13 Vitamin D and the Innate Immunity

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Abstract This chapter will examine the role of vitamin D in the innate immune system as a mediator of human host defense mechanisms against microbial disease, focusing on tuberculosis. The first section will examine tuberculosis and the innate immune response to the intracellular pathogen, *Mycobacterium tuberculosis* (*M. tuberculosis)*, the causative agent of tuberculosis. This is followed by a discussion of the known associations, genetic and mechanistic, between the vitamin D pathway and tuberculosis susceptibility. Finally, the chapter will conclude with a discussion on the potential for adjuvant treatment of tuberculosis with vitamin D.

Key Words: Extrarenal; 1,25-dihydroxyvitamin D; CYP27B1; proliferation; differentiation; parathyroid hormone; FGF23; immune function; cancer; keratinocytes

1. TUBERCULOSIS

1.1. Tuberculosis Overview

Tuberculosis has plagued humans throughout history with fossil evidence indicating tuberculosis infection of early hominids, such as the *Homo erectus,* and recordings of the disease by man as far back as ancient Egyptian and Chinese manuscripts *(1)*. The bacterium that causes tuberculosis, *M. tuberculosis*, was first described by Robert Koch in 1882. The bacterium primarily infects lung macrophages leading to pathogenesis of the disease. More than a century later, tuberculosis remains as a leading cause of morbidity and mortality worldwide, with one-third of the world's population infected and eight million new cases of tuberculosis each year *(2)*. Tuberculosis is one of the leading causes of death worldwide in women of reproductive age and in individuals infected with HIV *(3, 4)*. Even developed countries are not spared by this pandemic; estimates are that 10–15 million people residing in the United States are infected with *M. tuberculosis (5, 6)*. And, like the situation worldwide, mycobacterial infection is a leading cause of death among patients with AIDS in the United States *(5)*. The recent emergence of extensively drug-resistant (XDR) TB in HIV-infected individuals in KwaZulu Natal and its high mortality are an additional and urgent concern *(7)*.

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In addition to its importance with respect to global health, tuberculosis provides an important model for investigation of the human immune response to an intracellular pathogen and studies on tuberculosis have led to many basic immunological findings; these include [1] that the innate immune system recognizes microbial lipoproteins via Toll-like receptor 2 (TLR2) *(8)*; [2] that activation, via TLR2, of monocytes leads to instruction of the adaptive immune response via release of IL-12 *(8)*, dendritic cell differentiation *(9),* and maturation *(10)*; and [3] that the activation of monocytes via TLR2 triggers (i) macrophage differentiation *(9)*, (ii) a nitric oxide-dependent antimicrobial pathway in mice *(11)*, and (iii) a vitamin D-dependent antimicrobial pathway in humans *(12)*. TLR2 has been shown to be important for resistance to *M. tuberculosis* in mouse models *(13–15)* and polymorphisms in both the vitamin D receptor (VDR) and TLR2 are associated with susceptibility to TB in humans *(16–23)*. The role of the innate immune response and vitamin D will be discussed in detail below.

1.1.1. Innate Immunity

Metchnikoff originally described the key direct functions of cells of the innate immune system: (1) rapid detection of microbes; (2) phagocytosis of those microbes; and (3) antimicrobial activity. Charles Janeway advanced our thinking about how the mammalian innate immune recognizes microbial pathogens, proposing that it must involve evolutionarily primitive receptors that bind conserved microbial constituents, termed pattern recognition receptors *(24)*. *M. tuberculosis* is known to activate at least two different families of pattern recognition receptors: TLRs and the nucleotide oligomerization domain (NOD)-like receptors on macrophages. The TLR2 and TLR1 heterodimer recognizes a triacylated lipoprotein derived from *M. tuberculosis*, which results in activation of NF-κB leading to the production of inflammatory cytokines and direct antimicrobial activity *(8, 11, 12)*. NOD2 recognizes muramyl dipeptide (MDP), which is a peptidoglycan present on *M. tuberculosis (25, 26)*. Triggering NOD2 similarly leads to a NF-κB-mediated inflammatory response; however, in contrast to TLRs, NOD2 also results in activation of the inflammasome *(27)*. The inflammasome is a protein complex whose function is to cleave and activate the pro-IL-1β protein into the active IL-1β cytokine through the enzymatic actions of caspase-1.

