Harnessing dendritic cells to promote immune tolerance: Opportunities for allergen-specific immunotherapy
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Citation for published version (APA):
Bakdash, G. (2013). Harnessing dendritic cells to promote immune tolerance: Opportunities for allergen-specific immunotherapy
VITAMIN D3 METABOLITE CALCIDIOL PRIMES HUMAN DENDRITIC CELLS TO PROMOTE THE DEVELOPMENT OF IMMUNOMODULATORY IL-10-PRODUCING T CELLS

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Manuscript in preparation
ABSTRACT

Vitamin D is recognized as a potent immunosuppressive drug. The suppressive effects of vitamin D are attributed to its physiologically active final metabolite 1,25-dihydroxy vitamin D3 (calcitriol), which was shown, to prime dendritic cells (DCs) to promote the development of regulatory T (Treg) cells. Despite the potential benefit in treating autoimmune diseases, clinical application of calcitriol is hindered by deleterious side effects manifested by hypercalcemia and hypercalciuria. Conversely, the physiological precursors of calcitriol, vitamin D3 (cholecalciferol) and its first metabolite 25-hydroxy vitamin D3 (calcidiol) are widely applied in the clinic due to their low calcimic burden. However, the mechanisms by which cholecalciferol and calcidiol may modulate adaptive immunity remain elusive. This prompted us to unravel the immunosuppressive capacity of these precursors by assessing their influence on DC functions and the subsequent polarization of naïve CD4+ T cells. In this study we show that, whereas cholecalciferol has insignificant effects on DC maturation and cytokine production, it only weakly primed DCs to induce suppressive T cells. However, like calcitriol, calcidiol not only exerted an inhibitory effect on DC maturation and altered DC cytokine production, but also primed DCs to promote the development of IL-10-producing Treg cells that act as regulatory T cells suppressing the proliferation of bystander cells. Strikingly, in contrast to the population of IL-10-producing Treg cells induced by calcitriol-primed DCs, the IL-10-producing Treg cells induced by calcidiol-primed DCs exhibited sustained IFN-γ production in face of their suppressive capacity. Experiments with the steroid synthesis inhibitor ketoconazole indicated that the immunomodulatory features of the precursors are dependent on their conversion into calcitriol. Collectively, calcidiol is a potent immune modulator, which may be more adequate than calcitriol for the treatment of chronic inflammatory diseases, since it is less hypercalcimic. This may be of particular interest for the treatment of allergic disease, where concurrent suppression and sustained IFN-γ production by Treg cells effectively counterbalance the Th2-dominated immune responses.

ABBREVIATIONS

DC  dendritic cell
MF  maturation factors
Treg  regulatory T cell
SIT  allergen-specific immunotherapy
INTRODUCTION

Best known for its vital role in calcium homeostasis and bone metabolism, vitamin D influence extends to cover a broad spectrum of biological processes. Amongst others, vitamin D deficiency is correlated to high risk of developing certain chronic inflammatory disorders, including multiple sclerosis, type I diabetes and Crohn’s disease (1). In addition, the vitamin D active metabolite, 1,25-dihydroxyvitamin D3 (calcitriol) was shown to be a potent immunosuppressant with beneficial effects in mouse models of inflammatory diseases (2-6). In spite of its vast immunomodulatory effects, systemic application of calcitriol to prevent and cure chronic inflammatory disorders is hindered by deleterious side effects manifested mainly by hypercalcemia and hypercalciuria. Factually, clinical application of calcitriol and its analogues as immunosuppressants is limited to the topical treatment of psoriasis (7,8). This obstacle was resolved by using calcitriol-related forms of vitamin D, which may maintain the immunological effects of calcitriol, but bare minimal hypercalcemic liability. Among the closest calcitriol relatives are the two calcitriol precursors: vitamin D3 (cholecalciferol) and 25-hydroxyvitamine D3 (calcidiol). Calcitriol, the active form of vitamin D, is physiologically generated from cholecalciferol and calcidiol through a sequential hydroxylation process mediated by the P450 enzymes CYP27A1 and CYP27B1 respectively (9), which mainly takes place in the liver and kidneys. However, active calcitriol synthesis from its precursors can also happen in immune cells like the antigen-presenting dendritic cells (DCs) and macrophages, which were shown to express the required enzymatic machinery (10,11). The binding affinity of these precursors to the vitamin D receptor (VDR), through which calcitriol exerts its effects, is minimal in comparison to calcitriol (12). Although this suggests that they lack both beneficial and side effects, a growing number of studies demonstrated calcidiol-specific functionality, independent of its transformation into calcitriol (13-15). Furthermore, and due to its low calcimic burden, cholecalciferol is the most commonly used form of vitamin D in clinical trials, proving both safe and effective (16-18).

