The coming of age of 1,25-dihydroxyvitamin D_3 analogs as immunomodulatory agents

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The active form of vitamin D, 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], 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$(OH)_2D_3$ 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$(OH)_2D_3$ (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$(OH)_2D_3$ 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$(OH)_2D_3$ 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$(OH)_2D_3$ 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$(OH)_2D_3$ analogs, with a wider therapeutic window than 1,25$(OH)_2D_3$ itself, have been synthetized 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$(OH)_2D_3$ 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$(OH)_2D_3$ and its analogs in T cells

Soon after the discovery of VDR expression in T cells [6,14], 1,25$(OH)_2D_3$ 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$(OH)_2D_3$ and its analogs, in particular Th1 cytokines, such as interleukin (IL)-2 and

Glossary

1,25$(OH)_2D_3$: The di-hydroxylated, biologically active form of vitamin D_3, 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$(OH)_2D_3$ analogs: Chemically modified molecules derived from 1,25$(OH)_2D_3$. 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$(OH)_2D_3$-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.

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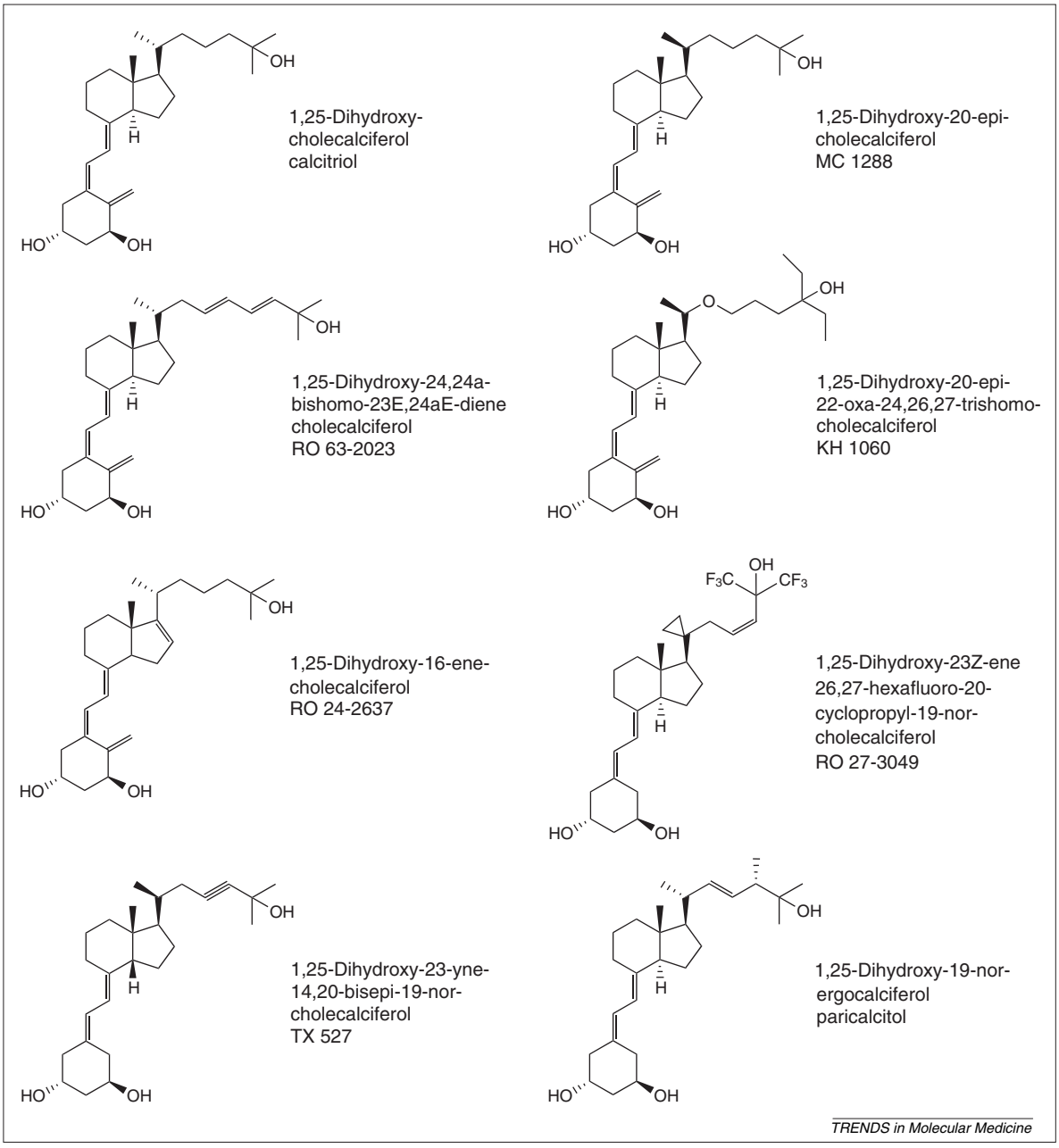