1.1.2. Toll-Like Receptors

Toll was first studied as a part of the dorsal ventral patterning system in *Drosophila melanogaster* embryogenesis. In 1996, Lemaitre et al. *(28)* reported that Toll-deficient adult *Drosophila* were more susceptible to fungal infection. Activation of Toll in flies results in production of antimicrobial peptides *(29)*, thus implicating Toll as a player in a primitive immune system. One year later, Medzhitov et al. *(30)* demonstrated that a constitutively active human Toll homologue, or a Toll-like receptor (TLR), modulates the adaptive immune response by inducing cytokine secretion as well as expression of co-stimulatory molecules. Together, these reports first established the importance of Toll and TLRs in host defense.

TLRs have been shown to have specificity in recognition of microbial ligands and mediate immune functions of the innate immune system. To date, 11 mammalian TLRs have been identified in both the human and murine genomes. Although, TLR1-9 are conserved between humans and mice, the murine *Tlr10* gene is nonfunctional, and the human *TLR11* gene harbors a premature stop codon which prevents its expression *(31)*. All the mammalian TLRs share a highly similar cytosolic Toll/IL-1 receptor (TIR) domain, which triggers several signaling pathways including the transcription factor: NF-κB *(32)*. The extracellular TLR domains include multiple leucine-rich repeat motifs and is responsible for recognition of conserved pathogen-associated molecular patterns (PAMPs). TLR2 heterodimerizes with TLR1 or TLR6, and the dimers mediate recognition of triacylated and diacylated bacterial lipoproteins, respectively *(33)*. The remainder of the known TLR ligands are as follows: viral dsRNA (TLR3), lipopolysaccharide (LPS) (TLR4), bacterial flagellin (TLR5), viral single-stranded RNA (ssRNA) (TLR7 and TLR8), bacterial unmethylated CpG DNA (TLR9), and protozoan profilin-like molecule (TLR11) *(31)*. The ligand for TLR10 is still unclear. Thus, TLRs provide a rapid first line of defense against a variety of microbial pathogens through the recognition of a milieu of pathogen-associated molecules.

TLR activation induces a variety of effects, including enhancement of macrophage phagocytosis *(34)*, endosomal/lysosomal fusion *(35)*, production of antimicrobial peptides *(36, 37)*, as well as induction of direct antibacterial *(11, 36)* and antiviral activity *(38–40)*. *M. tuberculosis*-infected macrophages can induce a direct antimicrobial activity upon TLR2/1 activation. In a murine macrophage cell line, this activity was dependent on the generation of nitric oxide (NO) through inducible nitric oxide synthase (iNOS) activity. Addition of the iNOS inhibitors L-NIL and L-NAME ablated the murine TLR2/1-mediated antimicrobial activity; however, neither had an effect on human monocytes, suggesting that human TLR2/1-induced antimicrobial activity is fundamentally different from murine cells *(11)*. This correlated with the finding that, upon TLR2/1 activation, human monocytes do not generate detectable levels of NO *(41)*. Accordingly, the mechanism by which human macrophages kill intracellular *M. tuberculosis* intrigued immunologists for many years; the surprising role of the vitamin D synthetic/metabolic pathway in this mechanism is detailed below.

1.1.3. Immunoactivity of 1,25-Dihydroxyvitamin D

There have been many studies on the role of active vitamin D, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], on innate and adaptive immune responses *(42–44)*. Insight into vitamin D-induced antimicrobial activity by human monocytes and macrophages against *M. tuberculosis* was first suggested by experiments in the labs of Rook in 1986 *(45)* and Crowle in 1987 *(46)*. These experiments were performed adding 1,25$(OH)_2$D to the extracellular medium of *M. tuberculosis*-infected human monocytes and macrophages in vitro with a resultant reduction of the intracellular bacterial load. Yet Crowle writes "concentrations of 1,25$(OH)_2$D near 4 μg/ml were needed for good protection, these levels seemed unphysiologically high compared with 26–70 pg/ml being in the normal circulating range." Nevertheless, these studies opened new questions

regarding [1] the role of vitamin D in the physiological response to *M. tuberculosis* and [2] the identity of the vitamin D-dependent antimicrobial effectors.