Studies on the mechanisms of immunosuppression of vitamin D have mainly been performed using calcitriol and demonstrated marked inhibitory effects on adaptive immune responses. This inhibition is exerted through two routes: direct effect on T cells and indirect inhibition via DCs. Calcitriol directly affects T cells by inhibiting their proliferation and expression of IL-2 and inflammatory cytokines (19-21), and promoting their development into regulatory T (Treg) cells from naïve precursors (22,23). Alternatively, calcitriol has an indirect effect on T cell function by inhibiting the expression of MHC-II and co-stimulatory molecules by DCs, leading to a reduced immunostimulatory capacity (24,25). Additionally, calcitriol endows DCs with tolerogenic qualities by inhibiting the secretion of pro-inflammatory IL-12 and increasing the secretion of anti-inflammatory IL-10 and the expression of immunoglobulin-like transcript 3 (ILT3) and programmed death-1 ligand (PD-L1), which inhibits the development of inflammatory of Th1 effector cells and promotes the development of Treg cells (26-29). On the other hand, little is known about the immunological implications of calcitriol’s precursors. Calcidiol was recently reported to inhibit DC maturation, inflammatory cytokine production and T cell stimulatory capacity (30). However, many questions concerning the putative role of cholecalciferol and calcidiol in inducing immune tolerance remain unanswered. This prompted us to investigate
whether calcidiol and cholecalciferol can modify DCs and grant them tolerogenic attributes allowing them to induce Treg cells. In this study we report that cholecalciferol had marginal inhibitory effects on DCs and the subsequently induced T cells. On the other hand, calcidiol-primed DCs were capable of promoting the development of IL-10-producing Treg cells, albeit these cells sustained their IFN-γ expression. The regulatory properties of induced T cells were completely dependent on transforming calcidiol into calcitriol in DCs. These findings may be of clinical significance in the treatment of allergic diseases, where Th2-dominated immune responses can be overridden by concurrent suppression and IFN-γ production resulting from cholecalciferol and calcidiol application.

MATERIALS AND METHODS

**In vitro generation and activation of DCs.** Monocyte-derived DCs were generated as described previously (31). Immature DCs were stimulated for 48h with IL-1β (10 ng/ml) and TNF-α (25 ng/ml), both purchased from Miltenyi Biotech (Bergisch Gladbach, Germany) in the presence or absence of optimal concentrations of: calcitriol (2.5 µM), calcidiol (0.1 µM) or cholecalciferol (0.1 µM), all purchased from Sigma-Aldrich (St. Louis, MO). When indicated Ketoconazole (5 µM) (Sigma-Aldrich) was also added. Mature DCs were analyzed for the expression of cell-surface molecules by flow cytometry with anti-CD86 allophycocyanin (APC), anti-CD83–phycoerythrin (PE), anti–HLA-DR–peridinin-chlorophyll-protein (PerCP) and anti-CD14–Fluorescein isothiocyanate (FITC); all purchased from BD Biosciences (San Jose, CA).

**Analysis of cytokine production by DCs.** Mature DCs (20x10³) were stimulated with 20x10³ CD40 ligand–expressing murine plasmacytoma cells (J558; a gift from Dr P. Lane, University of Birmingham, Birmingham, United Kingdom). ELISAs were performed to determine concentrations of IL-12p70 (Clone 20C2), IL-10 (BD Biosciences), and TNF-α (eBioscience, San Diego, CA) in 24-hour culture supernatants, as previously described (31).

