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Vitamin D immunoregulation through dendritic cells

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Summary

Vitamin D (VD3) has been linked to immunological processes, and its supplementation may have a role in treatment or prevention of diseases with underlying autoimmune or pro-inflammatory states. As initiators of the immune responses, dendritic cells (DC) are a potential target of VD3 to dampen autoimmunity and inflammation, but the role of DC in VD3-mediated immunomodulation *in vivo* is not understood. In addition to being targets of VD3, DC can provide a local source of bioactive VD3 for regulation of T-cell responses. Here we review existing studies that describe the tolerogenic potential of VD3 on DC, and discuss them in the context of current understanding of DC development and function. We speculate on mechanisms that might account for the potent but poorly understood tolerogenic activities of VD3 and the role of DC as both targets and sources of this hormone.

Keywords: dendritic cells; inflammation; vitamin D.

Dendritic cells (DC) orchestrate the immune response by integrating and balancing environmental signals. They assess imminent danger to the host, and then initiate, direct, amplify or limit subsequent adaptive immune reactions.[1] Historically, DC have been described as motile cells within tissues that present antigen to T cells. To detect invading microorganisms or viral infections, DC sense molecular patterns that are associated with pathogens or danger, such as bacterial cell-wall-derived lipopolysaccharides, necrotic cell particles or extracellular nucleotides.[2] Significantly, DC can also monitor host or commensal metabolism and dietary components, including short-chain fatty acids and vitamin derivatives.[3]

Vitamins are organic compounds that are usually obtained from the diet and are crucial to physiological processes.[4] The steroid vitamins, vitamin A (VitA) and vitamin D3 (cholecalciferol, here abbreviated as VD3) are precursors to host nuclear hormone receptor ligands that regulate transcription. Both VitA and VD3 and their deficiencies have a tremendous impact on the immune system.[5] Vitamin A is solely derived from nutritional sources, recirculates in the intestinal environment via bile and significantly shapes intestinal mucosal immune and barrier functions.[5–7] The source, distribution and specific immunomodulating effects of VD3 are less well understood. In addition to nutritional uptake of cholecalciferol, precursors can also be formed in superficial skin layers when UVB irradiation and spontaneous isomerization non-enzymatically convert 7-dehydro-cholesterol to VD3.[8] However, VD3 does not efficiently bind the intracellular receptor vitamin D receptor (VDR). Rather, cytochrome P oxidase Cyp27a1 converts it to 25-OH-VD3 which is then further metabolized by Cyp27b1 to 1,25-OH-VD3 (calcitriol), the VD3 derivative with the highest ability to activate transcriptional activity through the canonical VDR. Classical studies have shown that Cyp27a1 is highly expressed in liver, where much of 25-OH-VD3, the primary circulating form of the vitamin, is generated. Proximal renal tubular cells are a major site of Cyp27b1, generating the active metabolite. However,

Abbreviations: 1,25-OH-VD3, calcitriol; cDC1, classical DC 1; cDC2, classical DC2; DC, dendritic cell; GM-CSF, granulocyte–macrophage colony-stimulating factor; GM-DC, subpopulation of GM-CSF-DC with DC features; GM-Mac, subpopulation of GM-CSF-DC with macrophage features; GMP, granulocyte–monocyte precursors; IL-12, interleukin-12; MHCII, MHC class II; moDC, monocyte-derived DC; pre-cDC, classical DC precursor; pre-DC, DC precursor; pre-μDC, mucosal DC precursor; RA, retinoic acid; RAR, retinoic acid receptor; Treg, regulatory T; VD3, vitamin D (as cholecalciferol); VDR, vitamin D receptor; VitA, vitamin A

recent studies show that these enzymes are also present in various other tissues and cell types, including DC (discussed below), allowing local environmental regulation of 1,25-OH-VD3 levels and responses in the immune system. In spite of the generation of VD3 from sun exposed skin, VD3 bioavailability may be insufficient as the result of habits of sun avoidance or protection and concomitantly insufficient nutritional uptake. An estimated 40–100% of US or European elderly and 1 billion people worldwide do not have sufficient VD3 levels.[8] Although severe complications of childhood VD3 deficiency, most notably rickets, are rarely seen nowadays in countries with western lifestyle, the epidemiological associations of autoimmune diseases with VD3 insufficiency point to the importance of a mechanistic understanding of the interactions of the immune system and VD3.[8,9] Clinical trials are currently ongoing, designed to critically evaluate the efficacy of VD3 supplementation in multiple sclerosis (NCT01490502) and Crohn's disease (NCT02208310).

