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REVIEW

Vitamin D and macrophage functions in tuberculosis

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Mononuclear phagocytes like monocytes/macrophages engulf microbes and mediate intracellular killing through the activation of various antimicrobial activities such as synthesis of anti-microbial peptides, reactive oxygen/nitrogen intermediates and autophagy induction. However, intracellular pathogens like *M. tuberculosis* evade from macrophage defence mechanisms by various strategies to adapt the intracellular environment of macrophages and creating a major host cell niche for its growth and survival. 1, 25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] is the active metabolite of vitamin D, which modulates immune functions mediated by monocytes, macrophages, dendritic cells, T cells and B cells. Genomic actions of 1, $25(OH)_2D_3$ exert through the vitamin D receptor, which is expressed constitutively in macrophages. Various studies have shown that $1,25(OH)_2D_3$ enhances the macrophage phagocytosis by upregulating the surface receptors including CD14 and mannose receptor. Moreover, $1,25(OH)_2D_3$ enhances the antimicrobial effects of macrophages by upregulating the expression of cathelicidin antimicrobial peptide and defensin, which inhibit the intracellular growth of *M. tuberculosis*. 1, $25(OH)_2D_3$ mediated cathelicidin expression upregulates the autophagy genes and enhance the fusion of phagosome containing *M. tuberculosis* with lysosome. Apart from antimicrobial effects, $1,25(OH)_2D_3$ also modulates the antigen presentation and secretion of chemokines, cytokines and other factors of macrophages. In conclusion, it has been suggested that $1,25(OH)_2D_3$ enhances macrophage innate immune functions by upregulating the antimicrobial efficiency, which could be beneficial to the host during active tuberculosis disease. In addition, during anti-TB treatment, nutritional supplementation of vitamin D could be helpful to minimize the inflammation at the site of infection.

Keywords: 1,25-dihydroxyvitamin D_3; Macrophages; Phagocytosis; Anti-microbial peptides; Immuno-modulation; Tuberculosis

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Introduction

The resident alveolar macrophages and neutrophils are the primary immune cells that influx to the site of infection during early *M. tuberculosis* pathogenesis [1]. Macrophages are the main cells of the immune system which are differentiated from monocytes and they engulf microbes and other cellular debris through phagocytosis. Monocyte/macrophage phagocytosis of tubercle bacilli is mediated by a diverse array of receptors such as complement receptors, mannose receptor, dendritic cell-specific intercellular adhesion molecule (ICAM)-3-grabbing nonintegrin (DC-SIGN), toll like receptors, CD14 and Fc receptors [2-4]. Following phagocytosis, phagosomes are formed in a process called focal exocytosis [5] and these phagosomes mature by attaining low pH, degradative hydrolases and rapidly fuse with lysosomes to form phagolysosomes [6]. This creates a microenvironment where the bacteria subject to the action of hydrolytic enzymes such as hydrolases, proteases, superoxide dismutase and lysozymes, which are detrimental to the