**Isolation of naïve and memory CD4⁺ T cells.** Human PBMC were isolated from heparinized human peripheral blood by density gradient centrifugation on Lymphoprep (Nycomed, Glattpark-Opfikon, Switzerland). The total CD4⁺ T cell population was first isolated from PBMC by negative magnetic selection using MACS CD4⁺ T cell isolation kit (Miltenyi Biotech). Naïve CD45RA⁺CD45RO⁻CD4⁺ T cells were separated from memory T cells by applying anti-CD45RO-PE (Dako Cytomation, Glostrup, Denmark) and anti-PE beads (Miltenyi Biotech). Purity levels higher than 98% were achieved, determined by flow cytometry.

**Stimulation of naïve CD4⁺ T cells.** Primed DCs were washed extensively before co-culture with naïve CD4⁺ T cells at a ratio of 1:4 in Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 10% (v/v) fetal calf serum (FCS) (Lonza, Cologne, Germany) containing superantigen Staphylococcus aureus enterotoxin B (SEB, 10 pg/mL; Sigma), as described previously (31). At day 5 proliferating T cells were refreshed with medium supplemented with 20 U/ml of IL-2 (Chiron, Emeryville, CA). When resting (around day 11), the expression of Foxp3 was determined by performing an intracellular staining using Foxp3 staining kit (Biolegend, San Diego, CA) along with surface staining of CD127.
CALCIDIOL PROGRAMS DCs TO PROMOTE IMMUNEMODULATION

Furthermore, resting T cells were restimulated phorbol 12-myristate 13-acetate (PMA) (100 ng/mL)/ionomycin (1 µg/mL) in the presence of brefeldin (10 µg/ml) (all purchased from Sigma-Aldrich) and analyzed for expression of IFN-γ and IL-4 by using anti-IFN-γ-FITC and anti-IL-4-APC (both from BD Biosciences). In parallel, 100x10^3 T cells were restimulated with plate bound anti-CD3 (16A9, 1 µg/ml) and anti-CD28 (ISE8, 1µg/ml) (both purchased from Sanquin research Amsterdam, the Netherlands). 24 hours supernatants were taken for analysis of IL-10 and IFNγ (U-Cytech, Utrecht, the Netherlands) by ELISA.

To determine T cell proliferation induced by DCs, 11 KBq/well [3H]-TdR (Radiochemical Center, Amersham, Little Chalfont, U.K.) was added on day 3-5 of co-culture of naïve CD4+ T cells (50x10^3) with the indicated numbers of DCs. The incorporated [3H]-TdR was measured after 16h by liquid scintillation spectroscopy.

**T cell suppressor assay.** The T cells induced on co-culture with DCs (test cells) were harvested after 5 days, extensively washed, irradiated (30 Gy) to prevent expansion, and stained with the cell-cycle tracking dye PKH-26 (11.8 µM, Sigma-Aldrich). Memory T cells, from the same donor as test cells, were purified as mentioned above, labeled with 5,6-carboxy fluorescein diacetate succinimidyl ester (CFSE) (0.5 µM; Molecular Probes, Eugene, OR) and subsequently used as bystander target cells. Test cells (50x10^3) were co-cultured with 25x10^3 target cells and 1000 MF-matured DCs. After 5-7 days, the proliferation of the target T cells was determined by flow cytometry.

**Flow cytometry.** Flow cytometry analysis was performed on FACS Canto II (BD Biosciences, Franklin Lakes, NJ). Data analysis was done using FlowJo software (Tree Star, Ashland, OR).

**Statistics.** Data are presented as mean±SEM. Student t tests were performed for paired measurements with GraphPad Prism software (GraphPad, La Jolla, CA). Values of P < 0.05 were considered significant.