The primary function of DC, to initiate and refine adaptive immune responses, positions them as potential therapeutic tools in diseases with skewed or missing T-cell and B-cell responses. As such, DC therapy has successfully entered the clinic for cancers,[10] where cytotoxic T cells are primed by DC differentiated from easily available circulating $CD14^+$ blood monocytes by *ex vivo* incubation with the tumour antigen PAP fused to granulocyte–macrophage colony-stimulating factor (GM-CSF). Similarly, the feasibility of DC-based immunomodulation is being explored in the setting of autoimmune diseases, where detrimental immune reactions damage host tissue.[11] Clearly, a tolerogenic approach that limited or aborted T-cell or B-cell responses to self antigens would represent a significant advance in the treatment or prevention of autoimmune disease. Here we consider the physiology and potential therapeutic application of VD3 in the context of the tolerogenic programmes and functions of DC.

Insights from culture models of DC generation from monocytes

Vitamin D3 has long been proposed as programming DC for tolerance, dampening their ability to activate effector T-cell generation, while enhancing their potential to induce anti-inflammatory regulatory T (Treg) cells. This concept, extensively reviewed,[3,5,12–18] arose from studies of GM-CSF-driven differentiation of human blood $CD14^+$ monocytes into motile cells with dendrites (moDC), capable of presenting antigen on MHC class II (MHCII).[19] The properties of these *in-vitro*-generated moDC were substantially altered when 1,25-OH-VD3 was included during their differentiation: VD3-'tolerized' moDC were less effective in their induction of T-cell proliferation,[20] but rather induced Treg cells that promoted transplant tolerance.[21,22] MoDC generated in the presence of 1,25-OH-VD3 were less capable of producing interleukin-12 (IL-12) p70,[23] but rather secreted IL-10.[14,24] These cells also possess decreased densities of the co-stimulatory molecules CD80 and CD86 and of the antigen-presenting MHCII complex.[20,23] Hence, with all three pillars of T-cell activation being hampered by 1,25-OH-VD3 modulation of moDC development, its potential as a tolerance-inducing agent for DC therapy, and as a therapeutic immune modulator against diseases with underlying inappropriate or overwhelming inflammation, was recognized.[11,18]

VD3 in metabolic imprinting of DC

In addition to its effects on cytokine and co-stimulatory molecule expression, VD3 alters the metabolic profile of developing moDC. Maturation and activation of DC is a thermodynamically challenging process, where fatty acid synthesis provides crucial components of endoplasmic reticulum and Golgi organelles, and increased energy is necessary for migration and plasticity.[25] To meet these needs, glycolysis breaks down glucose for ATP generation. The resulting pyruvate can be metabolized to acetyl-CoA and subsequently used for fatty acid genesis, or it can fuel the tricarboxylic acid cycle to create CO_2 and proton donors. The latter then drive the respiratory chain to create ATP, a process called oxidative phosphorylation of glucose. However, energy can also be generated without an obligatory need for oxygen through anaerobic glycolysis and consequential excretion of glucose-derived lactate, useful particularly in relatively anaerobic (hypoxic) environments, as in sites of tissue damage. Rapidly growing tumour cells also show this method of glucose breakdown, but independently of the presence or absence oxygen – then termed aerobic glycolysis, or the Warburg effect.[26] The decisive triggers to induce oxidation-independent Warburg metabolism, and its benefits for a single cell or the organism are still not completely understood.[26] Generally, the anabolic needs to synthesize biomolecules during activation, cell division or expansion are supported by Warburg metabolism, converting glucose into carbon donors for fatty acid synthesis or pentose for nucleotide synthesis. At the same time, anabolic use of carbon or excretion of lactate rather than oxidative phosphorylation helps to avoid a build up of glycolysis metabolites. This Warburg metabolic pathway has therefore been proposed as a general anabolic principle of activated and proliferating cells, while glucose breakdown through oxidative phosphorylation is a characteristic of differentiated or resting cells – not only for tumours, but also in normal tissue and the immune system.[26,27] Indeed, in murine GM-CSF-DC (see below), Toll-like receptor stimulation promotes increased anabolic glycolysis rather than oxidative phosphorylation, which is crucial for DC

activation, survival and function in the face of increased membrane-forming demands due to secretory processes or migration.[28–31] However, when 1,25-OH-VD3 is given to human monocytes undergoing GM-CSF differentiation to create tolerogenic DC (as outlined above), an early transcriptional programme is started that engages oxidative phosphorylation.[32–34] By sustaining oxidative phosphorylation as a mode of glucose breakdown, the metabolic pattern used by quiescent cells, VD3 may support or favour immune quiescence and tolerance. This metabolic effect of VD3 could help to explain the association of VD3 deficiency with autoimmune syndromes, and the potentially beneficial effects of VD3 supplementation. Additional studies will be required to further elucidate the specific mechanisms and consequences of metabolism control by VD3.