Fig. 1. Structure of $1,25(OH)_2D_3$ (calcitriol) and of selected analogues with immunoregulatory properties. See Refs [11] and [12] for details.

interferon (IFN)-γ. $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ on IFN-γ production by T cells [22]. $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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, $1,25(OH)_2D_3$ *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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ and its analogs in antigen-presenting cells

APCs, and in particular dendritic cells (DCs) are key targets of $1,25(OH)_2D_3$ and its analogs, both *in vitro* and *in vivo*. Earlier indications for the capacity of

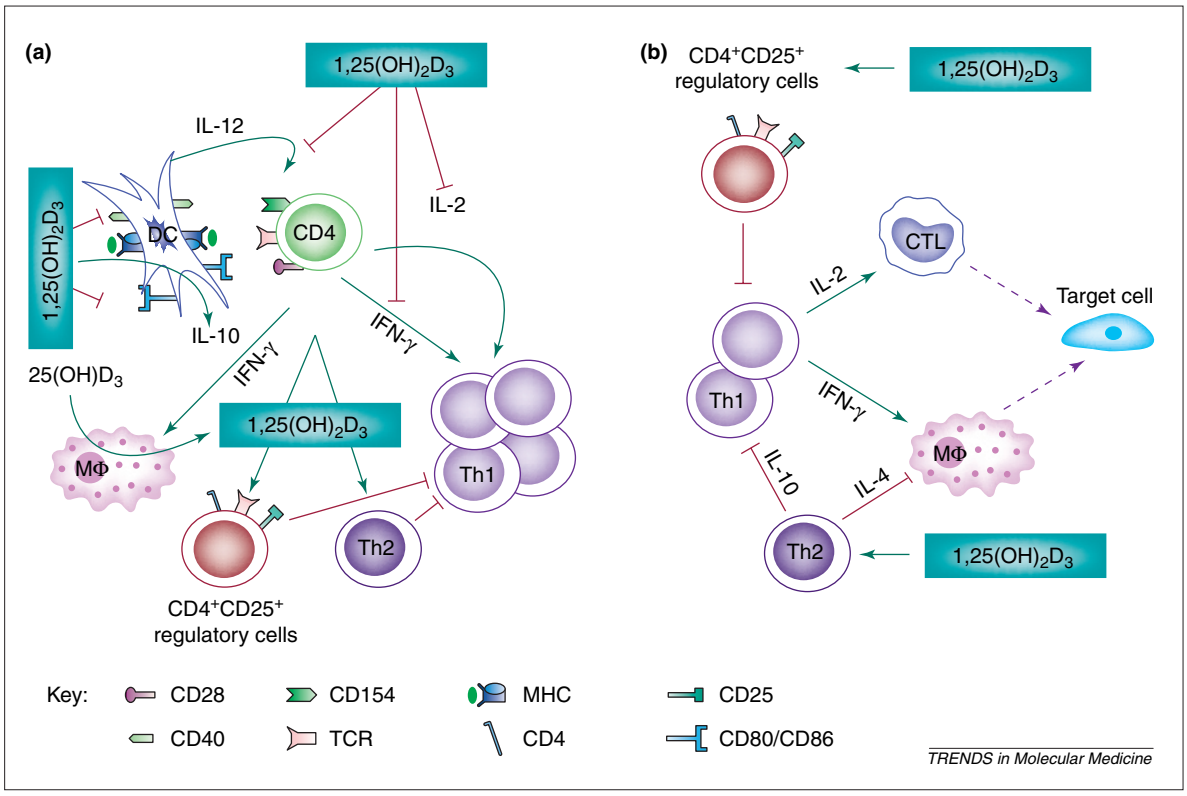

Fig. 2. Regulation of the immune response by $1,25(OH)_2D_3$. $1,25(OH)_2D_3$ and its analogues ($1,25(OH)_2D_3$) can modulate the immune response via several mechanisms in secondary lymphoid organs and in target tissues. (a) In secondary lymphoid organs, $1,25(OH)_2D_3$ inhibits interleukin (IL)-12 and stimulates IL-10 production and downregulates costimulatory molecule expression (CD40, CD80, CD86) expressed by dendritic cells (DC), thus inhibiting the development of T helper (Th) 1 cells along the Th1 pathway and favoring the induction of $CD4^+CD25^+$ regulatory T cells and of Th2 cells, both of them able to further inhibit Th1 cells. $1,25(OH)_2D_3$ also exerts direct effects on T cells by inhibiting IL-2 and interferon (IFN)-γ production. Macrophages (MΦ) can synthesize $1,25(OH)_2D_3$ and this might also contribute to the regulation of the local immune response. (b) In target tissues, pathogenic Th1 cells, that can damage target cells via induction of cytotoxic T cells (CTL) and activated macrophages, are reduced in number and their activity is further inhibited by $CD4^+CD25^+$ regulatory T cells and by Th2 cells that are induced by $1,25(OH)_2D_3$. Green arrows indicate stimulation, blunted red arrows inhibition, and broken arrows cytotoxicity.

$1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$. These effects are not limited to *in vitro* activity: $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ [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 $1,25(OH)_2D_3$ and its analogs: activated macrophages are also able to synthesize and secrete $1,25(OH)_2D_3$. These cells express 1α-hydroxylase, the enzyme responsible for the final hydroxylation step in the synthesis of $1,25(OH)_2D_3$ [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, $1,25(OH)_2D_3$, could be observed, explaining the hypercalcemia 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 $1,25(OH)_2D_3$. Therefore, this timing is compatible with its activity as a suppressive signal (Fig. 2). $1,25(OH)_2D_3$ 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, $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ and its analogs in animal models of autoimmunity and transplantation