It would be nearly a decade later before the molecular mechanism of the vitamin D-induced antimicrobial activity in macrophage began to be elucidated. One study by Sly et al. *(47)* reported $1,25(OH)_2D$-induced antimicrobial activity to be regulated by phosphatidylinositol 3-kinase and mediated through the generation of oxygen intermediates via NADPH-dependent phagocyte oxidase. Interestingly, the observed $1,25(OH)_2D$-induced oxidative burst occurred at a different time point than the antimicrobial activity, thus leading the authors to postulate that there had to be another key factor *(47)*. Another mechanism was proposed by Anand et al.; their study *(48), (49)* demonstrated that the $1,25(OH)_2D$-induced antimicrobial activity was associated with downregulated transcription of the host protein, tryptophan-aspartate-containing coat protein (TACO); this protein plays an important role in *M. tuberculosis* entry and survival in human macrophages. In 2005, using a genome-wide scan for vitamin D response elements (VDREs), Wang et al. *(50)* reported that the genes encoding antimicrobial peptides, cathelicidin and hBD2(DEFB4), were regulated by the VDR. Prior to this study, human macrophages were not thought to utilize antimicrobial peptides as a defense mechanism; however, it was demonstrated in the same year that human monocytes expressed cathelicidin at both the mRNA and protein levels when stimulated with $1,25(OH)_2D$ *(12, 50, 51)*. Although it was apparent that monocytes could express cathelicidin, whether or not it played a role in host defense against intracellular *M. tuberculosis* infection was not clear. Two years later, a critical role for cathelicidin in the $1,25(OH)_2D$-induced antimicrobial activity against intracellular *M. tuberculosis* was demonstrated in human monocytic cells using siRNA knockdowns *(52)*.

In contrast to its effects on macrophages, many studies have reported that $1,25(OH)_2D$ induces immunosuppressive effects, including but not limited to [1] inhibition of IL-12 secretion, [2] inhibition of lymphocyte proliferation and immunoglobulin synthesis, and [3] impairment of dendritic cell maturation, leading to the generation of tolerogenic dendritic cells and T-cell anergy *(53–56)*. In particular, it was suggested that $1,25(OH)_2D$ produced by the macrophage in granuloma-forming diseases, like tuberculosis and sarcoidosis, exerted a paracrine immunoinhibitory effect on neighboring, activated lymphocytes that express the VDR, and that this acts to slow an otherwise "overzealous" immune response that may be detrimental to the host *(57)*. The physiological significance of this has been highlighted by the recent development of $1,25(OH)_2D$-deficient mouse models in which the gene for the vitamin D-activating enzyme CYP27B1 has been knocked out *(58, 59)*. A notable feature of these animals is that they present with enhanced adaptive immunity signified by multiple enlarged lymph nodes.

1.1.4. Antimicrobial Peptides

Antimicrobial peptides consist of a highly diverse family of small peptides that can function as chemoattractants *(60, 61)*, dendritic cell activators *(62)*, and importantly, direct antimicrobial effectors *(63, 64)*. They exert microbicidal activity by disrupting the pathogen membrane through electrostatic interactions with the polar head groups of membrane lipids *(65)*, or the creation of membrane pores *(63)*; as such, they exhibit

a wide range of microbial targets including bacteria *(66)*, fungi *(67, 68)*, parasites *(69, 70)*, and enveloped virii *(71)*. Although epithelial cells at the interface between the outside and inside environment of the host express antimicrobial peptides *(72)*, it is the population of innate immune cells that buttress that external–internal barrier in the host, such as neutrophils *(73)*, mast cells *(74),* and monocytes/macrophages *(51, 75)*, that are recognized to be the major producers of antimicrobial peptides. Several antimicrobial peptides produced by macrophages have been demonstrated to have direct antimicrobial activity against *M. tuberculosis*, including but most likely not limited to LL-37 (cathelicidin) *(12, 76)*, hBD2 (DEFB4) *(77)*, and hepcidin *(78)*. In humans, cathelicidin and DEFB4 were found to contain activating VDREs in their promoter regions; whether or not hepcidin is vitamin D-regulated at the level of transcription is unknown *(50)*. Activation of the VDR in monocytes/macrophages results in the expression of cathelicidin at both the mRNA and protein levels *(12, 50, 76)*. siRNA knockdown of $1,25(OH)_2D$-induced cathelicidin in human monocytic cells resulted in complete loss of antimicrobial activity *(52)*, suggesting that antimicrobial peptides represent a major human macrophage host defense mechanism. Furthermore, macrophages can obtain and utilize neutrophil granules which can concentrate a variety of antimicrobial peptides against *M. tuberculosis (79, 80)*.

