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COMMENTARY

IMMUNOLOGICAL PROPERTIES OF VITAMIN D ANALOGUES AND METABOLITES

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The discovery of vitamin D_2 (ergocalciferol, from UV-irradiation of plant ergosterol) and D_3 (cholecalciferol, from UV-irradiation of 7-dehydrocholesterol in the skin) and the elucidation of their physiological role in stimulating intestinal calcium absorption and inducing bone mineralization are well known. For several decades it was thought that these vitamins *per se* were the active forms that mediated the biological activities. Later findings, however, have shown that both vitamin D_2 and D_3 are hydroxylated in the liver and in the kidney to their physiologically active forms [1, 2]. Figure 1 shows the transformations that lead to the formation of 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2$D$_3$]† from vitamin D_3.

Further studies have established that the biological actions of 1α,25(OH)$_2$D$_3$ on calcium metabolism are mediated by binding to specific receptors in the intestine, bone and kidney [3]. It has been shown that after binding to its receptor in the intestinal epithelial cells, 1α,25(OH)$_2$D$_3$ can induce the synthesis of calcium-transporting proteins, named *calbindins*, that mediate the uptake of calcium from the gut into the organism.

In the last 10 years, 1α,25(OH)$_2$D$_3$ has been shown to exert biological activities far beyond its effects on calcium uptake, control of serum calcium levels and regulation of bone mineralization [4]. The specific 1α,25(OH)$_2$D$_3$ receptor was found in many cells and tissues not traditionally involved in the regulation of calcium metabolism. This receptor was present in various cancer cell lines, skin cells, muscle cells, hematopoietic cells and also in the pancreas, the brain and the pituitary [3]. Initial observations in mouse myeloid leukemia cells suggested that 1α,25(OH)$_2$D$_3$ possessed a number of cell-regulating properties [5]. Since then, a large number of undifferentiated or low-differentiated cancer cell lines have been shown to differentiate towards the normal phenotype under the influence of 1α,25(OH)$_2$D$_3$ [4]. At the same time, their rate of proliferation was reduced markedly. The cells of the upper layer of the skin, the basal epithelial keratinocytes, also responded to 1α,25(OH)$_2$D$_3$ *in vitro* by inhibition of growth, coupled with an increased rate of terminal differentiation [6, 7]. Among the cells of the immune system, monocytes and macrophages have been found to express the 1α,25(OH)$_2$D$_3$ receptor constitutively [8], whereas lymphocytes require prior activation with antigen or mitogen before expressing the receptor [9]. The proliferation of activated T-lymphocytes *in vitro* was found to be inhibited profoundly by 1α,25(OH)$_2$D$_3$ [10].

These findings have stimulated an interest in the possible use of 1α,25(OH)$_2$D$_3$ in the treatment of proliferative diseases such as cancer and psoriasis and, quite recently, also in the treatment of autoimmune disorders and in the prevention of graft rejection. However, the use of 1α,25(OH)$_2$D$_3$ is limited by its potent effects on calcium metabolism which may result in the development of hypercalciuria, hypercalcemia and soft tissue calcifications [11]. This has led to an intensive search for new vitamin D analogues with an increased ability to regulate cell activation, proliferation and differentiation, but with a decreased risk of inducing calcemic side-effects.

The aim of the present commentary is to give an overview of the present state of knowledge regarding the effects of new vitamin D analogues on the immune system and to discuss their possible mechanisms of action and potential therapeutic use. A number of studies have dealt with the role of vitamin D analogues in the treatment of psoriasis [12, 13]. These analogues will only be discussed in the following to the extent that they contribute to our understanding of the role of 1α,25(OH)$_2$D$_3$ and its analogues in the regulation of immune responsiveness. The same applies to those analogues that have been reported to inhibit cancer cell growth *in vitro* or *in vivo*.

