin D induced intracellular killing of MTB by macrophages with the elevation of LL-37	Oral sodium Phenylbutyrate, cholecalciferol	NCT01580007 (120,122)
Vitamin D3 and the association with cathelicidin expression in patients with active TB*	Observational	Severe forms of intrathoracic TB may be associated with lower vitamin D3 status and lower of LL-37	Cross-sectional observation	(45)
The impact of vitamin D on TB among Koreans	Observational	Vitamin D deficiency is significantly prevalent in TB patients compared to people without TB	Cohort study	NCT01137370
Role of vitamin D in innate immunity to TB	Interventional	Vitamin D supplementation had significant favorable effects on serum 25(OH)D concentrations	Dietary supplement: vitamin D	NCT01244204 (130)
Trial of adjunctive vitamin D in TB treatment	Interventional (phase 3)	Administration of vitamin D increased serum 25(OH)D in TB patients.	Adjunctive vitamin D	NCT00419068 (131)
Replacement of vitamin D in patients with active TB	Interventional	Cytokine response	Intramuscular injection of cholecalciferol	NCT01130311
A study the effect of vitamin D to conventional treatment in new pulmonary TB patients	Interventional	Vitamin D supplementation did not reduce time to sputum culture conversion.	Supplemental high-dose oral vitamin D	NCT00366470 (132)
Pneumonia & peritonitis				
Study of vitamin D for the prevention of acute respiratory infections in children*	Interventional	Serum of cathelicidin, 25(OD)-D levels	Dietary supplement: vitamin D	NCT02046577
Vitamin D3 supplementation in adults with bronchiectasis*	Interventional	Serum of cathelicidin, 25(OD)-D levels	Dietary supplement: vitamin D	ACTRN12607000641493 (121)
Vitamin D supplementation prevent early pneumonia	Interventional	Prevalence of pneumonia, all-cause mortality	Dietary supplement: vitamin D	NCT00877422
Vitamin D supplementation and respiratory index of severity in children in pneumonia	Interventional (phase 4)	Respiratory index of severity in children	Supplemental cholecalciferol	NCT02936895
The effect of vitamin D supplement on the prevention of peritoneal dialysis-related peritonitis	Interventional	Change in serum 25(OH)-vitamin D level	Supplemental cholecalciferol	NCT03264625

Further details for trial with NCT numbers can be accessed at http://clinicaltrials.gov.
ICU, intensive care unit; NCT, national clinical trial; UMIN, University hospital Medical Information Network; ACTRN, Australian New Zealand Clinical Trials Registry.
*Clinical studies that measured the level of LL-37 in patients' samples.

therapeutic strategy, even against drug-resistant bacterial strains (129). Understanding the mechanisms by which vitamin D-cathelicidin regulates innate host defense systems, in the context of autophagy and immune pathways, is of prime importance for exploring strong candidates for host-directed therapeutics against diverse infectious diseases. Vitamin D is strongly clinically associated with acute respiratory tract infections, parallel to vitamin D

status and incidences of airway infections (16). Current findings provide promising evidence for the protective effects of vitamin D on respiratory tract infections, including TB (16). To date, vitamin D supplemental therapy has been the most widely used potential treatment in TB clinical trials. However, further evidence of the cathelicidin levels and its efficacy as a prerequisite before vitamin D supplementation move into clinical routine in infectious diseases. Further translational knowledge about vitamin D-cathelicidin axis should be accumulated to support the clinical application of vitamin D in various acute and chronic infectious diseases for prevention and/or to offer improved outcomes.

ACKNOWLEDGEMENTS

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