Potential relevance of culture models to inflammatory (monocyte-derived) DC *in vivo*

A potential problem with these seminal studies is that moDC may not be representative of DC *in vivo*. The artificial culture conditions used to generate them from blood-derived $CD14^+$ monocytes clearly lack many or most factors (cellular and soluble) that their precursors would experience *in vivo*. The moDC are perhaps most similar to human $CD14^+$or mouse $CX3CR1^{intermediate}$ $Ly6C^{high}$ monocyte-derived inflammatory DC[35] that differentiate from blood-circulating monocytes in target tissues.[36] Although these represent a minor subset of DC *in vivo* in most settings, they may be important targets of VD3 immunoregulation. Notably, steady-state intestinal lamina propria leucocytes also contain a population of $CD11b^+$/$Sirpa^+$ $CD103^-$ $MHCII^+$ 'DC' that originate from monocytes,[37] possibly differentiated in the low-grade and restricted inflammatory setting of the lamina propria (induced by the constant sensing of luminal bacteria). $CD11b^+$/$Sirpa^+$ $CD103^-$ DC were increased in frequency in human small intestinal lamina propria with gross findings of inflammation, and these DC showed transcriptional signatures consistant with monocyte derivation as well.[38] In experimental autoimmune encephalomyelitis (a murine multiple sclerosis model), central nervous system-infiltrating $CCR2^+$ monocytes differentiate into pathogenic moDC under the influence of endogenous GM-CSF and produce IL-1β to recruit further effector cells initiating and fuelling T-cell-mediated pathology. Specific deletion of the GM-CSF receptor on these monocytes, but not on DC, diminished disease severity.[39] Multiple sclerosis is associated with VD3 deficiency; its murine model experimental autoimmune encephalomyelitis, and possibly also multiple sclerosis itself, is ameliorated by VD3 supplementation.[40,41] Hence, we speculate that, paralleling the *in vitro* studies of moDC outlined above, high VD3 conditions *in vivo* may redirect monocyte differentiation into tolerogenic versus inflammatory DC and contribute to the potential therapeutic effects of such supplementation. It will be important to characterize moDC, generated under VD3-deficient versus VD3-sufficient settings *in vivo*, to validate this hypothesis.

VD3 and DC precursor (pre-DC) derived DC in the mouse

Recent studies refined our understanding about DC biology, in particular by dissecting the exact ontogeny of subsets that derive from specialized classical DC precursors (pre-cDC), such as cross-presenting classical DC 1 (cDC1) or CD4 T-cell-stimulating cDC2. Such DC are phenotypically and ontogenically distinct from monocyte-derived, redifferentiated *in vivo* moDC that originate from the committed monocyte progenitor[36,42,43] (Fig. 1). The great majority of 'professional' DC *in vivo* derive from bone marrow pre-DC.[1] In this regard, studies addressing the effects of VD3 on murine DC have mostly cultured whole bone marrow with GM-CSF or GM-SCF + IL-4 to generate $CD11c^+$ cells (GM-CSF-DC). In such systems, $CD11c^+$ $MHCII^+$ progeny (fulfilling classical DC definitions) comprise a significant portion of $MHCII^{intermediate}$ cells having a phenotypic, developmental and transcriptional profile reminiscent of macrophages ('GM-Mac'). The MHC-II^{high} 'GM-DC' are indeed bona fide DC, depend on interferon regulatory factor 4, are probably derived from pre-cDC and efficiently present antigen to CD4 T cells.[44–46] Using this differentiation protocol, a framework similar to that for human moDC was developed where murine DC are rendered tolerogenic by 1,25-OH-VD3 during differentiation. These cells secrete fewer pro-inflammatory cytokines, express lower levels of co-stimulatory molecules, and propagate Treg cell conversion and effector T-cell hyporesponsiveness.[47] According to microarray analyses, the above-mentioned MHC-II^{high} 'GM-DC' but not $MHCII^{intermediate}$ 'GM-Mac' express VDR,[44] so they are probably the subpopulation described as 1,25-OH-D3-responsive. However, as for human moDC, murine GM-DC have no exact *in vivo* counterpart. They show transcriptional hallmarks only partly overlapping with *in vivo* migratory DC, and are clearly distinct from *in vivo* lymph-node-resident or splenic DC.[46]

Hence, the recently improved understanding of DC biology raises questions of whether or not the effects seen in human moDC and in murine *in-vitro*-differentiated GM-CSF-DC truly reflect the biology of tissue-resident DC. The latter still receive multiple input signals from their microenvironment (such as Wnt proteins[48]) that can shape their capacity to stimulate T cells, and which are often absent or present at non-physiological levels in *in vitro* settings. Indeed, the VitA derivative retinoic acid (RA) has a crucial role in DC differentiation: not only does it induce the formation of intestine-homing pre-