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† Abbreviations: 1α,25(OH)$_2$D$_3$, 1α,25-dihydroxyvitamin D_3; 1α(OH)D$_3$, 1α-hydroxyvitamin D_3; IL, interleukin; TNF-α, tumor necrosis factor α; CyA, cyclosporin A; DBP, vitamin D binding protein; OCT, 22-oxa-1α,25(OH)$_2$D$_3$; LPS, lipopolysaccharide; PHA, phytohemagglutinin; and PMN, polymorphonuclear leucocyte.

THE IMMUNOLOGICAL PROFILE OF 1α,25(OH)$_2$D$_3$

To discuss the immunological properties of new vitamin D analogues, it is necessary to recall the main effects of 1α,25(OH)$_2$D$_3$, the physiologically active form of vitamin D_3, on the immune system. This will be done mainly with reference to previous reviews by Amento [14], Rigby [15] and Manolagas *et al.* [16]. Table 1 shows some of the key findings

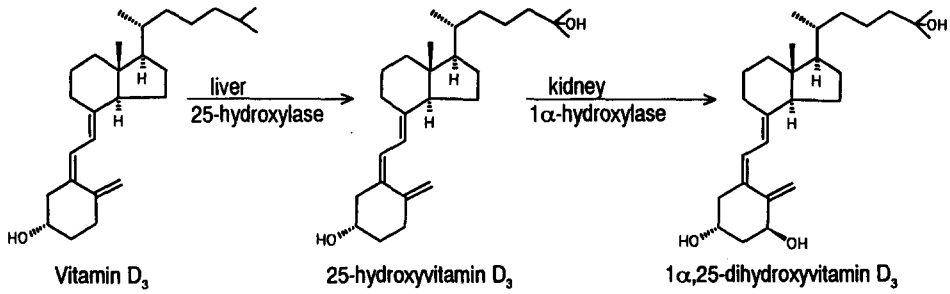

Fig. 1. Formation of 1α,25-hydroxyvitamin D_3 by hydroxylation of vitamin D_3 in the liver and in the kidney.

Table 1. Effects of 1α,25$(OH)_2D_3$ *in vitro* and *in vivo* on the immune system

In vitro:
Monocytes/Macrophages
Induction of monocyte differentiation.
Increased expression of MHC/class II antigens induced by γ-IFN.
Increased antigen presentation.
Enhancement of chemotaxis, phagocytosis and cytotoxic functions.
Modulation of lymphokine production.
T-lymphocytes
Decreased proliferation of activated lymphocytes (antigen-, mitogen- or cytokine-induced).
Inhibition of IL-2 production.
Inhibition of γ-IFN and GM-CSF production.
Decreased proliferation of IL-1/PHA activated thymocytes.
B-lymphocytes
Inhibition of IgM and IgG synthesis through effects on T-lymphocytes.
In vivo:
Inhibition of cytokine release from monocytes from human volunteers.
Inhibition of autoimmune encephalomyelitis and thyroiditis in mice.
Prolongation of skin allograft survival in mice.
Potentiation of primary immune responses and inhibition of hyperimmune responses in mice.
Restoration of defective macrophage and lymphocyte functions in vitamin D-deficient rats and in patients with vitamin D-resistant rickets.
Restoration of lymphocyte proliferation and IL-2 production in patients on hemodialysis.

with 1α,25$(OH)_2D_3$ *in vitro* and *in vivo*, which are briefly commented upon below.

Effects of 1α,25$(OH)_2D_3$ in vitro *on monocytes/macrophages*

It has been well documented that monocytes and macrophages from both human and animal sources constitutively express the 1α,25$(OH)_2D_3$ receptor [8]. Upon incubation with 1α,25$(OH)_2D_3$, monocytes acquire characteristics of functionally mature macrophages, and a general enhancement of the accessory cell functions is observed. The situation regarding cytokine production and release is less clear. Several studies indicate that 1α,25$(OH)_2D_3$ down-regulates cytokine production (IL-1, IL-6, TNF-α) by antigen or lipopolysaccharide (LPS)-stimulated monocytes [17, 18], whereas others have found unchanged or increased production [19], depending on the eliciting stimulus and the culture conditions.