	Main effects	Refs
Autoimmunity		
Autoimmune diabetes	Inhibition of insulitis, reduction of diabetes	[43,44,56]
Low-dose streptozotocin-induced diabetes mellitus	Decreased diabetes	[59]
Collagen-induced arthritis	Decreased incidence and severity of arthritis also when given at disease onset	[40,41]
Lyme arthritis	Prevents symptoms and progression to severe arthritis	[41]
Experimental allergic encephalomyelitis	Prevention and treatment of disease, inhibition of relapses	[18,25,38,60]
Experimental autoimmune thyroiditis	Reduction of thyroid histologic lesions, when administered together with CsA	[61]
Heyman nephritis	Reduction of proteinuria and autoantibodies	[62]
Lupus nephritis	Inhibition of proteinuria, prevention of skin lesions	[37,63]
Mercuric chloride-induced glomerulonephritis	Prevention of autoimmune manifestations including proteinuria, serum IgE, and serum anti-laminin antibodies	[64,65]
Inflammatory bowel disease	Significant amelioration of symptoms, block of disease progression	[42]
Transplantation		
Aorta	Reduced signs of chronic rejection, in particular intimal hyperplasia	[54]
Bone marrow	Decreased graft-versus-host disease	[66]
Heart	Marked prolongation of non vascularized and vascularized heart allografts	[46–48]
Liver	Prolonged graft survival by decreasing the severity of acute rejection	[49]
Pancreatic islets	Induction of transplantation tolerance, prevention of autoimmune diabetes recurrence	[31,50,51]
Skin	Prolonged graft survival	[52,53]
Small bowel	Reduced amounts of hyaluronan secreted into the intestinal lumen	[48]

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-γ is directly downregulated through interaction of the ligand-bound VDR with a VDRE [21].

Immunomodulatory effects of $1,25(OH)_2D_3$ and its analogs in autoimmune diseases and allograft rejection

The immunoregulatory properties of $1,25(OH)_2D_3$ and its analogs have been demonstrated in different models of autoimmune diseases and in experimental organ transplantation (Table 1). Notably, $1,25(OH)_2D_3$ 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]. $1,25(OH)_2D_3$ 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, $1,25(OH)_2D_3$ 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, $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ [55], further suggesting its capacity to inhibit chronic graft rejection.

An important property of $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ or its analogs and immunosuppressive agents, such as CsA and sirolimus [58]. These effects have been confirmed *in vivo* 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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.