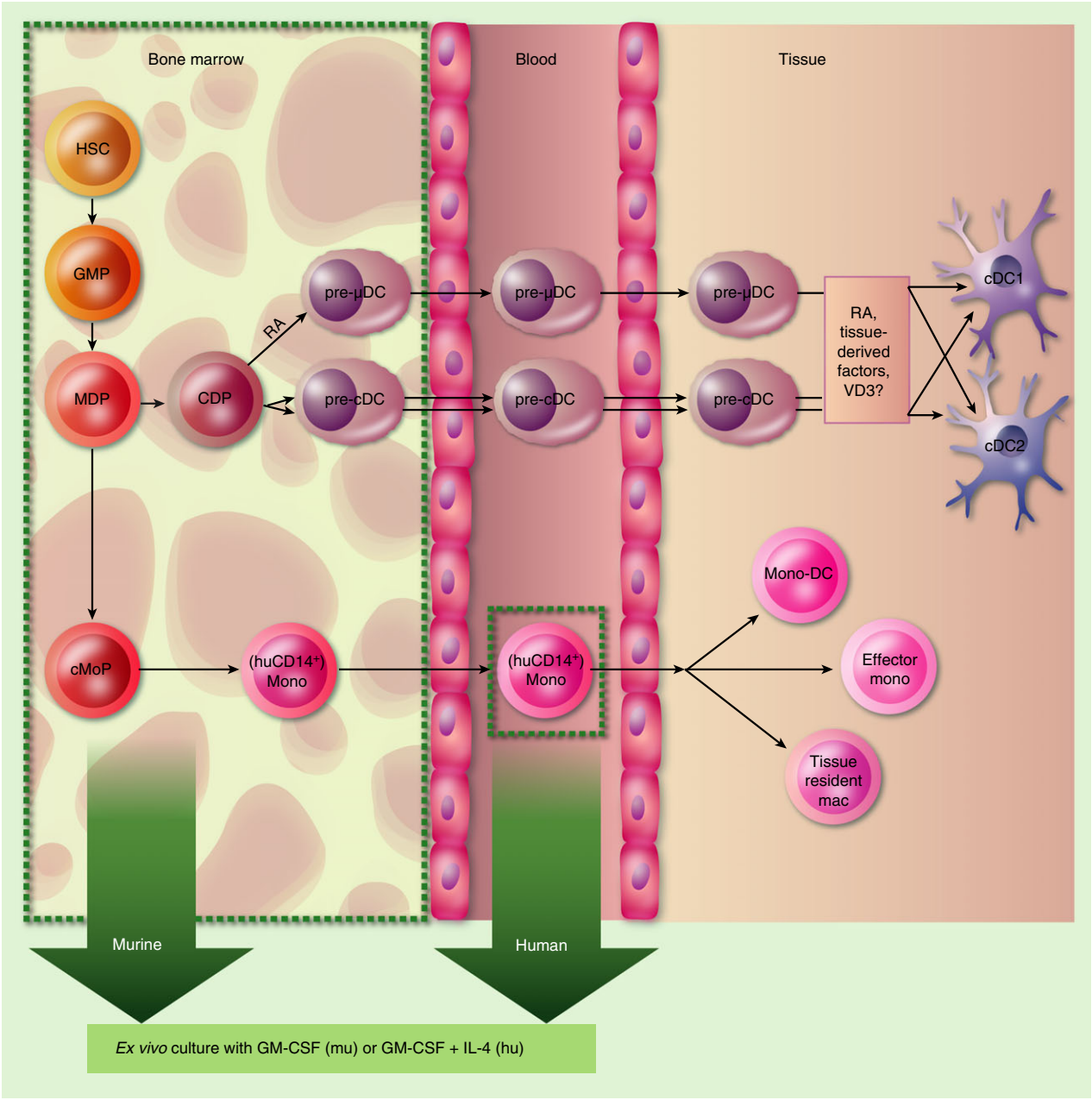


Figure 1. Overview of monocyte- and dendritic cell (DC) precursor-dendritic DC differentiation *in vitro* and *in vivo*. Haematopoeitic stem cell-derived granulocyte–monocyte precursors (GMP) give rise to monocyte-and-DC precursors (MDP), after which developmental pathways segregate into a DC lineage with common DC precursors (CDP) and into a monocytic lineage with common monocyte precursors (cMoP). CDP-derived precursors exhibit early specialization to gut-tropic mucosal DC precursors (pre-μDC), regulated by retinoic acid (RA)[6,49] and classical DC precursors (pre-cDC), and within the latter into cDC1 or cDC2 lineages in the bone marrow.[80] Pre-cDC and pre-μDC then circulate through blood and home to tissues where they complete their differentiation under tissue-specific influences.[1,6,38,49] The cMoP differentiate to human $CD14^+$/mouse $Ly6C^+$ monocytes and circulate through blood. Upon migration to tissues, they can fulfil various microenvironmentally specific functions.[36] Green boxes delineate populations used for GM-colony-stimulating factor (GM-CSF)-dependent differentiation protocols in most studies addressing DC–vitamin D3 (VD3) interactions.

mucosal DC (pre-μDC) in the bone marrow,[49] but it also shapes further differentiation and function in target tissues through its receptor RAR.[6,50–53] In contrast, a similar role of VD3 and VDR in this context is not known. VD3, Cyp27b1 or VDR deficiencies have a myriad of effects on immune cells and other compartments, such as an impaired intestinal epithelial barrier,[54] altered calcium homeostasis[55] and constant low-grade inflammation with

elevated IL-6,[56] which makes studies of DC biology in these mice problematic due to secondary effects. Future studies with novel genetic tools, single-cell-based, or single-population-based analyses and corresponding bioinformatics should help to clarify the cell-specific influence of VD3 on DC *in vivo*, with possible implications for patients. Specifically, they will have to determine: which DC *in vivo* are targeted by 1,25-OH-VD3, and at which developmental state; what their ensuing immune functions are; and how dietary supplemented or sun-induced VD3 participates.