Effects of 1α,25$(OH)_2D_3$ in vitro *on lymphocytes*

Lymphocytes, on the other hand, require activation in order to express the 1α,25$(OH)_2D_3$ receptor. Following this initial activation, incubation with 1α,25$(OH)_2D_3$ inhibits T-lymphocyte proliferation induced by antigens, cytokines and several mitogens [14, 15]. The role of accessory cells in this effect is under discussion. Some studies point to an important role for monocytes [19], whereas others have found that T-lymphocyte proliferation can be inhibited in the virtual absence of monocytes [20, 21]. Whatever the mechanism, IL-2 production by activated T-lymphocytes is strongly inhibited by 1α,25$(OH)_2D_3$ and this seems to be one of the crucial events in the immunoregulatory activities of 1α,25$(OH)_2D_3$, linking it to the immunosuppressive properties exerted by known immunosuppressants such as cyclosporin A (CyA) and FK 506 [22].

Effects of 1α,25$(OH)_2D_3$ *on immune functions* in vivo

In contrast to the *in vitro* situation, where 1α,25$(OH)_2D_3$ is mainly characterized by its immunosuppressive properties via inhibition of T-

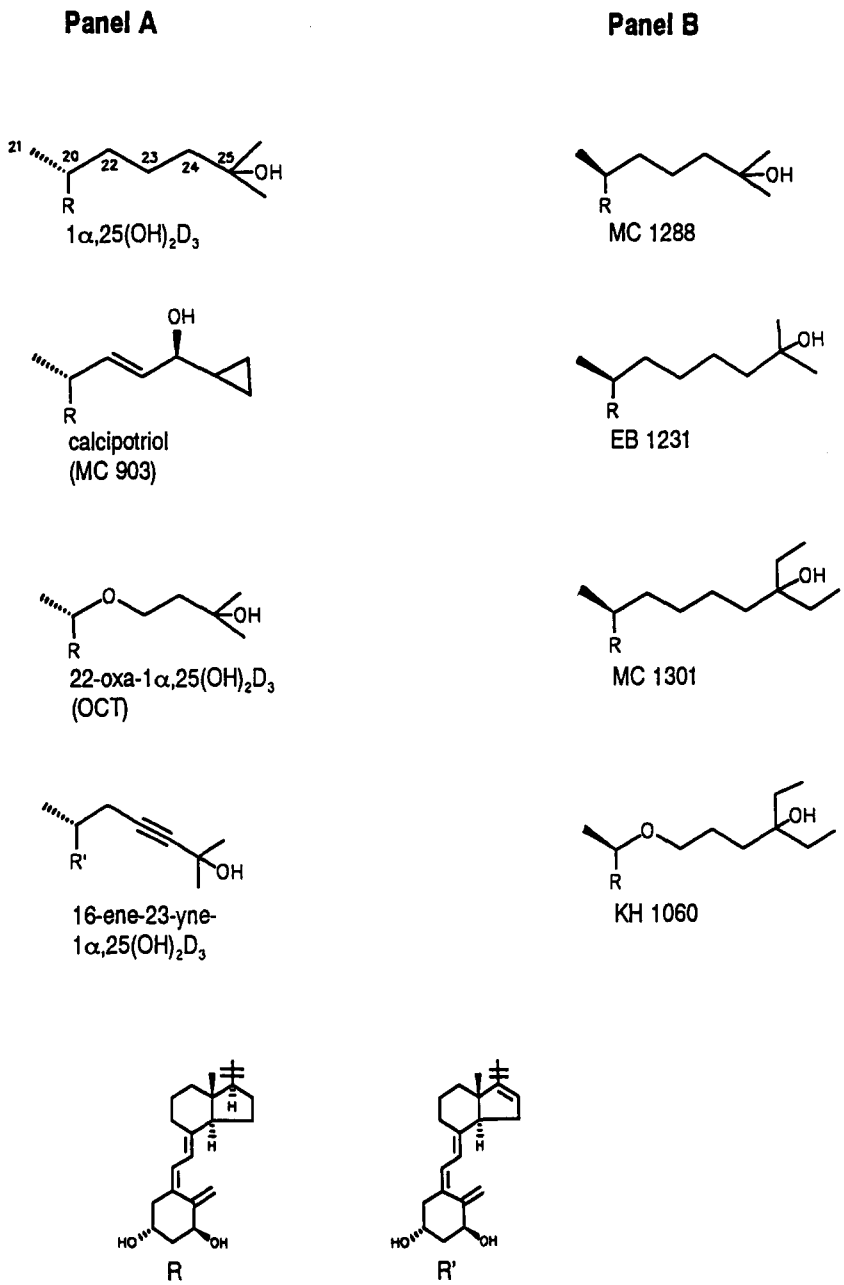

Fig. 2. New vitamin D analogues with altered side chain structures. Panel A shows structures with a stereochemical configuration at C20 identical to that of $1\alpha,25(OH)_2D_3$. Panel B shows analogues with altered stereochemistry at C20 (20-epi-vitamin D_3 analogues).

cell proliferation, the effects of $1\alpha,25(OH)_2D_3$ on the immune responses *in vivo* are more complex. Administration of $1\alpha,25(OH)_2D_3$ can prevent the development of experimental autoimmune encephalomyelitis and thyroiditis in mice [23, 24] and prolong the survival of transplanted skin allografts in mice [25]. A recent report describes reduced monocyte functions in human volunteers given 2 μg/day of $1\alpha,25(OH)_2D_3$ for 7 days [26]. In normal mice, administration of 1α-hydroxyvitamin D_3 [$1\alpha(OH)D_3$], a synthetic vitamin D analogue that is converted to $1\alpha,25(OH)_2D_3$ in the organism, leads to an increased primary antibody response, but the same treatment suppresses the hyperimmune response induced by concomitant administration of colchicine [27]. Several studies have shown that treatment with $1\alpha,25(OH)_2D_3$ or $1\alpha(OH)D_3$ can restore deficient macrophage and lymphocyte