References

1 Casteels, K. *et al.* (1995) Immunomodulatory effects of 1,25-dihydroxyvitamin D3. *Curr. Opin. Nephrol. Hypertens.* 4, 313–318
2 Deluca, H.F. and Cantorna, M.T. (2001) Vitamin D: its role and uses in immunology. *FASEB J.* 15, 2579–2585
3 Haussler, M.R. *et al.* (1998) The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J. Bone Miner. Res.* 13, 325–349
4 Norman, A.W. *et al.* (2001) Ligands for the vitamin D endocrine system: different shapes function as agonists and antagonists for genomic and rapid response receptors or as a ligand for the plasma vitamin D binding protein. *J. Steroid Biochem. Mol. Biol.* 76, 49–59
5 Carlberg, C. and Polly, P. (1998) Gene regulation by vitamin D3. *Crit. Rev. Eukaryot. Gene Expr.* 8, 19–42
6 Provvedini, D.M. *et al.* (1983) 1,25-dihydroxyvitamin D3 receptors in human leukocytes. *Science* 221, 1181–1183
7 Brennan, A. *et al.* (1987) Dendritic cells from human tissues express receptors for the immunoregulatory vitamin D3 metabolite, dihydroxycholecalciferol. *Immunology* 61, 457–461
8 Veldman, C.M. *et al.* (2000) Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. *Arch. Biochem. Biophys.* 374, 334–338
9 Mathieu, C. *et al.* (2001) *In vitro* and *in vivo* analysis of the immune system of vitamin D receptor-knock out mice. *J. Bone Miner. Res.* 16, 2057–2065
10 Panda, D.K. *et al.* (2001) Targeted ablation of the 25-hydroxyvitamin D1α-hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. *Proc. Natl. Acad. Sci. U. S. A.* 98, 7498–7503
11 Nagpal, S. *et al.* (2001) Vitamin D analogs: mechanism of action and therapeutic applications. *Curr. Med. Chem.* 8, 1679–1697
12 Verstuyf, A. *et al.* (2000) Recent developments in the use of vitamin D analogues. *Expert Opin. Invest. Drugs* 9, 443–455
13 Rochel, N. *et al.* (2000) The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand. *Mol. Cell* 5, 173–179
14 Bhalla, A.K. *et al.* (1983) Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. *J. Clin. Endocrinol. Metab.* 57, 1308–1310
15 Bhalla, A.K. *et al.* (1984) 1,25-Dihydroxyvitamin D3 inhibits antigen-induced T cell activation. *J. Immunol.* 133, 1748–1754
16 Rigby, W.F. *et al.* (1987) Regulation of lymphokine production and human T lymphocyte activation by 1,25-dihydroxyvitamin D3. Specific inhibition at the level of messenger RNA. *J. Clin. Invest.* 79, 1659–1664
17 Lemire, J.M. *et al.* (1995) Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions. *J. Nutr.* 125, 1704S–1708S
18 Mattner, F. *et al.* (2000) Inhibition of Th1 development and treatment of chronic-relapsing experimental allergic encephalomyelitis by a non-hypercalcemic analogue of 1,25-dihydroxyvitamin D_3. *Eur. J. Immunol.* 30, 498–508
19 Alroy, I. *et al.* (1995) Transcriptional repression of the interleukin-2 gene by vitamin D_3: direct inhibition NFATp/AP-1 complex formation by a nuclear hormone receptor. *Mol. Cell. Biol.* 15, 5789–5799
20 Takeuchi, A. *et al.* (1998) Nuclear factor of activated T cells (NFAT) as a molecular target for 1α,25-dihydroxyvitamin D_3-mediated effects. *J. Immunol.* 160, 209–218
21 Cippitelli, M. and Santoni, A. (1998) Vitamin D_3: a transcriptional modulator of the IFN-γ gene. *Eur. J. Immunol.* 28, 3017–3030
22 Cantorna, M. *et al.* (1998) 1,25-dihydroxyvitamin D_3 is a positive regulator for the two anti-encephalitogenic cytokines TGF-β1 and IL-4. *J. Immunol.* 160, 5314–5319
23 Boonstra, A. *et al.* (2001) 1alpha,25-Dihydroxyvitamin D3 has a direct effect on naive $CD4^+$ T cells to enhance the development of Th2 cells. *J. Immunol.* 167, 4974–4980
24 Overbergh, L. *et al.* (2000) 1alpha,25-dihydroxyvitamin D3 induces an autoantigen-specific T-helper 1/T-helper 2 immune shift in NOD mice immunized with GAD65 (p524-543). *Diabetes* 49, 1301–1307