**RESULTS**

**Calcitriol precursors inhibit DC maturation and alter DC cytokine production**

Previous studies have indicated that calcitriol inhibits DC maturation and modulates the cytokine profile of DCs. However, the effects of the two calcitriol precursors, calcidiol and cholecalciferol, on DCs remain elusive. To test their effect on DC maturation, human monocyte-derived DCs were activated by a combination of TNF-α and IL-1β, referred to as maturation factors (MF), in the presence or absence of calcitriol or its precursors. In line with the effects of calcitriol, both calcidiol and cholecalciferol inhibited DC maturation as reflected by reduced upregulation of MHC-II and the co-stimulatory molecule CD86, complete ablation of the induction of the maturation marker CD83, and partial persistence of the monocyte marker CD14 (Fig. 1A). Whereas the levels of inhibition by calcitriol and calcidiol were comparable, the effect of cholecalciferol was less pronounced for all parameters.

Subsequently, we determined the effect of DC priming by the vitamin D and its metabolites on the ability of DCs to produce cytokines following stimulation by CD40 ligation. Similar to the priming by calcitriol, priming by calcidiol significantly reduced the production of pro-
Figure 1. Calcitriol precursors inhibit DC maturation and alter DC cytokine production. (A) CD14, CD83, CD86, and HLA-DR expression by unstimulated (dashed histograms), MF-primed (open histograms) and MF/calcitriol-, MF/calcidiol-, MF/cholecalciferol-primed (filled histograms) DCs. (B) Production of IL-12, TNF-α and IL-10 by MF-, MF/calcitriol-, MF/calcidiol-, MF/cholecalciferol-primed DCs after 24 h stimulation by CD40 ligation. The upper panel shows IL-12 and TNF-α fold reduction and IL-10 fold induction compared to the MF condition. The lower panel is a representative experiment. Results are a representative out of 6 (A) or 5-8 (B lower panel) independent experiments, or the mean±SEM of 5-8 independent experiments (B upper panel). * P < 0.05, ** P < 0.01, *** P < 0.001.
inflammatory TNF-α and induced the production of anti-inflammatory IL-10. Surprisingly, in contrast to calcitriol priming, calcidiol priming did not affect the production of Th1-polarizing cytokine IL-12 (Fig. 1B). Notably, cholecalciferol priming did not affect the ability of production of any of these cytokines. Collectively these data show that calcidiol is as adequate as calcitriol in modulating MF-induced DC maturation and cytokine production, albeit a difference in the ability to produce IL-12, suggesting variations in the ability of calcitriol and calcidiol to prime DCs to induce the development of effector T cells.

**Calcidiol- or cholecalciferol-primed DCs induce the development of Treg cells**

To compare the effect of the priming of maturing DCs by the various vitamin D forms on the ability of DCs to support the T cell development, the differently primed DCs were co-cultured with allogeneic naïve CD4+ T cells. MF-activated DCs strongly promoted T cell proliferation, but the mere presence of calcitriol or calcidiol during DC maturation significantly hampered their T cell stimulating capacity of DCs (Fig. 2A). In line with data on the weak effects of cholecalciferol on the DC phenotype, the presence of cholecalciferol treatment during DC maturation did not significantly influence the capacity of DCs to induce T cell proliferation.

The reduced T cell stimulatory capacity displayed by calcidiol-treated DCs may be explained by the presence of Treg cells, as it was shown previously (32) for calcitriol-primed DCs. In order to determine to what extent T cells induced by calcidiol- or cholecalciferol-primed DCs have regulatory qualities, we tested their capacity to inhibit the proliferation of bystander activated CD4+ T cells. T cells generated by DCs primed by any of the three forms of vitamin D were able to suppress proliferation of bystander activated T cells. Compared to calcitriol, calcidiol was weaker and cholecalciferol was much weaker in their ability to prime DCs to promote Treg cells (Fig. 2B). Overall, this data suggest that calcitriol precursors can prime DCs to induce the development of Treg cells, albeit less efficiently compared to calcitriol itself.

**Calcidiol-primed DCs induce L-10-producing Treg cells that maintain IFN-γ production**

Calcitriol-primed DCs were shown to promote the development of IL-10 producing Treg cells (32). In order to determine whether calcitriol precursors had the same effect, the phenotype of induced Treg cells was characterized. First, the intracellular expression of the transcription factor Foxp3, which is associated with a subset of Treg cells, in induced T cells was assessed. Just like calcitriol, neither calcidiol- nor cholecalciferol-primed DCs were able to promote Foxp3+ Tregs (Fig. 3A). Next, we analyzed IL-10 levels in supernatants of these T cells upon restimulation when resting after a first cycle of expansion, with αCD3/αCD28 antibodies. Interestingly, calcitriol, and to a lesser extent, calcidiol could prime DCs to promote IL-10 expression in the induced effector T cells (Fig. 3B). In line with their weak suppressive capacity, T cells induced by cholecalciferol-primed DCs were poor producers of IL-10, which was not significantly higher compared to the MF control.

