The coming of age of 1,25-dihydroxyvitamin D₃ analogs as immunomodulatory agents

Chantal Mathieu and Luciano Adorini

The active form of vitamin D, 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], is a secosteroid hormone that regulates calcium and bone metabolism, controls cell proliferation and differentiation, and exerts immunoregulatory activities. This range of functions has been exploited clinically to treat a variety of conditions, from secondary hyperparathyroidism to osteoporosis, to autoimmune diseases such as psoriasis. Recent advances in understanding 1,25(OH)₂D₃ functions and novel insights into the mechanisms of its immunomodulatory properties suggest a wider applicability of this hormone in the treatment of autoimmune diseases and allograft rejection.

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The activated form of vitamin D, 1,25(α,β)-dihydroxyvitamin D₃ (see Glossary), has, in addition to its central function in calcium and bone metabolism, important effects on the growth and differentiation of many cell types, and intriguing immunoregulatory properties [1,2]. The biological effects of 1,25(OH)₂D₃ are mediated by the vitamin D receptor (VDR), a member of the superfamily of nuclear hormone receptors [3,4]. Ligand binding induces conformational changes in the VDR, which promote heterodimerization with the retinoid X receptor (RXR) and recruitment of several nuclear receptor coactivator proteins, including steroid receptor coactivator family members and a multimember coactivator complex, D receptor interacting proteins (DRIP). These coactivators induce chromatin remodeling through intrinsic histone-modifying activities and direct recruitment of key transcription initiation components at regulated promoters. Thus, the VDR functions as a ligand-activated transcription factor that binds to specific DNA sequence elements (vitamin D responsive element, VDRE) in vitamin D responsive genes and ultimately influences the rate of RNA polymerase II-mediated transcription [5]. The presence of VDR in most cell types of the immune system [6], in particular in antigen presenting cells (APCs) such as macrophages [6] and dendritic cells [7], as well as in both CD4⁺ and CD8⁺ T cells (reviewed in Ref. [8]), lead to the investigation of the potential for 1,25(OH)₂D₃ as an immunomodulatory agent [1,2]. Intriguingly, VDR-deficient mice fail to display major immune abnormalities, suggesting that VDR serves a redundant function in the immune system [9]. However, mice incapable of synthesizing 1,25(OH)₂D₃ because of the targeted ablation of the 25-hydroxyvitamin D 1α-hydroxylase enzyme show a significant reduction in CD4⁺ and CD8⁺ peripheral T cells [10].

VDR ligands have widespread clinical application [11], but hypercalcemia is a dose-limiting effect that prevents sustained systemic administration. To overcome this limitation, several 1,25(OH)₂D₃ analogs, with a wider therapeutic window than 1,25(OH)₂D₃ itself, have been synthesized and shown effective in experimental models of autoimmune diseases and allograft rejection [12] (Fig. 1). The recent elucidation of the crystal structure of the VDR bound to its natural ligand [13] will facilitate the development of 1,25(OH)₂D₃ analogs with enhanced potency, lower calcemic liability, and increased tissue specificity. This will be instrumental also for the generation of novel analogs with selective immunoregulatory properties.

Direct effects of 1,25(OH)₂D₃ and its analogs in T cells

Soon after the discovery of VDR expression in T cells [6,14], 1,25(OH)₂D₃ was shown to inhibit antigen-induced T-cell proliferation [15] and cytokine production [16]. Later studies demonstrated selective inhibition of Th1 cell development [17,18], although it was not clarified how much of this effect could be accounted for by modulation of APC functions. Indeed, several key cytokines in T cells are direct targets for 1,25(OH)₂D₃ and its analogs, in particular Th1 cytokines, such as interleukin (IL)-2 and

Glossary

1,25(OH)₂D₃: The di-hydroxylated, biologically active form of vitamin D₃, also known as calcitriol. It is a central hormone in calcium homeostasis and bone metabolism, but has also a number of other functions, and notably powerful immunomodulatory properties.

1,25(OH)₂D₃ analogs: Chemically modified molecules derived from 1,25(OH)₂D₃. Modifications have been made throughout the molecule, to obtain analogs with the desired properties. More than 1000 different vitamin D analogs have been synthesized worldwide.