Table 2. Effects of 20-epi-vitamin D_3 analogues on cancer cell proliferation and T-lymphocyte activation *in vitro*, and on calcium metabolism *in vivo*

Vitamin D analogue	Inhibition of cancer cell proliferation IC_{50} (M)	Inhibition of allogeneic T-cell activation IC_{50} (M)	Calcemic activity relative to $1\alpha,25(OH)_2D_3$
$1\alpha,25(OH)_2D_3$	1.4×10^{-8}	5.2×10^{-11}	1.0
MC 1288	2.8×10^{-10}	1.9×10^{-13}	2.3
EB 1231	4.4×10^{-11}	3.2×10^{-13}	5.0
MC 1301	8.2×10^{-11}	2.2×10^{-14}	1.2
KH 1060	1.0×10^{-12}	4.9×10^{-15}	1.3

Inhibition of cancer cell proliferation (U 937 hisitocytic lymphoma cells) and T-lymphocyte activation was measured *in vitro*. The calcemic activty of the vitamin D analogues was measured by the increase in calcium excretion in the urine after p.o. administration and calculated in relation to $1\alpha,25(OH)_2D_3$. For details of test systems, see Ref. 48.

functions in vitamin D-deficient rats [28], in patients with vitamin D-resistant rickets [29] and in patients on hemodialysis [30]. These findings suggest a dual role for $1\alpha,25(OH)_2D_3$ under physiological conditions: as an important factor in sustaining normal functions of the immune system (which can be compromised by vitamin D deficiency) and as a suppressor/regulator of activated lymphocytes, especially activated $CD4^+$ T-helper cells.

DEVELOPMENT OF NEW VITAMIN D ANALOGUES

With evidence accumulating to indicate that $1\alpha,25(OH)_2D_3$ could act as an important factor in immunobiology, the search for new analogues with increased cell-regulating properties and decreased risks of inducing calcemic side-effects has been intensified. Most of these analogues are characterized by structural changes in the side chain at carbon 17 (C17) in the $1\alpha,25(OH)_2D_3$ molecule, as shown by the two series of representative analogues in Fig. 2, panels A and B. The main difference between the two series lies in the stereochemical configuration of the methyl group at carbon 20 (C20), which is altered in the structures shown in panel B, compared to the configuration of $1\alpha,25(OH)_2D_3$. These 20-epi-vitamin D_3 analogues with altered stereochemistry differ markedly in potency from the analogues with "normal" C20 stereochemistry, as described below.