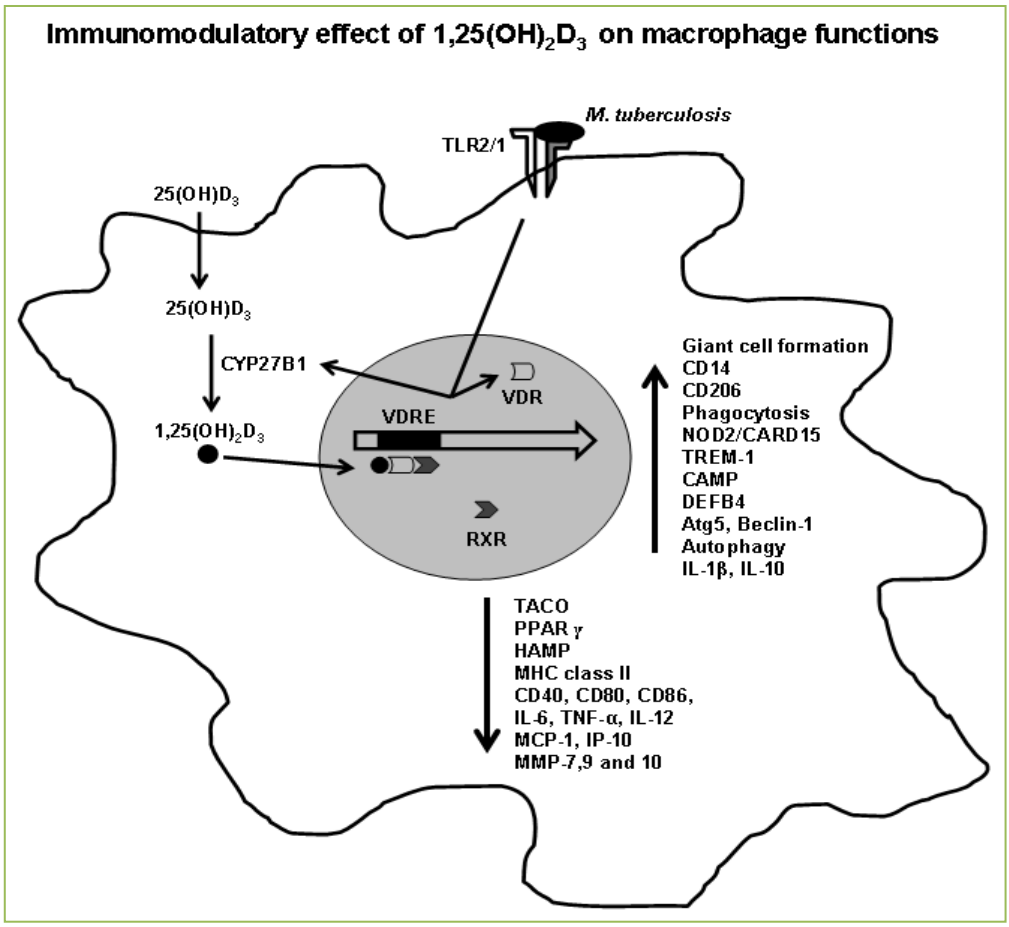

Figure 1. Toll-like receptor-2/1 (TLR-2/1) dimer recognizes *M. tuberculosis* and induces the expression of CYP27B1 and vitamin D receptor (VDR). CYP27B1 converts 25(OH)D_3 into 1,25(OH)$_2D_3$, which bind with VDR and induce the formation of VDR–RXR (retinoid X-receptor) complex that interact with vitamin D response elements (VDRE) in the promoter region and modulate various immune functions. 1,25(OH)$_2D_3$ upregulates the macrophage cell surface markers CD14 and CD206, phagocytosis, triggering receptor expressed on myeloid cells-1 (TREM-1), cathelicidin anti-microbial peptide (CAMP), beta-defensin-4 (DEFB4), autophagy-related genes such as Atg5 and Beclin-1, cytokines interleukin (IL)-1β and IL-10. On the other hand, 1,25(OH)$_2D_3$ downregulates the tryptophan-aspartate-containing coat protein (TACO), proadipogenic peroxisome proliferator–activated receptor-γ (PPAR-γ), hepcidin antibacterial protein (HAMP), major histocompatibility complex II (MHC class II), cytokines IL-6, IL-12, tumor necrosis factor-α (TNF-α), chemokines such as macrophage chemotactic protein-1 (MCP-1) and interferon-γ inducible protein-10 (IP-10), matrix metalloproteinases 7,8 and 10 (MMP-7,8 and 10).

bacteria. In response to the phagocytic stimuli, macrophages produce reactive nitrogen intermediates (RNI) as well as reactive oxygen intermediates (ROI) such as superoxides, hydrogen peroxide and hydroxyl radicals [7, 8], which inhibit the growth of intracellular bacteria.

1,25-dihydroxyvitamin D_3 [1,25(OH)$_2D_3$], the hormonally active metabolite of vitamin D, act as an immuno-modulator and regulate the various macrophage functions (Figure 1). A recent study demonstrated that vitamin D supplementation restored the impaired immune response and better clinical outcome in tuberculosis patients [9], which reveals that sufficient vitamin D level has an important role to control the intracellular infection like tuberculosis. In the current review, we focused on the immuno-modulatory effects of vitamin D_3 on macrophage functions mainly in tuberculosis.