Thus far, calcitriol precursors induced a pattern of effects similar, albeit less efficient, to calcitriol. Earlier studies clearly demonstrated that Treg cells promoted by calcitriol-primed DCs are characterized by abrogated expression of the inflammatory Th1 cytokine IFN-γ (26,29,32). Interestingly, in sharp contrast to calcitriol-priming of DCs, which strongly downregulated IFN-γ
Figure 2. Calcitriol precursors prime DCs to promote the development of suppressive T cells. (A) Proliferation of allogenic naïve CD4$^+$ T cells stimulated by DCs that were primed with MF in the presence or absence of calcitriol, calcidiol or cholecalciferol. Data are shown as the mean±SEM of triplicates of one representative experiment out of 5 independent experiments. (B) Suppressive capacity of T cells primed by MF/calcitriol, MF/calcidiol-, or MF/cholecalciferol-primed DCs expressed as the percentage of proliferating cells relative to the MF condition. Results are a representative (lower panel) or the mean±SEM (upper panel) of 9 independent experiments. **$P < 0.01$, ***$P < 0.001$. 
expression, calcidiol- or cholecalciferol-priming of DCs did not influence the percentages of IFN-γ+ T cells (Fig. 3C), or the levels of secreted IFN-γ (Fig. 3D), as they were both comparable to those of the MF control. Moreover, the same pattern in IFN-γ expression was found in memory CD4+ T cells when they were activated by calcitriol-, calcidiol- or cholecalciferol-primed DCs (Fig. 3C low panel). IFN-γ, the Th1-hallmark cytokine, is induced by several factors among which IL-12 is the most important. As shown above, compared to calcitriol, calcidiol and cholecalciferol did not significantly inhibit the production of IL-12 by MF-matured and CD40-activated DCs. This suggests that the induction of IFN-γ in T cells, induced by cholecalciferol- or calcidiol-primed DCs resulted from persistent IL-12 production by these DCs. In order to test this possible mechanism, IL-12 was neutralized in the DC and T cell co-cultures by applying αIL-12 antibody. IL-12 blocking crippled the ability of cholecalciferol- or calcidiol-primed DCs to induce IFN-γ in T cells as demonstrated by reduced of IFN-γ+ T percentages, which were comparable to those of calcitriol-primed DCs (Fig. 3 E). Altogether, these findings show that analogous to calcitriol, calcidiol programs DCs to induce the development of IL-10+ Treg cells, but does not regulate the induction of IFN-γ expression in these T cells.

**The immunomodulatory effects of calcidiol and cholecalciferol depend on their conversion into active calcitriol**

Although they vary in efficacy, the effects of calcidiol on DC phenotype and function in promoting IL-10-producing Treg cells bare a lot of similarities to those of calcitriol. In previous studies monocyte-derived DCs were shown to express the enzymatic machinery required to metabolize calcitriol precursors into calcitriol, the metabolically most active form of vitamin D (10,11). In order to test the possibility that calcidiol and cholecalciferol become active as a result of their conversion into calcitriol, we blocked CYP27B1, the enzyme responsible of transforming calcidiol into calcitriol, by the addition of ketoconazole during DC priming with the precursors. Strikingly, ketoconazole dramatically reversed the calcidiol and cholecalciferol-mediated inhibition of DC maturation as demonstrated by restored expression of CD86 and MHC-II and partially abolished CD14 expression (Fig. 4A). Moreover, ketoconazole treatment depleted calcidiol- or cholecalciferol-primed DCs of their capacity to induce Treg cells as reflected by abrogated suppressive activity of induced T cells (Fig. 4B). In addition to inhibited suppression, ketoconazole also blocked the ability of calcidiol-primed DCs to enhance IL-10 production by T cells (Fig. 4C). As anticipated, blocking calcidiol and cholecalciferol transformation into calcitriol did not have any influence on IFN-γ production by T cells as reflected by unaffected total IFN-γ amounts and the percentage of IFN-γ-expressing cells (Fig. 4D). Collectively, our findings imply that the immunomodulatory effects displayed by calcidiol and cholecalciferol are completely dependent on their transformation into calcitriol.