1.2. Vitamin D Pathway and Tuberculosis

Many studies have identified genes that may confer some degree of susceptibility to tuberculosis, including: HLA-DR alleles *(81–83)*, NRAMP-1 *(84)*, interferon-γ signaling *(85)*, SP110 *(86)*, complement receptor-1 *(87)*, and notably, the VDR *(19–23)*. However, these studies did not identify a clear cut host defense mechanism. Several studies have linked serum levels of the major circulating form of vitamin D, 25-hydroxyvitamin D [25(OH)D], to both tuberculosis disease progression and susceptibility *(23, 88)*. In 1985, a study reported that of 40 Indonesian patients with active tuberculosis and treated with anti-tuberculosis chemotherapy, 10 patients with the highest 25(OH)D levels at the outset of therapy had "less active pulmonary disease" *(88)*.

Another aspect of the vitamin D pathway that has been extensively studied is the VDR itself. There are two major VDR polymorphisms that have been studied in terms of tuberculosis susceptibility with conflicting results: TaqI *(20–22)* and FokI *(22, 89)*, located in exons nine and two of the VDR coding sequence, respectively *(90)*. Bellamy et al. conclude that the tt allele of the TaqI polymorphism protects against TB, however, studies by two other groups report no such association *(21, 89)*. Liu et al. *(22)* report that the FokI ff allele is associated with active TB among the Chinese Han population, but there are no other reports concluding an association for FokI ff and TB in any other population. These associations became clearer in a study examining the relationship between vitamin D deficiency and VDR polymorphisms with tuberculosis in the Gujarati Asians living in West London in the year 2000 *(23)*. The study reported that both the TaqI (Tt/TT) and FokI (ff) alleles were associated with tuberculosis only when the individual exhibited serum 25(OH)D deficiency *(23)*. Collectively, these studies have demonstrated that vitamin D plays an important role to host defense against *M. tuberculosis* in vivo. The problem in drawing conclusions between the studies in vitro with human inflammatory cells and these observations in vivo in humans with

tuberculosis resides in the fact that previous in vitro studies used the active, $1,25(OH)_2D$ metabolite to affect antimicrobial activity, while the association to tuberculosis was with serum levels of the $1,25(OH)_2D$ substrate, 25(OH)D.

Relatively little is known about the direct effects of 25(OH)D on innate immunity. Hewison et al. *(91)* found that 25(OH)D at physiologic levels (100 nM) suppressed CD40L-induced IL-12 production in day-7 GM-CSF/IL-4-derived DCs in vitro. Other studies in vitro have shown that intracrine metabolism of 25–$1,25(OH)_2D$ via endogenous expression of CYP27B1 is a more efficient mechanism for modulating the phenotype of either DCs or monocytes compared to the exogenous addition of active $1,25(OH)_2D$ itself *(92)*. In contrast to these in vitro analyses, there are little data on the effects of altering the 25(OH)D status in vivo on the immune status of the host. Yang et al. *(93)* showed that profound reduction in the serum 25(OH)D in mice resulted in significant blunting of the cell-mediated immune response to cutaneous dinitrofluorobenzene (DNFB) challenge. Administration of 25(OH)D to humans with head and neck squamous cell carcinoma increases plasma IL-12 and IFN-γ levels and improves T-cell blastogenesis *(94)*. In more recent studies, we have shown that the ability of monocytes from human subjects to mount a cathelicidin response following TLR challenge is directly proportional to circulating levels of 25(OH)D but not $1,25(OH)_2D$ *(95)*. Importantly, this study also showed that TLR-induction of cathelicidin was enhanced in subjects supplemented with vitamin D (500,000 IU vitamin D_2 over 5 weeks), indicating that the immunomodulatory effects of 25(OH)D also occur in vivo.