VD3-programmed DC for immune therapy

Although *in vivo* counterparts that mirror the VD3-dependent effects observed in cultured DC remain to be described, *in-vitro*-generated moDC are clearly capable of manipulating adaptive immune responses and are being used in the clinic.[10,57] Hence, the transfer of *in-vitro*-differentiated, VD3-primed DC bears potential to alter autoinflammatory diseases.[58,59] Protocols to differentiate moDC use purification of $CD14^+$ blood cells, and most likely omit dedicated DC precursors, which circulate in human peripheral blood in low numbers. These blood-borne pre-DC can be induced to differentiate into bona fide DC *ex vivo*.[60,61] For future DC-based immunotherapy, it will be of interest to compare the tolerogenic potential of preDC-derived DC and monocyte-derived DC generated *ex vivo* with or without VD3. Still, general mechanisms of adoptive DC therapy need to be addressed further, as tolerogenic DC may be harmful when broadly impairing immunosurveillance during malignancies or infection,[62] or when presenting loaded antigens in a pro-inflammatory setting.

DC as a source of 1,25-OH-VD3 for T cell programming

In addition to its potential to skew DC development, VD3 directly modulates T-cell responses. The presence of 1,25-OH-VD3 during T-cell activation inhibits their proliferation, favours Treg cell development, and alters trafficking receptor expression.[63–65] However, circulating levels of 1,25-OH-VD3 *in vivo* are too low to mediate these effects,[8,66] and 25-OH-VD3, the major circulating form of the vitamin, does not itself activate VDR-dependent transcription. Hence, 1,25-OH-VD3 must be generated locally to effect T-cell programming, and DC can fulfil this function: both *in-vitro*-derived human moDC and subsets of *in vivo* DC can generate and present the hormone to T cells.[67] At the level of gene expression, all physiological *in vivo* subsets of mouse DC, including plasmacytoid DC, and both cDC1 and cDC2 express the 25-hydroxylase Cyp27a1 (Immgen database, http://www.immgen.org/). In contrast, none of the analysed mouse DC subsets expressed Cyp27b1 above a threshold level of detection (Immgen). However, other studies have reported the expression and metabolic activity of Cyp27b1 in human moDC that subsequently influences T-cell activity, although the degree to which DC can produce 1,25-OH-VD3 *in vitro* varies with their activation and differentiation status.[23,67–69] Similarly to macrophages, direct stimulation of human and mouse GM-CSF-DC with pathogen-associated molecular pattern or pro-inflammatory cytokines triggers Cyp27b1 expression, as does T-cell contact, especially in a pro-inflammatory cytokine milieu.[23,69–71] Moreover, DC isolated from skin-draining afferent lymph of sheep have also been shown to metabolize VD3 to 1,25-OH-VD3.[67] Interestingly, human T cells also express Cyp27b1 upon activation and so can carry out the final VD3 conversion step on their own.[67] Hence, when Cyp27b1 is not expressed by DC, activated T cells and DC together can still convert VD3 to 1,25-OH-VD3 through cross-cellular metabolism, a process that has been demonstrated experimentally in IL-12-stimulated co-cultures of human moDC and naive peripheral blood T cells.[67]

In differentiating moDC, 25-OH-VD3 at concentrations similar to those in serum (10–100 nM) is sufficient for DC generation of 1,25-OH-VD3 and autocrine imprinting of a tolerogenic phenotype (i.e. T-cell hyporesponsiveness induction or metabolic switches),[23,32] but these serum 25-OH-VD3 levels are too low for the autocrine/paracrine induction of CCR10 on T cells in moDC : T-cell co-cultures[67] (Fig. 2). We speculate that local concentrations above those present in circulation are induced in skin and draining lymph by UVB irradiation and confine epidermotropism in T cells through expression of CCR10, a chemoattractant receptor for the keratinocyte-expressed chemokine CCL28 while suppressing gut-homing properties (integrin $\alpha_4\beta_7$ and CCR9 expression).[67] In contrast, tolerogenic imprinting of DC might be a broader, not skin-specific mechanism of immunomodulation. If the hormone were presented in a targeted fashion at the immunological synapse, its action could be limited to responding T cells. Local diffusion could affect nearby bystander cells such as stromal fibroblasts or endothelial cells. Such paracrine secretion would therefore influence the tissue-specific properties of immune instruction. Perhaps skin-draining DC, which can synthesize 1,25-OH-VD3 from D3,[67] also transport VD3 after sun exposure, creating a characteristic environment in skin-draining lymph nodes. This may provide a mechanism to direct skin-homing T cells to the epidermis in response to sun-induced damage, where VD3 is high due to UVB-induced conversion of 7-dehydro-cholesterol and subsequent isomerization. Indeed, in the parallel VitA/RA system, DC draining the intestine are thought not only to express RA synthetic capability, but also to transport RA or its