**DISCUSSION**

In this study we show for the first time that not only calcitriol, but also the calcitriol precursor calcidiol is capable of modifying DC functions to promote the development of T cells that suppress the proliferation of bystander T cells, the functional hallmark of Treg cells. Despite
their suppressive qualities, Treg cells induced by DCs primed by calcidiol, and in particular cholecalciferol were less efficient suppressors in comparison to those induced by calcitriol-primed DCs. These varying suppressor capabilities can be attributed to the variable effects of the different forms of vitamin D on DC maturation, cytokine production and function, in which the effects of cholecalciferol were mostly insignificant.

There is no consensus on the underlying mechanisms of the cellular effects of calcidiol. Despite the low binding affinity of calcidiol to VDR, in comparison to calcitriol (12), some studies suggest that calcidiol may exert its effects by directly binding to VDR (14,15). For example, Lou et al. showed that calcidiol remained efficient in primary prostate cells of Cyp27b1-deficient mice, but lost its effect in Vdr knock-out mice (13). Alternatively, calcidiol can act after being converted into calcitriol under the effect of CYP27B1, which was also found to be expressed in immune cells of monocytic origin i.e. macrophages and DCs (10,11). This suggests that treating DCs with calcidiol will eventually lead to the production of calcitriol, which would exert its inhibitory effect on DCs in an autocrine fashion. In order to assess to what extent the DC-mediated conversion of calcidiol into calcitriol accounts for the observed effects of calcidiol, CYP27B1 was blocked using ketoconazole. This blockade not only reversed DC maturation inhibition but also abolished the induction of Treg cells and IL-10 production in primed T cells. Expectedly, IFN-γ production by T cells was not influenced by this blocking. Overall, this indicates that the immunomodulatory effects accomplished by priming DCs with calcidiol are actually dependent on its transformation into calcitriol.

Similar to calcidiol, cholecalciferol may also be expected to affect DCs since these cells express both CYP27A1 and CYP27B1 (10,11), required to convert cholecalciferol into calcitriol according to a two-step procedure. The marginal effects of cholecalciferol may point a very low yield of such a two-step conversion. Unfortunately, we could not test this hypothesis by priming DCs with higher doses of cholecalciferol, since cholecalciferol concentrations higher than applied in our experimental setup were cytotoxic to DCs in culture (data not shown).

The phenotype of the T cells induced by the DCs primed with different vitamin D precursors is related to cytokine profile of these DCs. The present experiments extend our previous finding
that calcitriol-primed monocyte-derived DCs express elevated IL-10 levels and promote the development of IL-10-producing Treg cells (32) by showing that calcidiol-primed DCs have similar properties. A striking difference, however, is that, in contrast to calcitriol-primed DCs that cannot produce IL-12 and induce IFN-γ-negative T cells, calcidiol-primed DCs are still able to produce IL-12 and induce the expansion of T cells with normal IFN-γ expression in an IL-12 dependent manner. The mechanisms underlying the inability of calcidiol to inhibit IL-12 production are unclear, but may arise from differential kinetics between the two forms of vitamin D3. Calcitriol was shown to inhibit induced IL-12 transcription as early as 1 hour following calcitriol application by introducing repressive epigenetic changes (33). However, calcidiol needs first to be converted into calcitriol, which will lead to a delay in the repression of IL-12 transcription. Such mechanism cannot be extended to IL-10 expression, which takes up to 24 hours to be upregulated under the effect of calcitriol (34), allowing enough time for calcidiol to be converted into calcitriol and consequently upregulate IL-10 expression. Further investigation is required to explain the difference in the effects of calcidiol and calcitriol on IL-12 production by DCs.