Analogues with normal C20 stereochemistry

Some of the main representatives of this type of analogues are shown in Fig. 2, panel A.

Calcipotriol (MC 903) was first described as a novel vitamin D analogue able to inhibit proliferation and induce differentiation of cancer cells [31] and skin cells [32] *in vitro*, with a potency similar to that of $1\alpha,25(OH)_2D_3$. *In vivo*, however, calcipotriol was 100–200 times less active than $1\alpha,25(OH)_2D_3$ in promoting intestinal calcium absorption and in inducing bone mineralization in rachitic rats [31]. The low calcemic activity *in vivo* was associated with a decreased binding to serum proteins, in particular to the vitamin D binding protein (DBP) that serves to transport 25-hydroxyvitamin D_3 and $1\alpha,25(OH)_2D_3$ [33, 34], and with a rapid metabolic clearance, leading to the formation of biologically inactive metabolites [35, 36]. Due to this rapid inactivation *in vivo*, calcipotriol was considered a promising candidate for the topical treatment of hyperproliferative disease, with a low risk of inducing systemic side-effects. Recent studies have shown that calcipotriol is an effective and safe treatment of psoriasis vulgaris [13]. In addition, it may induce regression of cutaneous metastatic breast cancer in patients whose tumours express the vitamin D receptor [37]. *In vitro*, calcipotriol exerts immunological effects which both qualitatively and quantitatively are similar to those of $1\alpha,25(OH)_2D_3$. These effects include inhibition of thymocyte proliferation induced by IL-1 [38] and reduction of immunoglobulin production through interference with T-helper cell functions [18]. *In vivo*, topical treatment with calcipotriol has been shown to decrease polymorphonuclear leucocyte (PMN) and T-lymphocyte accumulation and to reduce IL-6 expression in psoriatic lesions [39, 40]. In addition to its effect on keratinocyte proliferation and differentiation, calcipotriol may thus play a role in down-regulating the immunoinflammatory processes in psoriatic skin.

22-Oxa-$1\alpha,25(OH)_2D_3$ (OCT) is another novel analogue of $1\alpha,25(OH)_2D_3$. It is characterized by the introduction of an oxygen atom in the side chain (in the 22-position) (Fig. 2, panel A) [41]. This analogue is interesting for its ability to augment the primary immune response in mice immunized with sheep erythrocytes, without inducing calcemic side-effects [42]. Like calcipotriol, OCT is effective in inhibiting cancer cell proliferation *in vitro* [41]. Compared to $1\alpha,25(OH)_2D_3$, OCT has a reduced binding to DBP and a decreased serum half-life [43].

The last structure shown in Fig. 2, panel A, is $1\alpha,25$-dihydroxy-16-ene-23-yne-vitamin D_3. In addition to the introduction of a triple bond in the side chain, it is characterized by a double bond between C16 and C17 in the sterol moiety. This analogue slightly increases the clonal growth of normal human myeloid clonogenic cells, whereas clonal growth of myeloid leukemia cells is potently

inhibited [44]. *In vivo*, it prolongs survival of leukemic mice, while being approximately 10 times less calcemic than $1\alpha,25(OH)_2D_3$ [45].

A great number of other interesting analogues with structural changes in the side chain, but with the stereochemistry at C20 characteristic of $1\alpha,25(OH)_2D_3$, have been described in the past few years. These analogues, however, fall outside the scope of this commentary, as their immunological properties *in vitro* and/or *in vivo* have not been investigated. Interested readers are referred to the comprehensive review by Ikekawa [46].