Vitamin D: Mode of action

Vitamin D belongs to the class of secosteroids, mainly involves in the homeostasis of calcium, magnesium and phosphate and regulates bone metabolism. There are two major forms of vitamin D metabolites; Vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D is synthesised from 7-dehydrocholestrol by the action of ultraviolet B (UVB- spectrum 280–320 nm) in the skin or it can be attained from food such as fatty fish and mushroom. Vitamin D_3 includes both calcidiol (25-hydroxyvitamin D_3) and calcitriol (1,25-dihydroxyvitamin D_3).

25-hydroxyvitamin D_3 [25(OH)D_3] is considered as major circulating form of vitamin D metabolite and serum 25(OH)D_3 level less than 20 nmol/L is often considered as vitamin D deficiency [10]. The genomic actions of 1,25$(OH)_2D_3$ are initiated after binding to the nuclear vitamin D receptor (VDR), a member of the nuclear receptor superfamily, which acts as a transcription factor and interact with vitamin D response elements (VDRE) through various mechanisms and influence the expression of various genes and microRNAs [11-13]. It has been shown that 1,25$(OH)_2D_3$ treatment appreciably increases the number of VDR binding sites and alter the expression of various genes [14, 15]. In addition, 1,25$(OH)_2D_3$ mediated gene expression profile may vary in different cell types and depends on a duration of 1,25$(OH)_2D_3$ treatment [16]. Moreover, 1,25$(OH)_2D_3$ mediated antimicrobial activity in monocytes depends on the bioavailability of 25(OH)D_3, which is inversely correlated with vitamin D binding protein (DBP) levels as well as binding affinity between 25(OH)D_3 and DBP [17, 18].

Vitamin D and macrophage phagocytosis

Phagocytosis is the vital defence mechanism of monocyte-derived macrophages through its cell surface receptors. Several studies demonstrated that 1,25$(OH)_2D_3$ induces the differentiation of precursor monocytes into mature macrophages [19, 20]. Moreover, macrophages upon treatment with 1,25$(OH)_2D_3$ was shown to induce the formation of multinucleate giant cells which control the dissemination of *M. tuberculosis* and prevent the loss of macrophages during infection [21-23]. 1,25$(OH)_2D_3$ while upregulates the expression of CD14 and mannose receptor in monocytes, it suppresses the generation of dendritic cells from monocytes [24-26]. Another study demonstrated that a combination of vitamin D_3 and retinoic acid treatment upregulate the mannose receptor and Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN) expression on THP1 macrophage cell line that enhance the bacterial uptake and intracellular killing of mycobacteria by triggering the synthesis of reactive oxygen species and the induction of autophagy [23]. It has been shown that 1,25$(OH)_2D_3$ augments the chemotactic potential of monocytes and upregulates bacterial uptake in a complement-dependent way [27]. Our previous study has shown that 1,25$(OH)_2D_3$ enhances macrophage phagocytosis of live *M. tuberculosis* [28] and this enhanced monocyte/macrophage phagocytic potential is positively correlated with the upregulated expression of cathelicidin antimicrobial peptide (CAMP) [29]. Monocytes and macrophages also express various defensins [30] and these defensins also play an important role in the control of mycobacterial growth [31]. The influence of 1,25$(OH)_2D_3$ on antimicrobial peptide synthesis is discussed in a separate section. A recent study has shown that 1,25$(OH)_2D_3$ amplifies the innate immune responses of monocytes/macrophages by upregulating the expression of 'triggering receptor expressed on myeloid cells-1' (TREM-1) [32]. These studies suggest that 1,25$(OH)_2D_3$ enhances intracellular killing of pathogens by upregulating the phagocytic potential of macrophages as well as activating the various anti-microbial mechanisms.

Microbicidal function of macrophages

Upon activation of macrophages by suitable agents such as lipo-polysaccharides (LPS), interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) generate an inducible enzyme called nitric oxide synthase (iNOS), which cleaves L- arginine into L- citrulline and generate reactive nitric oxide [33] and this reactive nitric oxide is involved in killing of *M. tuberculosis*. It has been shown that 1,25$(OH)_2D_3$ enhances human monocyte anti-mycobacterial activity by enhancing the synthesis of ROI and RNI in *M. tuberculosis* infected macrophages as well as THP1 cells via NADPH oxidase system and is regulated by phosphatidyl inositol 3-kinase (PI 3-K) signalling pathways [23, 34]. Apoptosis is another defence mechanism associated with reduced pathogen viability in infected macrophages [35]. Moreover, macrophage phagocytosis of *M. tuberculosis* results in the secretion of various cytokines such as TNF-α, interleukin-12 (IL-12) and IFN-γ, which play an important role against tuberculosis [36]. It has been shown that IFN-γ activates various macrophage anti-microbial mechanisms such as anti-microbial peptide synthesis and autophagy induction during *M. tuberculosis* infection; however, sufficient vitamin D level in the system is required for inducing optimal immune responses [37].