Vitamin D receptor (VDR): A member of the superfamily of nuclear receptors for steroid hormones, thyroid hormone, and retinoic acid. The VDR functions as a 1,25(OH)₂D₃-activated transcription factor that ultimately influences the rate of RNA polymerase II-mediated transcription. VDRs are present not only in cells typically involved in calcium and bone metabolism, but also in other cell types, such as cells of the immune system.
interferon (IFN-γ) 1,25(OH)2D3 inhibits IL-2 secretion via impairment of transcription factor NF-AT complex formation, because the ligand-bound VDR complex itself binds to the distal NF-AT binding site of the human IL-2 promoter [19,20]. Another key T-cell cytokine, IFN-γ, has been found directly inhibited by 1,25(OH)2D3 through interaction of the ligand-bound VDR complex with a VDRE in the promoter region of the cytokine [21]. Progressive deletion analysis of the IFN-γ promoter revealed that negative regulation by 1,25(OH)2D3 is also exerted at the level of an upstream region containing an enhancer element [21]. However, some in vivo studies have failed to support a direct effect of 1,25(OH)2D3 on IFN-γ production by T cells [22]. 1,25(OH)2D3 has been recently shown to enhance the development of Th2 cells via a direct effect on naïve CD4+ cells [23], and this could also account for the beneficial effect of VDR ligands in the treatment of autoimmune diseases and possibly also allograft rejection. The capacity of 1,25(OH)2D3 to skew T cells towards the Th2 pathway had been previously suggested [22,24], but could not be confirmed by other studies [18,25]. In conclusion, 1,25(OH)2D3 in vivo appears primarily to inhibit Th1 cells and, under appropriate conditions, might favor a deviation to the Th2 pathway. These effects reflect, in part, a direct activity of 1,25(OH)2D3 and its analogs on T cells, but modulation of APC function by these compounds certainly plays an important role in shaping the development of T cell responses.

**Regulatory effects of 1,25(OH)2D3 and its analogs in antigen-presenting cells**

APCs, and in particular dendritic cells (DCs) are key targets of 1,25(OH)2D3 and its analogs, both in vitro and in vivo. Earlier indications for the capacity of
1,25(OH)\(_2\)D\(_3\) to target APCs were corroborated by its ability to inhibit the production of IL-12 [17,26], an APC-derived cytokine critical for Th1 cell development. More recent work has demonstrated that 1,25(OH)\(_2\)D\(_3\) and its analogs inhibit the differentiation and maturation of DCs [27–30], a crucial APC in the induction of T-cell-mediated immune responses. These studies, performed either on monocyte-derived DCs from human peripheral blood or on bone-marrow derived mouse DCs, have consistently shown that in vitro treatment of DCs with 1,25(OH)\(_2\)D\(_3\) and its analogs leads to downregulated expression of the costimulatory molecules CD40, CD80, CD86 and to decreased IL-12 and enhanced IL-10 production, resulting in decreased T-cell activation. The inhibition of IL-12 production and the enhanced production of IL-10, an important immunoregulatory factor, highlight the important functional effects of 1,25(OH)\(_2\)D\(_3\) and its analogs on DCs. The prevention of DC differentiation and maturation as well as the modulation of their activation and survival leading to DCs with tolerogenic phenotype and function, and to T-cell hyporesponsiveness, certainly play an important role in the immunoregulatory activity of 1,25(OH)\(_2\)D\(_3\). These effects are not limited to in vitro activity: 1,25(OH)\(_2\)D\(_3\) and its analogs can also induce DCs with tolerogenic properties in vivo, as demonstrated in models of allograft rejection [31,32]. Tolerogenic DCs induced by a short treatment with 1,25(OH)\(_2\)D\(_3\) are probably responsible for the capacity of this hormone to induce CD4+CD25+ regulatory T cells that are able to mediate transplantation tolerance [31]. Rag-1-dependent regulatory cells have also been implicated in the prevention of experimental allergic encephalomyelitis (EAE) induced by 1,25(OH)\(_2\)D\(_3\) [25]. However, in this study neither an effect on APCs nor a deviation to the Th2 pathway could be demonstrated.

