*European Journal of Microbiology and Immunology 3 (2013) 4, pp. 237–240*
*DOI: 10.1556/EuJMI.3.2013.4.1*

# MECHANISMS UNDERLYING EFFECTS OF 1,25-DIHYDROXYVITAMIN $D_3$ ON THE TH17 CELLS

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Received: July 17, 2013; Revised: August 18, 2013; Accepted: August 21, 2013

Th17 cells, a class of $CD4^+$ T cells, have been identified as novel effector cells, which play a pivotal role in several inflammatory and autoimmune diseases. 1,25-Dihydroxyvitamin $D_3$ ($1,25(OH)_2D_3$), the active form of vitamin D, has emerged as a direct regulator of immune system function in humans. Accumulating reports demonstrated that $1,25(OH)_2D_3$ possessed anti-inflammatory activity on Th17 cells to maintain immunologic homeostasis. This report will review the novel immune regulatory role of $1,25(OH)_2D_3$ in its potential use for Th17 cell-related inflammatory and autoimmune conditions.

**Keywords:** $1,25(OH)_2D_3$, VDR, Th17, immune response

## Introduction

1,25-Dihydroxyvitamin $D_3$ ($1,25(OH)_2D_3$) is a central regulator of mineral absorption among its other pleiotropic functions including immunomodulatory activities. $1,25(OH)_2D_3$, the active vitamin D metabolite, has been rediscovered as a modulator of a variety of cells, including cells of the immune system, especially the $CD4^+$ T cells. In relation to the Th1/Th2 balance, $1,25(OH)_2D_3$ showed its immunomodulating activities through their direct effect on naive $CD4^+$ T cells and their indirect effect on dendritic cells (DCs) [1, 2]. It has also been demonstrated that the administration of $1,25(OH)_2D_3$ prevented Th1-mediated autoimmune diseases in animal models [3]. Interleukin 17 (IL-17)-producing helper T cells (Th17 cells) are often present at the sites of tissue inflammation in autoimmune diseases, which has led to the conclusion that Th17 cells are main drivers of autoimmune tissue injuries [4]. Although it is also known that $1,25(OH)_2D_3$ modulates the inflammation and Th17-mediated autoimmunity, little is known about the molecular mechanisms mediating the effects of $1,25(OH)_2D_3$. Here, we described the mechanisms of the inhibitory effects by $1,25(OH)_2D_3$ on Th17 cells, indicating that a promising therapy using $1,25(OH)_2D_3$ would be a novel and beneficial strategy to overcome many of the autoimmune diseases.

## The role of Th17 cells in mucosal inflammation

Th17 cells are a subset of $CD4^+$ T helper cells. Th17 cells produce IL-17A and to a lesser extent IL-17F, IL-22, IL-21, tumor necrosis factor (TNF-α), and IL-6. Studies have shown that IL-6 and transforming growth factor-beta1 (TGF-β1) can promote murine Th17 cells development while IL-23 maintains these cells to the Th17 lineage. Orphan nuclear receptor (RORγt, also described as RORC) is the master transcription factor guiding Th17 differentiation. RORγt also synergizes with other transcription factors including STAT3, ROR-α for appropriate development of Th17 cells [5]. Th17 cells have a critical role in host defense against infection by recruiting neutrophils and macrophages to infected tissues and have also been considered as a key player in autoimmune inflammatory diseases, especially in inflammatory bowel disease (IBD). Cross talk between Th1 and Th17 cells effector responses also occurs since Th17 associated cytokines (IL-21 and IL-22) can promote Th1 responses and exacerbate intestinal inflammation. IL-23 was another cytokine of Th17 cells, which can enhance immune responses in Th17 and Th1 cells, resulting in gastrointestinal tract inflammation [6]. In addition to Th1 and Th2 cells effector immune

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*ISSN 2062-509X / $ 20.00* 

response, Th17 cells also play an important role in IBD pathogenesis.

## Effects of vitamin D on the immune system

Recently, important progresses have been made in understanding the noncanonical activities of vitamin D in the immune cells function, especially in the $CD4^+$ T cells [7]. The effects of $1,25(OH)_2D_3$ on immune cells are mediated through vitamin D receptor (VDR), a nuclear receptor, which is expressed by macrophages, dendritic cells, and activated T cells. Among the various T cells, elevated VDR expression is found on differentiated Th17 cells [8]. $1,25(OH)_2D_3$ is able to prevent autoimmune inflammatory diseases, such as multiple sclerosis, type 1 diabetes, and IBD, partially owing to its suppressive effect directly on Th17 cells [9]. As a consequence of these observations, much attention has focused on a possible role of VD/VDR as a target in Th17 cells.

