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Immunomodulatory Actions of 1,25-Dihydroxyvitamin D_3

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The sterol, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), has immunosuppressive activity. The hormone inhibits the production of lymphokines (IL-2, IFN-γ) and monocyte-derived cytokine (IL-12) leading to inhibition of helper T cell subset type 1 (Th_1). When given *in vivo*, the hormone prevents the development of spontaneous and induced models of autoimmunity. Analogs of $1,25(OH)_2D_3$, with reduced hypercalcemic effects, display an enhanced activity in autoimmunity compared to the sterol and prolong graft survival in experimental transplantation. This paper reviews our understanding of the cellular actions of the hormone and the therapeutic application of $1,25(OH)_2D_3$ and analogs in autoimmunity and transplantation.

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INTRODUCTION

In addition to its traditional role as a calcium regulating hormone, the active vitamin D metabolite, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), exerts anti-proliferative, pro-differentiating and immunosuppressive properties. The development of vitamin D_3 analogs with equipotent or enhanced activity but with reduced hypercalcemic effects compared to the natural compound, has allowed for newer applications *in vivo*. For example, the anti-proliferative effect of the drug has been applied for the treatment of psoriasis [1]. The analog Calcipotriene is now commonly used in humans for treatment of this condition. While such an application for its pro-differentiating and immunosuppressive effects in humans remains to be shown, the development of various analogs have allowed for a therapeutic use in animal models. For instance, the same analog, 1,25-dihydroxy-16ene-vitamin D_3 ($1,25(OH)_2$-16ene-D_3), can be an antileukemic agent as well as an immunosuppressant [2, 3]. Therefore, the same compound may display more than one action at any one time. We will summarize the properties of $1,25(OH)_2D_3$ and its analogs at a cellular and molecular levels and review the *in vivo* applications of the compounds with particular emphasis on the immunosuppressive effects.

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VITAMIN D_3 AND CELLS OF IMMUNE LINEAGE *IN VITRO*

Recent reviews [4–6] have outlined the various properties of $1,25(OH)_2D_3$ and its analogs. One decade ago, $1,25(OH)_2D_3$ was shown to inhibit lymphocyte function [7, 8]. The metabolite had an antiproliferative effect on lymphocytes and suppressed the immunoglobulin production after antigenic or mitogenic activation [7]. Further studies revealed the T helper cell to be particularly suppressed by the compound [9]. Despite their ability to express VDR upon activation, the B cells appeared to be more resistant to the direct effect of the drug [9]. Contrary to T cells, B cells do not up-regulate 24-hydroxylase activity mRNA expression [10] and it was suggested that the action of $1,25(OH)_2D_3$ on immunoglobulin production by B cells was mediated through monocytes [11].

Recent advances have contributed to improve our understanding of the action of the sterol. Based upon their lymphokine secreting pattern, helper T cells have been characterized into type 1 (Th_1) and type 2 (Th_2). Th_1 cells secrete IL-2 and IFN-γ, transfer delayed-type hypersensitivity (DTH) and provide help for IgG2a production by B cells [12]. Th_2 cells produce IL-4 and IL-10 and help B cells to produce IgE and IgG2b antibodies. By its ability to inhibit the production of IL-2 [13], IFN-γ [14], the transfer of DTH [15] and the production of antigen-specific IgG2a [5], $1,25(OH)_2D_3$ appears to target Th_1 cells

for its inhibitory effect. While the sterol has an antiproliferative effect on Th_2, when measured by thymidine incorporation after antigenic stimulation, the secretion of IL-4 is not inhibited in the presence of $1,25(OH)_2D_3$ [5]. Further evidence for a pro-Th_1 effect of Vitamin D_3 has been provided by the analog $1,25(OH)_2$-16ene-D_3. Using rye-grass antigen-specific human T cells clones, the analog was 100 times more potent than the natural hormone in inhibiting IFN-γ production by Th_1 cells but did not inhibit IL-4 secretion by Th_2 cells [5].