APCs are not only sensitive to 1,25(OH)\(_2\)D\(_3\) and its analogs: activated macrophages are also able to synthesize and secrete 1,25(OH)\(_2\)D\(_3\). These cells express 1α-hydroxylase, the enzyme responsible for the final hydroxylation step in the synthesis of 1,25(OH)\(_2\)D\(_3\) [33]. Although the macrophage enzyme is identical to the renal form, its regulation seems to be under a different control system, mediated by immune signals, with IFN-γ being a powerful stimulator [33]. In macrophages, no clear downregulation of the enzyme by the end-product, 1,25(OH)\(_2\)D\(_3\), could be observed, explaining the hypercalcaemia occurring in situations of macrophage overactivation such as tuberculosis or sarcoidosis [34]. The secretion of classical macrophage products such as cytokines [IL-1, tumor necrosis factor (TNF)-α, and IL-12] precedes the transcription of 1α-hydroxylase and, as a consequence, the secretion of 1,25(OH)\(_2\)D\(_3\). Therefore, this timing is compatible with its activity as a suppressive signal (Fig. 2). 1,25(OH)\(_2\)D\(_3\) also influences the secretion of other cytokines secreted by monocyte-derived cells: the suppressive PGE\(_2\) is stimulated, whereas the monocyte-recruiter granulocyte-macrophage colony-stimulating factor (GM-CSF) is suppressed [35,36]. Interestingly, 1,25(OH)\(_2\)D\(_3\) utilizes different mechanisms to regulate cytokine production. IL-12 secretion is inhibited by targeting the nuclear factor (NF)-κB pathway [26], whereas suppression of GM-CSF is achieved by interaction of ligand-bound
Table 1. Beneficial effects of 1,25(OH)\textsubscript{2}D\textsubscript{3} and its analogs in animal models of autoimmunity and transplantation

<table>
<thead>
<tr>
<th>Autoimmunity</th>
<th>Main effects</th>
<th>Refs</th>
</tr>
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<tbody>
<tr>
<td>Autoimmune diabetes</td>
<td>Inhibition of insulitis, reduction of diabetes</td>
<td>[43,44,56]</td>
</tr>
<tr>
<td>Low-dose streptozotocin-induced diabetes mellitus</td>
<td>Decreased diabetes</td>
<td>[59]</td>
</tr>
<tr>
<td>Collagen-induced arthritis</td>
<td>Decreased incidence and severity of arthritis also when given at disease onset</td>
<td>[40,41]</td>
</tr>
<tr>
<td>Lyme arthritis</td>
<td>Prevents symptoms and progression to severe arthritis</td>
<td>[41]</td>
</tr>
<tr>
<td>Experimental allergic encephalomyelitis</td>
<td>Prevention and treatment of disease, inhibition of relapses</td>
<td>[18,25,38,60]</td>
</tr>
<tr>
<td>Experimental autoimmune thyroiditis</td>
<td>Reduction of thyroid histologic lesions, when administered together with CsA</td>
<td>[61]</td>
</tr>
<tr>
<td>Heyman nephritis</td>
<td>Reduction of proteinuria and autoantibodies</td>
<td>[62]</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>Inhibition of proteinuria, prevention of skin lesions</td>
<td>[37,63]</td>
</tr>
<tr>
<td>Mercuric chloride-induced glomerulonephritis</td>
<td>Prevention of autoimmune manifestations including proteinuria, serum IgE, and serum anti-laminin antibodies</td>
<td>[64,66]</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>Significant amelioration of symptoms, block of disease progression</td>
<td>[42]</td>
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<tr>
<td>Transplantation</td>
<td></td>
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<tr>
<td>Aorta</td>
<td>Reduced signs of chronic rejection, in particular intimal hyperplasia</td>
<td>[54]</td>
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<tr>
<td>Bone marrow</td>
<td>Decreased graft-versus-host disease</td>
<td>[66]</td>
</tr>
<tr>
<td>Heart</td>
<td>Marked prolongation of non vascularized and vascularized heart allografts</td>
<td>[46–48]</td>
</tr>
<tr>
<td>Liver</td>
<td>Prolonged graft survival by decreasing the severity of acute rejection</td>
<td>[49]</td>
</tr>
<tr>
<td>Pancreatic islets</td>
<td>Induction of transplantation tolerance, prevention of autoimmune diabetes</td>
<td>[31,50,51]</td>
</tr>
<tr>
<td>Skin</td>
<td>Prolonged graft survival</td>
<td>[52,53]</td>
</tr>
<tr>
<td>Small bowel</td>
<td>Reduced amounts of hyaluronan secreted into the intestinal lumen</td>
<td>[48]</td>
</tr>
</tbody>
</table>

VDR monomers with functional repressive complexes in the promoter region of the cytokine, instead of the typical formation of VDR-RXR heterodimers [36]. Also T-cell-derived cytokines are inhibited via different mechanisms: IL-2 secretion is inhibited by direct interference with the binding of NF-AT to the promoter region of the cytokine [19,20], and IFN-\(\gamma\)-is directly downregulated through interaction of the ligand-bound VDR with a VDRE [21].