25 Nashold, F.E. *et al.* (2001) Rag-1-dependent cells are necessary for 1,25-dihydroxyvitamin D3 prevention of experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 119, 16–29
26 D'Ambrosio, D. *et al.* (1998) Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvement of NF-κB downregulation in transcriptional repression of the p40 gene. *J. Clin. Invest.* 101, 252–262
27 Penna, G. and Adorini, L. (2000) 1,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation and survival of dendritic cells leading to impaired alloreactive T cell activation. *J. Immunol.* 164, 2405–2411
28 Piemonti, L. *et al.* (2000) Vitamin D3 affects differentiation, maturation, and function of human monocyte-derived dendritic cells. *J. Immunol.* 164, 4443–4451
29 Griffin, M.D. *et al.* (2000) Potent inhibition of dendritic cell differentiation and maturation by vitamin D analogs. *Biochem. Biophys. Res. Commun.* 270, 701–708
30 Berer, A. *et al.* (2000) 1,25-Dihydroxyvitamin D(3) inhibits dendritic cell differentiation and maturation *in vitro*. *Exp. Hematol.* 28, 575–583
31 Gregori, S. *et al.* (2001) Regulatory T cells induced by 1α,25-Dihydroxyvitamin D_3 and mycophenolate mofetil treatment mediate transplantation tolerance. *J. Immunol.* 167, 1945–1953
32 Griffin, M.D. *et al.* (2001) Dendritic cell modulation by 1alpha,25 dihydroxyvitamin D3 and its analogs: A vitamin D receptor-dependent pathway that promotes a persistent state of immaturity *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 98, 6800–6805
33 Overbergh, L. *et al.* (2000) Identification and immune regulation of 25-hydroxyvitamin D-1-α-hydroxylase in murine macrophages. *Clin. Exp. Immunol.* 120, 139–146
34 Dusso, A. *et al.* (1994) Extrarenal production of calcitriol. *Semin. Nephrol.* 14, 144–155
35 Koren, R. *et al.* (1986) 1,25-Dihydroxyvitamin D3 enhances prostaglandin E2 production by monocytes. A mechanism which partially accounts for the antiproliferative effect of 1,25(OH)2D3 on lymphocytes. *FEBS Lett.* 205, 113–116
36 Towers, T.L. and Freedman, L.P. (1998) Granulocyte-macrophage colony-stimulating factor gene transcription is directly repressed by the vitamin D3 receptor. Implications for allosteric influences on nuclear receptor structure and function by a DNA element. *J. Biol. Chem.* 273, 10338–10348
37 Koizumi, T. *et al.* (1985) Effects of corticosteroid and 1,24R-dihydroxy-vitamin D3 administration on lymphoproliferation and autoimmune disease in MRL/MP-lpr/lpr mice. *Int. Arch. Allergy Appl. Immunol.* 77, 396–404
38 Lemire, J.M. and Archer, D.C. (1991) 1,25-dihydroxyvitamin D3 prevents the *in vivo* induction of murine experimental autoimmune encephalomyelitis. *J. Clin. Invest.* 87, 1103–1107
39 Cantorna, M.T. *et al.* (1996) 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* 93, 7861–7864
40 Larsson, P. *et al.* (1998) A vitamin D analogue (MC 1288) has immunomodulatory properties and suppresses collagen-induced arthritis (CIA) without causing hypercalcaemia. *Clin. Exp. Immunol.* 114, 277–283
41 Cantorna, M.T. *et al.* (1998) 1,25-Dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. *J. Nutr.* 128, 68–72
42 Cantorna, M.T. *et al.* (2000) 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. *J. Nutr.* 130, 2648–2652
43 Mathieu, C. *et al.* (1994) Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3. *Diabetologia* 37, 552–558
44 Mathieu, C. *et al.* (1995) Prevention of type I diabetes in NOD mice by nonhypercalcemic doses of a new structural analog of 1,25-dihydroxyvitamin D3, KH1060. *Endocrinology* 136, 866–872
45 Casteels, K. *et al.* (1998) Prevention of autoimmune destruction of syngeneic islet grafts in spontaneously diabetic nonobese diabetic mice by a combination of a vitamin D3 analog and cyclosporine. *Transplantation* 65, 1225–1232