The effect of $1,25(OH)_2D_3$ on Th_1 cells may also be mediated through monocytes/macrophages. The cytokine IL-12, initially called natural killer cell stimulatory factor, is produced by B cells and macrophages and stimulates the production of IFN-γ from T cells [16]. IL-12 enhances the expansion of human Th_1 cells *in vitro* [17]. In murine transgenic mice, IL-12 induced the development of Th_1 cells from naive CD4 + cells in the presence of Listeria [18]. Therefore this cytokine may play a role in the initiation of cellular immunity [19]. Recently, in collaboration with Dr M. Gately, Hoffmann-LaRoche, Nutley, NJ and Dr Hans Spiegelberg, Department of Pediatrics, UCSD, we studied the effect of $1,25(OH)_2D_3$ on the production of IL-12 by normal human B cells and monocytes (Table 1). Cells were isolated from normal human volunteers, separated into B cells and monocytes by standard techniques, and incubated at 10^6 cells/ml, alone or with Staph. aureus Cowan strain I (SAC) 0.01% w/v for 18 h in the presence or absence of $1,25(OH)_2D_3$. Cells were then harvested and assayed for IL-12 by ELISA. A significant inhibition of IL-12 was observed for both monocytes and B cells. The hormone exerts two additional suppressive actions on monocytes/macrophages. A reduction of class II antigens on monocytes is seen in the presence of $1,25(OH)_2D_3$ without significant reduction of class I antigen expression [20, 21]. The accessory activity of monocytes, assessed by allogeneic mixed lymphocyte reaction, was also reduced in the presence of the sterol [22]. While $1,25(OH)_2D_3$ promotes phagocytosis and intracellular killing by macrophages [23], the resultant action of the compound is immunoinhibitory.

Table 1. Effect of $1,25(OH)_2D_3$ on IL-12 production by human monocytes and B cells

	Monocytes	B cells
Cells alone	<60*	<60
Cells + ETOH (vehicle)	<60	<60
Cells + SAC	448	260
Cells + SAC + ETOH	445	222
Cells + SAC + $1,25(OH)_2D_3$ 10^{-7} M	<60	77
Cells + SAC + $1,25(OH)_2D_3$ 10^{-8} M	92	120

*Data expressed as pg/ml of cytokine.

$1,25(OH)_2D_3$ AS AN IMMUNOMODULATOR *IN VIVO*

It soon became evident that the immunosuppressive effects of the hormone observed *in vitro* would find an application in animal models *in vivo*. The first trials were done using skin grafts in mice and cardiac allografts in rats; a prolongation of graft survival was observed in both instances but with significant toxicity due to hypercalcemia [24]. However, the immunosuppressive effect of the sterol in autoimmunity was more successful. In the animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), immunization of naive mice with neuroantigen induces a clinical paralysis within 10–15 days and a rise in antigen-specific titers within a month. We found that the administration of 0.1 μg (5 μg/kg/2 days) $1,25(OH)_2D_3$ intraperitoneally (i.p.) every other day starting 3 days prior to and for up to 15 days (subsequently up to 5 days) post-immunization significantly prevented the expression of EAE and the rise in antibody titers [25]. In other experimentally induced autoimmune diseases, $1,25(OH)_2D_3$ has also shown effectiveness. In autoimmune thyroiditis, a reduction of the severity of histologic lesions was observed with treatment with up to 0.2 μg/kg/day [26]. In active Heymann nephritis in Lewis rats, a reduction in proteinuria resulted from treatment with 0.5 μg/kg given every other day [27].

In spontaneous models of autoimmunity, the sterol was also quite immunosuppressive. In experimental murine lupus, female MRL/l mice treated with 0.1 μg $1,25(OH)_2D_3$ i.p. every other day, from 4 weeks of age, showed a prolongation of mean survival rate: 32 weeks vs 20 weeks (treated vs controls respectively). Treatment with the sterol was extremely effective in preventing the skin lesions [28]. A reduction of proteinuria and autoantibody titers was also seen in the animals treated with $1,25(OH)_2D_3$. In the spontaneous diabetes of NOD mice, with insulitis, the histopathological lesion of type 1 diabetes, the administration of 5 μg/kg i.p., given every other day, from 21 days of age, reduced the incidence of insulitis and the clinical onset of diabetes [29]. Therefore, when treatment is initiated at an early age, $1,25(OH)_2D_3$ can prevent the autoimmune process.