**Immunomodulatory effects of 1,25(OH)\textsubscript{2}D\textsubscript{3} and its analogs in autoimmune diseases and allograft rejection**

The immunoregulatory properties of 1,25(OH)\textsubscript{2}D\textsubscript{3} and its analogs have been demonstrated in different models of autoimmune diseases and in experimental organ transplantation (Table 1). Notably, 1,25(OH)\textsubscript{2}D\textsubscript{3} and its analogs can prevent systemic lupus erythematosus in \(lpr/lpr\) mice [37], EAE [18,38,39], collagen-induced arthritis [40,41], Lyme arthritis [41], inflammatory bowel disease [42] and autoimmune diabetes in non-obese diabetic (NOD) mice [43,44]. 1,25(OH)\textsubscript{2}D\textsubscript{3} analogs are able not only to prevent but also to treat ongoing autoimmune diseases, as demonstrated by their ability to inhibit the recurrence of autoimmune disease after islet transplantation in the NOD mouse [45], and to ameliorate significantly the chronic-relapsing EAE induced in Biozzi mice by spinal cord homogenate [18]. In addition, 1,25(OH)\textsubscript{2}D\textsubscript{3} and its analogs prolong allograft survival in a variety of experimental models (Table 1), including heart [46–48], liver [49], pancreatic islets [31,50,51], skin [52,53] and small-bowel allografts [48]. Importantly, 1,25(OH)\textsubscript{2}D\textsubscript{3} analogs can inhibit, in association with cyclosporin A (CsA), not only acute but also chronic allograft rejection, as documented by inhibition of adventitial inflammation and intimal hyperplasia in rat aortic allografts [54]. Renal graft loss has been found decelerated also in patients treated with 1,25(OH)\textsubscript{2}D\textsubscript{3} [55], further suggesting its capacity to inhibit chronic graft rejection.

An important property of 1,25(OH)\textsubscript{2}D\textsubscript{3} and its analogs is their capacity to modulate not only T cells but also APCs. The induction of tolerogenic DCs, which leads to an enhanced number of CD4\(^+\)CD25\(^+\) regulatory T cells [31], renders them appealing for clinical use, especially for the control of allograft rejection and for the prevention and treatment of autoimmune diseases. In the NOD mouse, treatment with 1,25(OH)\textsubscript{2}D\textsubscript{3} and its analogs prevents the development of autoimmune diabetes, and is associated with an increased number of regulatory T cells [43] and a shift from the Th1 to the Th2 phenotype in the target organ [24,56]. In addition, the pro-apoptotic activity of 1,25(OH)\textsubscript{2}D\textsubscript{3} and its analogs can restore the defective sensitivity to apoptosis of NOD lymphocytes [57], leading to a more efficient elimination of potentially dangerous autoimmune effector cells. The increased apoptosis induced by 1,25(OH)\textsubscript{2}D\textsubscript{3} and its analogs in DCs [27] and T cells [57] has been observed after different apoptosis-inducing signals, and could help to explain why short-term treatments with these agents afford long-term protection and promote tolerance induction. Additive and even synergistic effects have been observed between 1,25(OH)\textsubscript{2}D\textsubscript{3} or its analogs and immunosuppressive agents, such as CsA and sirolimus [58]. These effects have been confirmed in vitro in models of autoimmune diabetes and EAE, and in graft rejection [45].

**Conclusions**

VDR ligands have pleiotropic activities in immune regulation. It is intriguing that several different molecular mechanisms of cytokine inhibition by 1,25(OH)\textsubscript{2}D\textsubscript{3} exist. APCs and T cells can be direct
targets of the hormone, leading to the inhibition of pathogenic effector T cells and enhancing the frequency of T cells with regulatory properties, largely via induction of tolerogenic DCs. These immunoregulatory activities, coupled with the absence of major side effects once calcemia is under control, have been translated into effective immunointervention in a variety of models of autoimmune diseases and graft rejection.

This body of knowledge, documenting the coming of age of 1,25(OH)\(_2\)D\(_3\) and its analogs as immunomodulatory agents, represents a sound basis to further explore their immunoregulatory properties in the development of therapies for autoimmune diseases and allograft rejection.

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