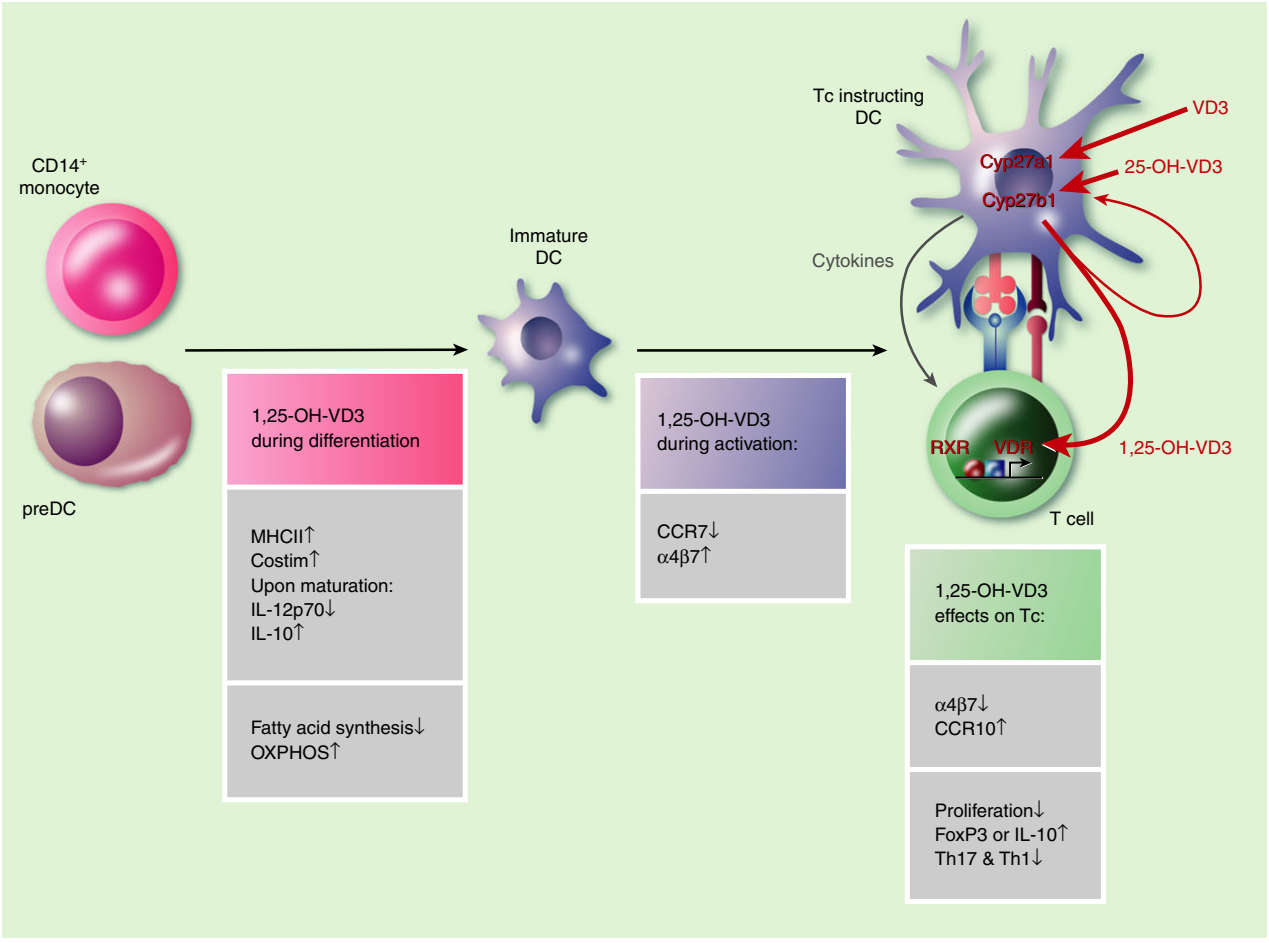
precursors for processing and presentation in the draining mesenteric lymph node.[5,66,72]