$1,25(OH)_2D_3$ ANALOGS AS IMMUNOMODULATORS *IN VIVO*

The availability of $1,25(OH)_2D_3$ analogs with less hypercalcemic effects have enhanced the potential therapeutic application of the sterol *in vivo*. In addition to suppressing the onset of autoimmune processes without significant hypercalcemia, the analogs have now revived the potential of Vitamin D_3 in the arsenal of immunosuppressants of transplantation.

Various analogs have been effective in inhibiting the development of autoimmune encephalomyelitis [30]. One in particular, $1,25(OH)_2$-16ene-D_3 (Hoffmann-La

Roche Inc., Nutley, NJ) suppressed the disease with less hypercalcemia than observed with $1,25(OH)_2D_3$. Recently, we found that a renal metabolite of that analog, $1,25(OH)_2$-24-OXO-16ene-D_3, was as immunosuppressive as the parent compound without inducing hypercalcemia *in vivo* [31]. Another analog, KH 1060 (Leo Pharmaceutical Products, Ballerup, Denmark), at 0.1 μg/kg/day, was effective in preventing the nephritis of mercuric chloride-induced autoimmune disease in BN rats [32]. This analog, when given every other day, was less effective in preventing Heymann nephritis of Lewis rats [27].

In transplantation, the analog $1,25(OH)_2$-16ene-D_3 prolonged survival of hearts between histoincompatible mice. The administration of 0.2 μg i.p., starting 3 days prior to transplantation and every other day until rejection, significantly prolonged graft survival; 27.2 ± 4 vs 11.57 ± 0.5 days respectively [3]. The other analog, KH 1060, prolonged skin allograft survival in mice [33]. Recently, the analog MC 1288 (Leo Pharmaceutical Products, Ballerup, Denmark), given at 0.1 μg/kg/day, had immunosuppressive effects on cardiac and small bowel grafts in rats [34].

The exact mechanism of action of the analogs remains to be elucidated. Potential mechanisms include conversion of the compounds into an intermediary metabolite with longer half-life *in vivo*, induction of conformational changes in the receptor complex with subsequent different biological responses or the nongenomic actions such as rapid calcium transport leading to different responses.

CONCLUSIONS

The sterol, $1,25(OH)_2D_3$, exerts immunosuppressive properties *in vitro* and *in vivo*. At a cellular level, the hormone prevents the expression of Th_1 directly or indirectly through inhibition of monocyte-derived IL-12. When used *in vivo*, the hormone inhibits spontaneous or induced models of autoimmunity, presumably by preventing the activation of Th_1 cells.

The natural hormone cannot, however, prolong graft survival without significant toxicity. Various analogs display reduced hypercalcemic activity compared to the sterol. However, they differ in their immunosuppressive effects. In general, the analogs' effects on the immune system is similar to $1,25(OH)_2D_3$ but their activity is enhanced 10–100-fold. Further studies on receptor configuration after binding to VDR, natural metabolites of the drugs and affinity to Vitamin D binding protein may help to select the "optimal" compound.

While the immunosuppressive effect of $1,25(OH)_2D_3$ cannot be dissociated from its hypercalcemic activity, other actions of this pleiotropic compound cannot be excluded. For example, $1,25(OH)_2D_3$ and analogs have been used for their anti-proliferative effect in the treatment of psoriasis. There is now suggestion that psoriasis may be an immunologically mediated process and that the T cells involved in the lesions belong to the Th_1 subset [35, 36]. Therefore, the vitamin D_3 compounds may not only suppress the proliferation of keratinocytes but prevents the expression of pathogenic Th_1 cells. Our further understanding of the immune process and the molecular and cellular actions of $1,25(OH)_2D_3$ and analogs, should lead to newer and better therapeutic application of the drugs.

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