In this study we demonstrated that calcidiol-treated DCs prime naive T cells to produce IL-10 without inhibiting IFN-γ production in the primed T cell population. The population of IL-10-producing T cells resemble Tr1 cells that can be found in a variety conditions in vitro and in vivo at variable percentages (35). CD4+ T cells coproducing IL-10 and inflammatory cytokines, such as IFN-γ or IL-17, with regulatory properties have also been extensively described (36-38). The concurrent production of IFN-γ in the regulatory T cells may be further helpful in the treatment of allergies, which result from immune responses dominated by Th2 cells, the development and function of which is counteracted by IFN-γ. Currently the only disease-modifying treatment of mono allergies is allergen specific immunotherapy (SIT) which is based on desensitization by repetitive administration of allergens to patients (39). A substantial effort is being invested in enhancing SIT efficacy through co-administration of Th1- or Treg-driving adjuvants to counter-balance Th2 responses (5,40). Applying calcidiol as an adjuvant may potentiate SIT by tipping the immunological balance against Th2 responses through both anti-inflammatory cytokines and IFN-γ. In this respect, calcidiol may prove more beneficial than calcitriol, which...

Figure 4. The immunomodulatory effects of calcidiol and cholecalciferol depend on their conversion into active calcitriol. (A) CD14, CD83, CD86, and HLA-DR expression by MF-primed DCs (dashed histogram) and MF/calcitriol-, MF/calcidiol-, MF/cholecalciferol-primed DCs in the presence (open histogram) or absence (filled histogram) of ketoconazole. (B) Suppressive capacity of T cells primed by MF/calcidiol-, or MF/cholecalciferol-primed DCs in the absence (black bars) or presence (white bars) of ketoconazole, expressed as the percentage of proliferating cells relative to the MF condition. (C) IL-10 production by T cells induced by MF/calcidiol- or MF/cholecalciferol-primed DCs in the absence (black bars) or presence (white bars) of ketoconazole was measured upon restimulation of resting T cells by αCD3/αCD28 in 24-h supernatants. The upper panel shows the fold induction of IL-10 production compared to the MF condition. The lower panel is a representative experiment. (D) IFN-γ production (upper panel) by induced T cells was determined in 24h supernatants following restimulation by αCD3/αCD28. The percentages of IFN-γ and IL-4 producing cells (lower panel) were determined by intracellular staining of IFN-γ and IL-4, following restimulation of resting cells with PMA/ionomycin in the presence of brefeldin. Results are a representative out of 5 (A), 6 (C lower panel) or 3 (D lower panel) independent experiments, or the mean±SEM 4 (B), 6 (C upper panel) or 3 (D upper panel) independent experiments, *P < 0.05.
was shown to potentiate SIT solely through the regulatory route (5). However, harnessing the immunomodulatory effects of calcidiol in autoimmune diseases in which Th2 pathogenesis is not involved, such as multiple sclerosis and rheumatoid arthritis, may not be suitable due to the persisting IFN-γ production in primed T cells.

Calcitriol precursors are the main physiologically available forms of vitamin D. Whereas cholecalciferol is the prevailing form in the skin where vitamin D metabolism is initiated; calcidiol is the dominant form in circulation. The most advantageous serum concentration of calcidiol begins at 75 nM and the best are between 90-100 nM (41). Whereas vitamin D deficiency is defined by calcidiol serum concentrations below 50 nM, vitamin D intoxication occurs when this concentration exceeds 374 nM (1). In this study, calcidiol-induced immunomodulatory effects were achieved with concentrations falling within the physiological, non-toxic range. Our findings support previous studies correlating low calcidiol concentrations with autoimmune diseases (42-44). A major concern about clinical application of vitamin D is the calcemic liability. Several clinical trials applying calcidiol or high doses of cholecalciferol revealed no signs of hypercalcemia or hypercalceuria in treated subjects (16,18,45,46). Collectively, calcidiol may provide a safe, albeit slightly less effective, alternative to calcitriol. This precursor form would be selectively transformed into calcitriol in DCs, eventually leading to the development of Treg cells.
REFERENCE LIST