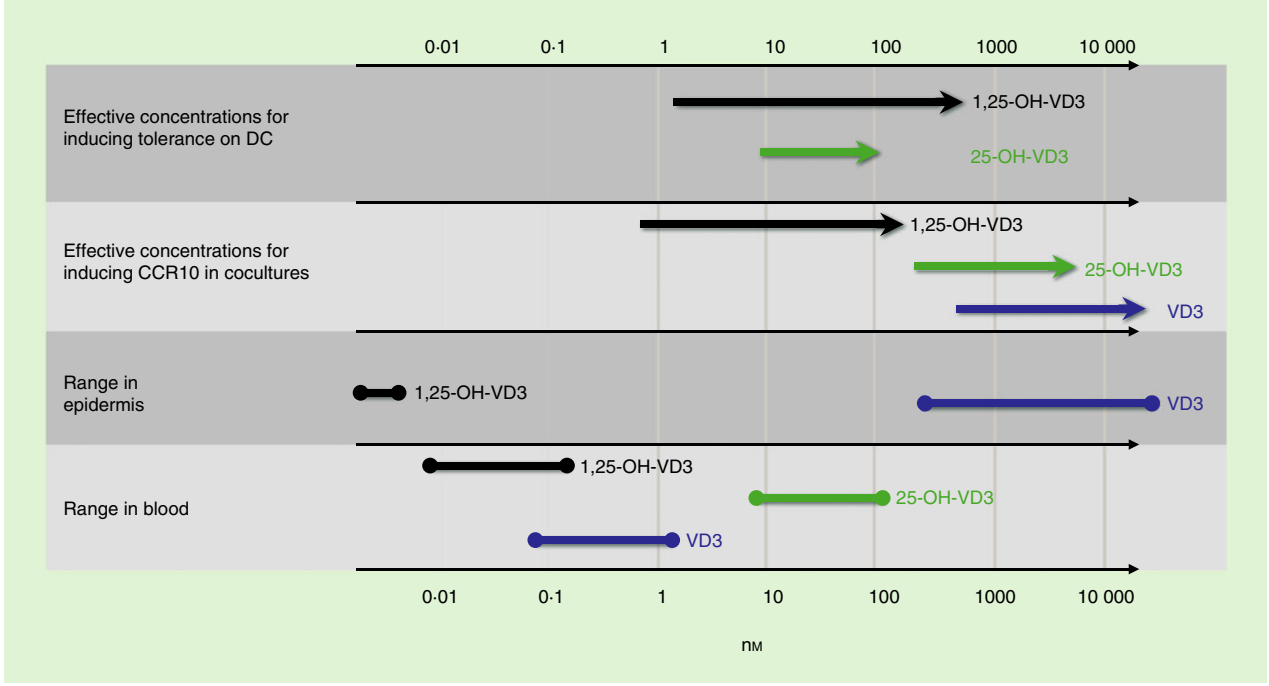
1,25-OH-VD3 can be locally inactivated by Cyp24a1-mediated hydroxylation to a metabolite without VDR-dependent transcriptional activity. In this way, 1,25-OH-VD3-induced autocrine Cyp24a1 induction can form a negative feedback loop of local VD3 activity *in vivo*. Although differential Cyp24a1 levels have been reported for *in-vitro*-differentiated moDC versus macrophages,[68] the relevance of this loop is unclear for the control of immune cell trafficking. Future studies will be required to elucidate potential region-specific T-cell trafficking patterns induced by VD3 generation and degradation, similar to those outlined for VitA/RA and gut trafficking in recent years.

VD3 influences the migratory patterns of DC

Vaccination has proven to be the most successful disease-preventing medical intervention. Although discovered centuries ago, the exact mechanisms responsible for an induction of competent T-cell and B-cell responses have been subject to continuous debate. Generally, a major pitfall is the poor induction of cross-compartment specific immune responses, specifically the protection of mucosal surfaces (a main entry site for many pathogens) after subcutaneous or intramuscular vaccinations. In contrast, oral or intranasal application together with cholera toxin induces potent mucosal protection, but toxicity limits its clinical use. 1,25-OH-VD3, when co-administered subcutaneously with the adjuvant alum has been shown to overcome this limitation in a murine model, initiating an intestinal and pulmonary IgA response after subcutaneous vaccination.[73] One hypothesis for these observations is an altered migratory pattern of DC originating at the site of vaccination. Bone-marrow-derived GM-CSF-DC that were matured (after
GM-CSF differentiation) with lipopolysaccharide and concomitant 1,25-OH-VD3 showed an altered migratory pattern when injected subcutaneously.[74] Whereas control DC readily migrated to the draining lymph node, their CCR7 expression sequesters them at sites of CCL19/21 production in the first lymph node they encounter, where they then fulfil their bona fide role of presenting antigen. In contrast, maturation in the presence of 1,25-OH-VD3 rapidly but transiently decreased the surface CCR7 expression of GM-CSF-DC, allowing them to migrate beyond this initial encounter to non-draining lymph nodes and Peyer's patches through a transient up-regulation of intestinal-targeting integrin $\alpha_4\beta_7$ and associated with expression of the intestinal cDC2 markers CD103 and DCIR2/Clec4a4.[74,75] Notably, such altered trafficking patterns were also observed for endogenous tissue-resident DC following subcutaneous injection of fluorescent microspheres and concomitant 1,25-OH-VD3 injection.[74] These migrated DC then fulfilled their T-cell instructing role in the respective sites: while activated T cells specific for the vaccinating antigen were only found in draining lymph nodes in the absence of 1,25-OH-VD3 co-vaccination, 1,25-OH-VD3 co-vaccination resulted in DC-instructed antigen-specific T cells in both draining and non-draining lymph nodes as well as in Peyer's patches.[74] In related studies of a human skin explant model, intradermally injected 1,25-OH-VD3 increased the migration of dermal $CD14^+$ DC, a monocyte-derived DC population,[76] while repressing the T-cell stimulatory capacities of total migrating DC.[77] Hence, the presence of 1,25-OH-VD3 during DC activation shapes their migratory capacity upon antigen uptake, and potentially modulates the scope of their induced T-cell responses not with respect to antigen specificity, but rather to T-cell skewing and trafficking potential. 1,25-OH-VD3 regulation of DC migratory properties during antigen responses may therefore represent an additional mechanism for regulation of immune responses by local VD3 metabolism. Similarly to UVB-induced VD3 conversion in skin, intestinal infection, epithelial barrier breach and inflammation might induce local Cyp27b1 in lamina propria DC or macrophages, which could result in conversion of dietary or systemic VD3 metabolites and act on intestinal DC during their activation. In this scenario, VDR signalling in DC could compete with VitA effects. Indeed, the canonical nuclear receptors for VD3 and RA, VDR and RARα, often counteract each other's signalling, potentially through competition for their common heterodimeric partner, RXR. Such competition has been documented for T cells: RA induction of integrin $\alpha_4\beta_7$-mediated and CCR9-mediated intestinal T-cell