46 Lemire, J.M. *et al.* (1992) Prolongation of the survival of murine cardiac allografts by the vitamin D3 analogue 1,25-dihydroxy-delta 16-cholecalciferol. *Transplantation* 54, 762–763

47 Hullett, D.A. *et al.* (1998) Prolongation of allograft survival by 1,25-dihydroxyvitamin D3. *Transplantation* 66, 824–828

48 Johnsson, C. and Tufveson, G. (1994) MC 1288 – a vitamin D analogue with immunosuppressive effects on heart and small bowel grafts. *Transpl. Int.* 7, 392–397

49 Redaelli, C.A. *et al.* (2001) 1alpha,25-Dihydroxycholecalciferol reduces rejection and improves survival in rat liver allografts. *Hepatology* 34, 926–934

50 van Etten, E. *et al.* (2001) Immunomodulatory properties of a 1,25(OH)(2) vitamin D(3) analog combined with IFNbeta in an animal model of syngeneic islet transplantation. *Transplant. Proc.* 33, 2319

51 Gysemans, C. *et al.* (2001) A combination of KH1060, a vitamin D(3) analogue, and cyclosporin prevents early graft failure and prolongs graft survival of xenogeneic islets in nonobese diabetic mice. *Transplant. Proc.* 33, 2365

52 Veyron, P. *et al.* (1993) Two novel vitamin D analogues, KH 1060 and CB 966, prolong skin allograft survival in mice. *Transpl. Immunol.* 1, 72–76

53 Bertolini, D.L. *et al.* (1999) Immunomodulatory effects of vitamin D analog KH1060 on an experimental skin transplantation model. *Transplant. Proc.* 31, 2998–2999

54 Raisanen-Sokolowski, A.K. *et al.* (1997) A vitamin D analog, MC1288, inhibits adventitial inflammation and suppresses intimal lesions in rat aortic allografts. *Transplantation* 63, 936–941

55 Aschenbrenner, J. *et al.* (2000) 1,25-dihydroxyvitamin D3 (1,25$(OH)_2D_3$) has salutary effects on renal allograft function. *Transplantation* 69, S448

56 Casteels, K.M. *et al.* (1998) Prevention of type I diabetes in nonobese diabetic mice by late intervention with nonhypercalcemic analogs of 1,25-dihydroxyvitamin D3 in combination with a short induction course of cyclosporin A. *Endocrinology* 139, 95–102

57 Casteels, K.M. *et al.* (1998) Sex difference in resistance to dexamethasone-induced apoptosis in NOD mice: treatment with 1,25(OH)2D3 restores defect. *Diabetes* 47, 1033–1037

58 van Etten, E. *et al.* (2000) Analogs of 1,25-dihydroxyvitamin D3 as dose-reducing agents for classical immunosuppressants. *Transplantation* 69, 1932–1942

59 Inaba, M. *et al.* (1992) Partial protection of 1 alpha-hydroxyvitamin D3 against the development of diabetes induced by multiple low-dose streptozotocin injection in CD-1 mice. *Metabolism* 41, 631–635

60 Branisteanu, D. *et al.* (1995) Prevention of murine experimental allergic encephalomyelitis: cooperative effects of cyclosporine and 1 α,25-$(OH)_2D_3$. *J. Neuroimmunol.* 61, 151–160

61 Fournier, C. *et al.* (1990) *In vivo* beneficial effect of cyclosporine A and 1,25-dihydroxyvitamin D_3 on the induction of experimental autoimmune thyroiditis. *Clin. Immunol. Immunopathol.* 54, 53–63

62 Branisteanu, D.D. *et al.* (1993) Partial prevention of active Heymann nephritis by 1α,25-dihydroxivitamin D_3. *Clin. Exp. Immunol.* 94, 412–417

63 Lemire, J.M. (1992) Immunomodulatory role of 1,25-dihydroxyvitamin D3. *J. Cell. Biochem.* 49, 26–31

64 Lillevang, S.T. *et al.* (1992) Single and combined effects of the vitamin D analogue KH1060 and cyclosporin A on mercuric-chloride-induced autoimmune disease in the BN rat. *Clin. Exp. Immunol.* 88, 301–306

65 Vendeville, B. *et al.* (1995) Effects of vitamin D3 and cyclosporin A on HgCl2-induced autoimmunity in brown Norway rats. *Nephrol. Dial. Transplant.* 10, 2020–2026

66 Pakkala, I. *et al.* (2001) MC1288, a vitamin D analog, prevents acute graft-versus-host disease in rat bone marrow transplantation. *Bone Marrow Transplant.* 27, 863–867