Invasive mechanisms of *M. tuberculosis* in macrophage

Although macrophages employ different mechanisms to kill the engulfed microbes, but bacteria like *M. tuberculosis* have developed different approaches to stay alive within the hostile environment of the phagocytes. Mycobacterial antigens induce the expression of various anti-inflammatory cytokines like IL-10 and transforming growth factor-β (TGF-β) in monocytes and dendritic cells and these cytokines in turn downregulate the protective macrophage functions [38]. The virulent strains of *M. tuberculosis* prevent phagosome fusion with lysosome and avert the phagosomal acidification [39] thus favour the survival of bacteria inside the macrophages. Mycobacterial sulfatides [40] and ammonia production by *M. tuberculosis* under *in vitro* conditions have been reported to inhibit phagolysosomal fusion [41]. Moreover, *M. tuberculosis* suppresses the formation of phago-lysosome by inducing the expression of TACO (tryptophan aspartate-containing coat) protein that creates a coat around the phagosome and prevents its fusion with lysosomes [42]. 1,25$(OH)_2D_3$ exerts various anti-invasive mechanisms to

control mycobacterial growth in macrophages. It has been shown that $1,25(OH)_2D_3$ downregulates the transcription of TACO gene and inhibit the survival of *M. tuberculosis* in human macrophages [43]. Further, another study reported that $1,25(OH)_2D_3$ augments the fusion of phagosomes with lysosomes in the infected macrophages and suppresses the viability of *M. tuberculosis* [44].

In addition, *M.tuberculosis* infection downregulates the MHC class II molecule expression in macrophages thereby inhibit the pathogen recognition by CD4+ T-cells [45]. Further, *M. tuberculosis* inhibits macrophage apoptosis by triggering the synthesis of lipoxin A4 (LXA4) (pronecrotic), which inhibit prostaglandin E2 (PGE2) (proapoptotic) synthesis that leads to necrosis of infected macrophages and mycobacterial spread [46]. The modulation of cytokine production by microRNAs (miRNAs) may be an effective escape mechanism of *M. tuberculosis* in macrophages. A study reported that higher expression of miR-125b expression results in destabilization of TNF-α mRNA and its level in *M. tuberculosis* infected human macrophages [47]. A study has shown that *M. tuberculosis* infected macrophages differentiate into lipid rich foam cells by accumulating lipid droplets that are required for its intracellular growth [48, 49]. Another recent study reported that vitamin D_3 treatment inhibited the accumulation of lipid droplets in infected macrophages by downregulating the expression of proadipogenic peroxisome proliferator–activated receptor-γ (PPAR-γ) and control the growth of intracellular *M. tuberculosis* [50].

Vitamin D and antimicrobial peptide synthesis

Antimicrobial peptides have an important role in host innate immunity and have wide antimicrobial activity against microbes such as bacteria, virus and fungi [51]. Antimicrobial peptides such as cathelicidin antimicrobial peptide (CAMP) and beta-defensin-4 (DEFB4) are expressed by various immune cells including macrophages, which contribute to antimicrobial activity. $1,25(OH)_2D_3$ triggered anti-microbial activity was first suggested by Rook and Crowle. They have shown that intracellular replication of *M. tuberculosis* are suppressed in monocytes that cultured in the presence of $1,25(OH)_2D_3$ [52, 53]. It has been shown that $1,25(OH)_2D_3$ interacts with three VDREs located in the promoter region of CAMP gene and induces its expression in monocytes, neutrophils, keratinocytes and human cell lines [54-56]. *M. tuberculosis* derived lipopeptide triggers TLR2/1 signalling in macrophages and upregulate the expression of VDR and 1α-hydroxylase (CYP27B1) that induce the expression of CAMP [57]. Subsequent studies have revealed that increased CAMP expression mediated by $1,25(OH)_2D_3$ is involved in the intracellular killing of *M. tuberculosis* in macrophages [58, 59]. $1,25(OH)_2D_3$-mediated CAMP expression is higher in monocytes/macrophages of pulmonary tuberculosis patients with less severe forms of tuberculosis than cavitary disease [29], who have a higher bacterial load in the lung. Another study has shown that $1,25(OH)_2D_3$-mediated cathelicidin expression depends on the stimulation of NADPH oxidase (NOX)2 signalling pathway [60]. LL-37 is the active form of CAMP, generated by enzymatic cleavage of human cathelicidin antimicrobial peptide-18 (hCAP18) by proteinase-3 [61]. Earlier studies have shown that LL-37 can directly kill the bacteria by disrupting the structure of microbial membrane [62]. Since LL-37 restricts the replication of drug sensitive and multi-drug resistant (MDR) *M. tuberculosis* [63], the application of antimicrobial peptides for the management of MDR-TB is a recently achieved research interest.