## The suppressive effects of $1,25(OH)_2D_3$ on Th17 cells

### *$1,25(OH)_2D_3$ suppresses the bioactivity of Th17 cells*

Recent studies have highlighted a central role of the Th17 cells cytokine network in mediating the immune responses. The IL-17 cytokines, IL-17A to IL-17F, are critical players in the innate and adaptive immunity. Substantial data support the role of these cytokines in host defense and inflammatory diseases. Of these family members, IL-17A is the main cytokine produced by Th17 cells. IL-17A is upregulated at sites of inflammation in several autoimmune diseases including multiple sclerosis, rheumatoid arthritis, psoriasis, and IBD. IL17A also synergizes with other cytokines, such as TNF-α, to augment the pro-inflammatory responses [10]. In addition, numerous preclinical and clinical studies have shed a new light on the link between IL-17A and the pathogenesis of inflammatory diseases, and therefore, a number of therapeutic measures targeting IL-17A are promising in the future.

$1,25(OH)_2D_3$ may be a promising tool to suppress IL-17A production. $1,25(OH)_2D_3$ inhibits IL-17A production in T cells from multiple sclerosis and from early rheumatoid arthritis patients [11]. The ability of $1,25(OH)_2D_3$ to reduce colitis has also been correlated to a suppression of IL-17A induction in a mouse model, such as 2,4,6-trinitrobenzene sulfuric acid (TNBS) colitis and early rheumatoid arthritis [12, 13]. $1,25(OH)_2D_3$ attenuated the production of IL-17A in *Candida albicans* stimulated peripheral blood mononuclear cell (PBMC) [14]. The immunosuppressive function of $1,25(OH)_2D_3$ may be through downregulation of IL-17A, IL-6, and IL-22 expression from both naive and memory human $CD4^+$ T cells [15]. All-trans retinoic acid (ATRA) and $1,25(OH)_2D_3$ could effectively inhibit the generation of IL-17-producing $CD4^+$ T cells during the development from naive T cells [16]. Additionally, murine recombinant IL-17A (mIL-17A) secretion was also inhibited by $1,25(OH)_2D_3$ in $CD8^+$T cells and in NKT cells, which also produce IL-17A [17].

### *$1,25(OH)_2D_3$ inhibits the differentiation and maintenance of Th17 cells*

TGF-β1 induces the Treg-specific transcription factor forkhead box P3 (Foxp3) expression and is required for the differentiation and maintenance of regulatory T cells (Treg cells). However, addition of IL-6 to TGF-β1 inhibits the generation of Treg cells and induces Th17 cells to generate [18]. On the basis of these data, IL-6 is proposed as a pivotal factor in dictating the balance between Treg cells and Th17 cells. A large body of reports indicated that $1,25(OH)_2D_3$ is able to suppress the productions of IL-6. The study of Khoo using human PBMC stimulated by *C. albicans* found that $1,25(OH)_2D_3$ had a distinct propensity to attenuate the production of IL-6 in the PBMC as well as IL-17A [14]. In Th17-rheumatoid arthritis synovial fluid (Th17-RASF) cocultures, Hamburg observed that the proinflammatory cytokine IL-6 production, together with IL-17A, was reduced by $1,25(OH)_2D_3$ [19, 20]. Daniel and his colleagues found that proinflammatory cytokines, such as IL-6 and IL-17A, were significantly up-regulated in acute TNBS colitis, and both dexamethasone and calcitriol significantly down-regulated the levels of IL-6 and IL-17A, and whereas, furthermore, the calcitriol–dexamethasone combination resulted in a strong inhibition of the two cytokines expressions [21]. However, data showed that $1,25(OH)_2D_3$ remarkably blocked IL-17A expression in human memory $CD4^+$ T cells, but did not suppress IL-6R messenger RNA (mRNA) expression. IL-6 was not detected [15]. Therefore, whether $1,25(OH)_2D_3$ downregulated IL-17A level when IL-6 was suppressed has not been completely understood.

TGF-β1 has dose-dependent effects on Th17 cells development, as it promotes Th17 cells differentiation at lower concentrations, while suppresses them at higher concentrations by inducing Foxp3 expression [21]. Several lines of evidence indicated that by up-regulating TGF-β1 level, $1,25(OH)_2D_3$ suppressed the development of Th17 cells. With a model of TNBS-induced colitis, Daniel indicated that calcitriol immunotherapy lead to a significant and distinct induction of TGF-β1 as well as IL-10 and Foxp3 levels, resulting in promoting the function of Treg cells, which in turn inhibited Th17 cells function [22].