1.2.1. Role of 25-Hydroxyvitamin D on the Innate Immune Response

In 2006, a potential mechanism by which the 25(OH)D status of an individual may alter their ability to mount an innate immune response against *M. tuberculosis* was reported. In humans, activation of TLR2/1 results in the induction of key genes in the vitamin D pathway (Fig. 1), including the VDR and CYP27B1. Under conditions where the extracellular concentration of 25(OH)D is present at sufficient levels, TLR2/1 activation of monocytes results in a CYP27B1- and VDR-dependent expression of the antimicrobial peptide, cathelicidin, and direct microbicidal activity against intracellular *M. tuberculosis*. The induction of CYP27B1 and VDR in monocytes was subsequently demonstrated to be mediated through the actions of TLR2/1-induced IL-15 expression *(96)*. Interestingly, the human but the not murine cathelicidin promoter contains an activating VDRE *(51)*, perhaps suggesting a point of divergent evolution between mice and humans in the antimicrobial effectors used by the TLR-mediated innate immune response. Inhibition of the VDR resulted in ablation of the TLR2/1-induced antimicrobial activity, implicating that VDR activation is a critical step in the innate immune response against *M. tuberculosis* and potentially explaining the association of 25(OH)D serum levels with susceptibility to tuberculosis; e.g., where low 25(OH)D levels in the circulation cannot provide sufficient substrate 25(OH)D for CYP27B1-mediated production of $1,25(OH)_2D$ to activate the VDR-dependent antimicrobial response.

This requirement of adequate 25(OH)D in the extracellular environment of the human macrophage for the induction of host defense mechanisms via TLR2/1 provided a link between two well-documented clinical observations: compared to lightly pigmented

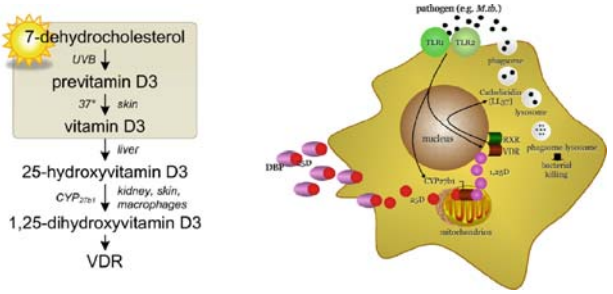

Fig. 1. Synthesis and innate immunoaction of vitamin D. The *left panel* shows synthetic/metabolic pathway of vitamin D3 beginning in the skin. The initial step is the UVB-mediated, non-enzymatic conversion of 7-dehydrocholesterol to previtamin D3. This is followed by thermal isomerization of previtamin D3 to vitamin D_3. Vitamin D_3 gains access to the circulation for oxidative metabolism first to 25-hydroxyvitamin D_3 in the liver followed by conversion via the CYP27B1 hydroxylase in the kidney, skin, and macrophage to hormone 1,25-dihydroxyvitamin D3. The *right panel* schematic recapitulates the events post interaction of the human TLR2/1 dimer with pathogen-associated membrane patterns (PAMPs) from *Mycobacterium tuberculosis* (*M.tb.*). TLR activation results in (1) induction of expression of the CYP27B1 hydroxylase and VDR genes; (2) intracrine generation of 1,25-dihydroxyvitamin D [1,25(OH)D], if and only if sufficient substrate 25-hydroxyvitamin D (25(OH)D) has been delivered to the macrophage bound to the serum vitamin D-binding protein (DBP); (3) transactivation of the cathelicidin gene via interaction of the $1,25(OH)_2$D-VDR-retinoid X receptor (RXR) with an enhancer element in its promoter; (4) induction of expression of the cathelicidin gene product LL37; and (5) killing of ingested mycobacteria.

human populations, darkly pigmented black individuals are [1] more susceptible to virulent infections with tuberculosis and [2] have lower circulating, serum 25(OH)D levels owing to their relatively diminished capacity to synthesize vitamin D in their skin during sunlight exposure. The biosynthetic pathway of 25(OH)D in humans (Fig. 1) involves the absorption of ultraviolet B (UVB) photons from sunlight by 7-dehydrocholesterol (7HDC) in the basal layer of the epidermis and its non-enzymatic conversion to a pre-vitamin D_3 precursor in the skin; in fact, the melanin in pigmented skin will competitively absorb these UVB rays preventing this photoreaction *(97)*. In human monocytes cultured in sera from pigmented African American subjects and stimulated with a TLR2/1 ligand, there was no upregulation of cathelicidin mRNA, whereas the same human monocytes conditioned in sera from lightly pigmented subjects did *(12)*. Moreover, supplementation of the African American sera with exogenous 25(OH)D restored the induction of cathelicidin mRNA. This implies that an individual's serum 25(OH)D level may affect their ability to combat infection and that returning circulating levels of 25(OH)D to normal could potentially restore their host defense mechanisms *(12)*.