Defensins are another group of antimicrobial peptides associated with antimicrobial activity against drug sensitive and drug resistant *M. tuberculosis* thus play a crucial role in the control of mycobacterial growth [31, 64]. In addition to CAMP, it is reported that $1,25(OH)_2D_3$ also enhances the expression of beta-defensin-4 (DEFB4) in monocytes/macrophages [37, 65]. Another study demonstrated that $1,25(OH)_2D_3$ robustly induces pattern recognition receptor nucleotide-binding oligomerization domain-containing protein 2 (NOD2)/caspase recruitment domain-containing protein 15 (CARD15) gene expression that recognize muramyl dipeptide (MDP), and upregulate the expression of DEFB4 through the activation of NF-κB signaling pathway [66]. Recent studies reported that $1,25(OH)_2D_3$ enhances the IL-1β production in macrophages infected with *M. tuberculosis* and induces the expression of DEFB4, which kills intracellular mycobacteria [65, 67, 68]. In contrary to CAMP and defensin, the anti-microbial protein hepcidin (HAMP) favour the survival as well as the growth of *M. tuberculosis* in macrophages by suppressing the ferroportin-mediated export of cellular iron, an essential mineral required for bacterial growth [69, 70]. It has been shown that $1,25(OH)_2D_3$ downregulates HAMP in hepatocytes and monocytes and decreases the availability of iron for intracellular bacteria [71]. This study suggests that $1,25(OH)_2D_3$ control intracellular growth of mycobacteria by regulating the iron concentration inside the macrophages.

Vitamin D and autophagy

Autophagy is a process of lysosomal self digestion, essential for cellular homeostasis and plays a significant role in the control of intracellular infection. Autophagy functions as an intracellular innate defence mechanism where phagosome containing intracellular pathogen fuses with lysosome and undergoes degradation [72]. Intracellular bacteria such as *M. tuberculosis* inhibits the phagosome fusion with

lysosome thus survive inside the macrophages [73]. Vitamin D sufficiency is a critical factor to activate autophagy pathways. It has been shown that vitamin D sufficiency is a very important factor for IFN-γ induced antimicrobial activities such as phagosome maturation, antimicrobial peptide synthesis and autophagy induction in macrophages [37]. It is demonstrated that $1,25(OH)_2D_3$-mediated production of hCAP18 upregulates the expression of autophagy-related genes such as Atg5 and Beclin-1 in monocytes/macrophages and enhances the fusion of phagosome containing *M. tuberculosis* with lysosome [59]. Similarly, another study has reported that mycobacterial lipoprotein LpqH stimulates the synthesis of $1,25(OH)_2D_3$ and production of cathelicidin as well as autophagy induction through the activation of TLR2/1 signalling in monocytes [74]. A recent study revealed that $1,25(OH)_2D_3$ induces autophagy in human immunodeficiency virus-1 (HIV-1) infected macrophages by upregulating the expression of Atg5 and Beclin-1 through the activation of phosphatidylinositol 3-kinase signaling pathways [75]. Further, $1,25(OH)_2D_3$ mediated cathelicidin production act as a crucial factor for autophagic flux, which reduces the survival of *M. tuberculosis* and HIV replication in macrophages [76]. The bacterial degradation product following autophagy is loaded on the MHC class II molecule, which may help to develop efficient adaptive immunity by stimulating CD4+ cell response against pathogens [77]. These studies suggest that $1,25(OH)_2D_3$ help to remove pathogen mediated block in phagosome-lysosome fusion by activating autophagy pathways in macrophages and play a potent role in the control of intracellular growth of *M. tuberculosis*.