Although Th17 cells are thought to be pathogenic, accumulating data indicate the existence of nonpathogenic IL-17-producing Th17 cells [4]. TGF-β1 plus IL-6 differentiated naive T cells into Th17 cells; these T cells were not pathogenic unless they were further exposed to IL-23, which has a profound impact on the maintenance of Th17 cells. It has been shown that T cells cultured in the presence of TGF-β1 plus IL-6 did not induce tissue inflam-

mation unless they are further cultured in the presence of IL-23. The development of gut inflammation in T cell-deficient mice was dependent on IL-23; the inhibition of IL-23 mediated by $1,25(OH)_2D_3$ led to a decrease in gut inflammation [8]. Also, lymph nodes cells of mice treated with calcitriol had reduced expression of message for IL-23p19 compared with control [11].

It is shown that the endogenous cytokine TGF-β3 was produced specifically by developing Th17 cells in an IL-23-dependent manner that was important for driving the pathogenic Th17 phenotype. Similarly to TGF-β1-induced Th17 cells, TGF-β3 also induced Th17 cells production in combination with IL-6. Studies have shown that IL-23 was critical for enhancing the expression of and/or maintaining the endogenous amount of TGF-β3 in developing Th17 cells. Therefore, Th17 cells induced by TGF-β3 and IL-6 were pathogenic [4]. However, little evidence demonstrates the effect of $1,25(OH)_2D_3$ on TGF-β3.

### *$1,25(OH)_2D_3$ inhibits the transcription of Th17 cells*

IL-17A, similar to most T cell-derived cytokines, is regulated at least in part at the level of transcription. Thus, $1,25(OH)_2D_3$ may mediate the transcription factor expression to suppress Th17 cells functions, which is also the possible mechanisms involved in the repression of Th17 cells functions by $1,25(OH)_2D_3$ [17].

Many reports have indicated that RORγt, mainly located in the nuclear of the immune cells, is critical for the differentiation of mouse and human Th17 cells. Previous studies have suggested a supporting role for vitamin D in reducing the production of RORγt. $1,25(OH)_2D_3$ is known to inhibit the development of experimental autoimmune encephalomyelitis (EAE; a mouse model of multiple sclerosis), and recent studies have correlated a suppressive effect of $1,25(OH)_2D_3$ on mouse Th17 cells with a suppression of RORγt, finally to protect against EAE *in vivo* [23]. In addition, studies have found that RORC mRNA expression was suppressed by $1,25(OH)_2D_3$ in human T cells [24].

Other transcription factors may also participate in $1,25(OH)_2D_3$-mediated suppression of IL-17A expression. Recent data have indicated that Runt-related transcription factor 1 (Runx1) binds to and acts together with RORγt during mIL-17A transcription [25]. It was also demonstrated that Foxp3 can interact with RORγt as well as with Runx1 to inhibit the production of IL-17A [26]. Thus, by stimulating the expression of Foxp3, $1,25(OH)_2D_3$ may also result in suppression of IL-17A production by increasing Foxp3 interaction with RORγt, which was consistent with the previous papers indicating that $1,25(OH)_2D_3$ and ATRA promoted Treg differentiation. In addition, VDR can sequester the RORγt coactivator, Runx1, and thereby it inhibits the transcriptional activity of RORγt [18].

It has been reported that the transcription factors nuclear factor for activated T cells (NFAT) is important for the T cell receptor (TCR)-mediated transcriptional regulation of IL-17A. NFAT plays a key role in the regulation of endogenous human recombinant IL-17A (hIL-17A). Data have shown that the negative effect of $1,25(OH)_2D_3$ on IL-17A involves blocking of NFAT level [27].

## Therapeutic perspective

Deficiency in vitamin D is associated with numerous health conditions ranging from bone health to cancer. However, a renewed interest in the impact of vitamin D on Th17 cells has ensued. Considering the broad physiological relevance of vitamin D, it may potentially become a treatment method for autoimmune diseases, especially for IBD. A recent report indicated that the ability of $1,25(OH)_2D_3$ to reduce the expression of IL-17A, IL-22, IL-23, and RORγt depended on the concentration of $1,25(OH)_2D_3$. At physiological concentration (0.1–10 nM), $1,25(OH)_2D_3$ could not suppress these inflammatory cytokines. Although higher concentration of $1,25(OH)_2D_3$ (above 10 nM) reduced the factors, $1,25(OH)_2D_3$ circulates in very low concentration (about 25–275 pM). Even with dietary vitamin supplement or sun exposure, they rarely reach 10 nM of concentration [12]. Therefore, it is too hard to reach such high levels of $1,25(OH)_2D_3$ in circulation. Furthermore, if we get to adequate serum levels throughout life to alleviate many of the autoimmune diseases that befall us as we use, the biggest obstacle to clinical use of vitamin D is its potent hypercalcemic effect. In conclusion, interventional studies to further quantify proper dosage of vitamin D of immunomodulatory effects or develop its analogues without hypercalcemia in humans badly needs to be done.

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