Through reostatic regulation of CYP27B1 activity and conversion of substrate 25(OH)D to product $1,25(OH)_2$D, the macrophage directly controls its intracellular level of $1,25(OH)_2$D *(98)*. It is also now recognized that TLR-induced antimicrobial activity can be inhibited by blocking CYP27B1 activity *(12)*. These data suggest that it is serum 25(OH)D and not the $1,25(OH)_2$D concentration that [1] controls the intracellular $1,25(OH)_2$D level and [2] is essential for the TLR-induced antimicrobial activity. This explains why in previous experiments in vitro, a supraphysiologic concentration of

$1,25(OH)_2D$ in the conditioning extracellular media was required to generate sufficient intracellular levels of the metabolite to affect the VDR and to achieve an antimicrobial effect in human macrophages.

1.2.2. History of Vitamin D, Sunshine, and Tuberculosis

Establishment of vitamin D's role in host defense against tuberculosis provides new insights into the historical understanding of tuberculosis treatment prior to the advent of antibiotics. In the late nineteenth century, two young physicians, who themselves had contracted tuberculosis, were instructed by their physicians to travel to mountainous regions of Europe during the summertime as part of their attempt to recover. Their trek into this high UVB environment led to the "remission" of their disease. As a consequence of this success, Hermann Brehmer built the world's first high-altitude tuberculosis sanitorium in Germany, designed to allow patients to be exposed to "fresh air and sunlight." At about the same time in the United States, Edward Livingston Trudeau of New York published his original scientific finding that rabbits infected with tuberculosis had a more severe course of disease if caged indoors in the dark as opposed to being kept outdoors on a remote island. These experimental observations led him to build the first sanitorium at Saranac Lake, NY. In fact, it was the success of treatment facilities like these that paved the way to the 1903 Nobel Prize in Medicine awarded to the Danish physician, Niels Ryberg Finsen, for demonstrating that UV light was beneficial to patients with lupus vulgaris, a form of cutaneous *M. tuberculosis* infection. Despite widespread skepticism about the value of sanitoria at the time and since then, it is quite possible that the prolonged exposure to sunlight increased cutaneous vitamin D production, increased substrate 25(OH)D levels, and enhanced innate immunity to combat tuberculosis. Clearly, the harmful effects of sunlight have been well documented and emphasized, particularly its strong association with melanomas and squamous cell carcinomas *(99, 100)*. However, there is also convincing epidemiologic evidence that vitamin D has a positive association with lower incidences of colorectal and prostate cancers *(101)*. While heliotherapy is not likely to re-emerge as a useful intervention for human disease, other than for perhaps seasonal depression, vitamin D supplementation could represent an inexpensive adjuvant therapeutic approach to correcting the worldwide prevalence of vitamin D insufficiency and enhancing innate immunity to microbial infections, especially in individuals of pigmented African and South Asian descent in whom tuberculosis is rampant.

1.2.3. Treatment of Tuberculosis with Vitamin D

There is a long history of using vitamin D to treat mycobacterial infections with apparent success. In 1946, Dowling et al. *(102)* reported the treatment of patients with lupus vulgaris with oral vitamin D. Eighteen of 32 patients appeared to be cured, nine improved. Morcos et al. *(103)* treated 24 newly diagnosed cases of tuberculosis in children with standard chemotherapy with and without vitamin D; they noted more profound clinical and radiological improvement in the group treated with vitamin D. Nursyam et al. *(104)* administered vitamin D or placebo to 67 tuberculosis patients following the

sixth week of standard treatment. Out of 60 total patients, the group with vitamin D had higher sputum conversion rate and radiological improvement (100%) than placebo group (76.7%). This difference was statistically significant ($p = 0.002$). Despite the clear benefits of vitamin D treatment for tuberculosis, the mechanism of action had not been elucidated. The fact that TLR-activated macrophages can convert vitamin D to produce antimicrobial peptides could be a possible mechanism by which supplementation of patients with inactive vitamin D leads to a positive therapeutic outcome.

Progress in curtailing the human death rate from tuberculosis has been hampered by access to, cost and effectiveness of current antibiotic regimens *(105)*. Some of these problems could potentially be overcome by adding vitamin D to the treatment regimen of tuberculosis. Although the currently published studies on the effects of vitamin D supplementation are generally inadequate to evaluate the efficacy of such treatment *(106)*, a single oral dose of 50,000 IU of vitamin D has been shown to enhance killing of mycobacteria by whole blood of healthy volunteers *(107)*. As such, knowledge of the role of human vitamin D metabolism and action in the basic innate immune defense mechanisms against mycobacterial infection provides hope in the development of safe, simple, and cost-effective strategies in the near future to prevent and treat tuberculosis.

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