Vitamin D and antigen presentation

Based on the interaction between macrophages with specific cytokines, macrophages are classified into two groups such as classically activated macrophages (CAMs) and alternatively activated macrophages (AAMs) [78, 79]. CAMs interact with IFN-γ and TNF-α and mediate more efficient antigen presentation as well as release of pro-inflammatory mediators. [79]. AAMs are generated by Th2 cytokines such as IL-4 and IL-13 [80] and are less efficient antigen presenting cells due to reduced MHC class II expression and mediate anti-inflammatory response by producing IL-10 and TGF-β [78, 79, 81]. Antigen presenting cells such as macrophages are the principal target for $1,25(OH)_2D_3$ mediated actions. $1,25(OH)_2D_3$ suppresses the antigen-presenting capacity of monocytes and macrophages by downregulating the expression of MHC class II and co-stimulatory molecules such as CD40, CD80 and CD86 and inhibits the T cell activation [27]. It has been shown that $1,25(OH)_2D_3$ downregulate the differentiation of monocytes into dendritic cells and its maturation [25, 82]. A recent clinical study revealed that nutritional supplementation of vitamin D enhanced the antigen presenting potential of monocytes of TB contacts; however, a similar result was not observed in active TB patients [83].

Effect of vitamin D on inflammatory responses

The pro-inflammatory and anti-inflammatory functions of macrophages are determined by the interactions with Th1 or Th2 cytokines. During infection, various pro-inflammatory cytokines such as IL-1, IL-6, TNF-α, IL-8, IL-12 and IFN-γ are produced by macrophages and T-cells. These cytokines activate macrophages to eliminate the intracellular pathogen and enhance Th1 and Th17 immune responses against *M. tuberculosis* [84, 85]. It has been shown that $1,25(OH)_2D_3$ suppresses the production of pro-inflammatory cytokines such as IL-1, IL-6, TNF-α, IL-8, IL-12 and IFN-γ by upregulating the expression of MAPK phosphatase-1 (MKP-1) and IκBα, which inhibit the nuclear factor-kappa B (NF-κB) activity in monocytes/ macrophages [86-90], whereas it upregulates the expression of anti-inflammatory cytokine IL-10 [91] and may enhance the emergence of anti-inflammatory alternatively activated macrophages. It has been reported that *M. tuberculosis* infected macrophages enhance the expression of chemokines such as monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), MIP-1β and regulated upon activation, normal T-cell expressed and secreted (RANTES), monokine induced by interferon-γ (MIG) and interferon gamma inducible protein-10 (IP-10) [92,93], that are involved in the recruitment of immune cells at the site of infection and granuloma formation in tuberculosis. However, it is reported that $1,25(OH)_2D_3$ treatment diminished the *M. tuberculosis* culture filtrate antigen stimulated MIG and IP-10 chemokine mRNA expression in monocytes/macrophages cultures [93] and this decreased expression could be due to $1,25(OH)_2D_3$ mediated downregulation of IFN-γ production. In addition, it is demonstrated that $1,25(OH)_2D_3$ also suppresses the expression of matrix metalloproteinase-7 (MMP-7), MMP-9 and MMP-10 in monocytes [91] that might aid to reduce the pathogen-mediated tissue injury and inflammation.

Conclusion

1,25-dihydroxyvitamin D_3 regulates immune functions of macrophages through binding with vitamin D receptor. The anti-microbial functions of macrophages such as phagocytosis, production of reactive oxygen/nitrogen intermediates, antimicrobial peptide synthesis and autophagy has been upregulated by $1,25(OH)_2D_3$ thus help to inhibit the growth of intracellular mycobacteria. In addition, $1,25(OH)_2D_3$ also suppresses the macrophage inflammatory response by downregulating the production of pro-inflammatory cytokines and chemokines. This suggests

that adjunct vitamin D supplementation along with a standard TB regime could enhance the innate immune functions of the macrophage and accelerate the early resumption from the active disease.

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Conflict of interest

This work was not funded by any International Agencies. The authors do not have any financial conflict of interest related to this manuscript.

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