Figure 2. Calcitriol (1,25-OH-VD3) and dendritic cell (DC) function during different stages of immune activation. Upper panel, left: 1,25-OH-VD3 acting on granulocyte–macrophage colony-stimulating factor (GM-CSF) -differentiating precursors renders DC 'tolerogenic' by inhibiting T-cell stimulation pillars, especially after DC maturation (e.g. performed with tumour necrosis factor-α or lipopolysaccharide). Also, profound metabolic patterns are induced to promote oxidative phosphorylation and reduce fatty acid synthesis. Middle: 1,25-OH-VD3 during DC maturation alters trafficking receptor profiles to redirect migration (see text). Right: Maturation induces DC-intrinsic up-regulation of Cyp27b1. Paracrine DC-derived 1,25-OH-VD3 serves as a fourth pillar of DC : T-cell interaction and promotes regulatory T-cell generation, inhibits effector T-cell proliferation and imprints trafficking patterns in instructed T cells. Autocrine 1,25-OH-VD3 possibly has effects on DC themselves as well. Lower panel: range of concentrations of VD3 and metabolites in blood and epidermis *in vivo* and required for immune effects *in vitro*.


CD14+ monocyte
preDC
1,25-OH-VD3 during differentiation
MHCII↑
Costim↑
Upon maturation:
IL-12p70↓
IL-10↑
Fatty acid synthesis↓
OXPHOS↑
Immature DC
1,25-OH-VD3 during activation:
CCR7↓
α4β7↑
Tc instructing DC
VD3
Cyp27a1
25-OH-VD3
Cyp27b1
Cytokines
RXR
VDR
1,25-OH-VD3
T cell
1,25-OH-VD3 effects on Tc:
α4β7↓
CCR10↑
Proliferation↓
FoxP3 or IL-10↑
Th17 & Th1↓


0·01
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1
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Effective concentrations for inducing tolerance on DC
1,25-OH-VD3
25-OH-VD3
Effective concentrations for inducing CCR10 in cocultures
1,25-OH-VD3
25-OH-VD3
VD3
Range in epidermis
1,25-OH-VD3
VD3
Range in blood
1,25-OH-VD3
25-OH-VD3
VD3
nM

homing is antagonized by 1,25-OH-VD3 *in vitro*.[67] However, VD3 can also cooperate with RA to induce VitA metabolizing enzymes in human (but not mouse) intestine-derived or blood-derived DC subsets,[78] whereas it suppresses them in mouse GM-CSF-DC.[79] Hence, the interplay of vitamins A and D and their role in shaping immune responses still requires further investigation; it is likely to be context-, cell-type- and species-dependent.

In conclusion, seminal studies show that DC can metabolize VD3 for programming of T cells, and suggest that 1,25-OH-VD3 also interacts directly with DC to influence their migration and their capacity to instruct T cells and hence to initiate, fine tune or dampen immune reactions. However, our understanding of the complexity of both VD3 and DC biology has grown considerably in recent years, and additional studies are required to address the role of DC–VD3 interactions in the potentially beneficial effects of VD3 supplementation reported in some autoimmune and inflammatory diseases. Understanding the diverse mechanisms of VD3 action will be crucial for the appropriate application of VD3 supplementation for therapy or prophylaxis that might evolve from currently ongoing clinical trials in autoimmune disease.

Disclosures

The authors disclose no conflicts.

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