20-*Epi-vitamin* D_3 *analogues*

Recently, the synthesis of a new class of vitamin D_3 analogues, the 20-epi-analogues, has been described [47]. As shown in Fig. 2, panel B, the common feature of these analogues is the altered stereochemistry at C20. In addition, various modifications have been made in the side chain. The effects of the 20-epi-vitamin D_3 analogues on cancer cell growth and differentiation, and on T-lymphocyte activation *in vitro*, have been described recently [48]. A brief summary of these findings is shown in Table 2. Many studies have shown that a number of vitamin D analogues are potent regulators of cell growth and differentiation, but the results obtained with the 20-epi-analogues suggest, for the first time, that such analogues may exert strong immunosuppressive activities at concentrations well below those that induce changes in calcium metabolism. Detailed investigations of the *in vivo* immunological profile of the 20-epi-analogues, are, by nature of their novelty, not yet available in published form. Preliminary studies with KH 1060, the most potent of the 20-epi-analogues, indicate, however, that this analogue is able to prolong significantly mouse skin allograft survival at doses as low as 0.02 μg/kg/day*. In addition, KH 1060 at 0.03 μg/kg/day prevents the development of autoimmune glomerulonephritis in BN rats injected with $HgCl_2$ [49]. In both studies, concomitant treatment with suboptimal doses of KH 1060 and CyA resulted in additive or synergistic effects.

Studies in progress include prevention of graft rejection in rats with kidney and heart allografts, prevention of rejection of transplanted pancreatic islets and prevention of development of insulitis in NOD mice.

Mechanisms of action of 20-*epi-vitamin* D_3 *analogues*

So far, *in vitro* studies with the 20-epi-vitamin D_3 analogues, and in particular with KH 1060, have been directed at establishing the mechanism(s) of action leading to the inhibition of proliferation of activated T-lymphocytes. These studies have shown that the production and release of lymphocyte-activating cytokines from LPS-stimulated human monocytes (IL-1β, IL-6 and TNF-α) are not affected by incubation with the analogues for up to 24 hr [48]. In contrast, a potent inhibition of IL-2 release from PHA-stimulated human peripheral blood lymphocytes is observed, and the inhibitory effects of the analogues on T-lymphocyte proliferation are reversed by addition of IL-2 [48]. These findings suggest that the 20-epi-analogues inhibit T-lymphocyte activation by a mechanism involving inhibition of IL-2 production and/or release. This is similar to what has been described previously for $1\alpha,25(OH)_2D_3$ [15, 16], but the 20-epi-vitamin D_3 analogues exert their suppressive effects at concentrations several orders of magnitude below those of $1\alpha,25(OH)_2D_3$.

This increased potency of the 20-epi-vitamin D_3 analogues raises a number of questions. The first one is whether these analogues act via binding to the $1\alpha,25(OH)_2D_3$ cellular receptor. Studies with a number of receptor preparations from various tissues have shown that the analogues are able to bind to the receptor, with an affinity close to that of $1\alpha,25(OH)_2D_3$ itself [48]. In my experience with more than 200 new vitamin D analogues with various structural modifications, failure to bind to the $1\alpha,25(OH)_2D_3$ receptor is always accompanied by a loss of cell-regulating activities. These findings, of course, do not exclude the possibility of additional non-receptor-mediated effects, but show that receptor-binding has to be considered as a crucial event in the regulation of cell proliferation by vitamin D analogues and metabolites in many cell types, including cells of the immune system.

The binding of an analogue with altered side chain stereochemistry to the receptor for $1\alpha,25(OH)_2D_3$ may induce conformational changes in the resultant receptor complex. This, in turn, may lead to alterations in the ability to interact with the DNA-binding site, to an altered pattern of gene expression and/or to changes in the intracellular clearance of the receptor complex. Each of these changes may alter the magnitude or type of biological response. In this respect, it is interesting to note that a hexafluoro-analogue of $1\alpha,25(OH)_2D_3$ forms receptor complexes that bind more tightly to DNA than the $1\alpha,25(OH)_2D_3$ receptor complex itself. This analogue is known to be approximately 10 times more potent than $1\alpha,25(OH)_2D_3$ in suppressing proliferation of leukemic cells [50].

Another important question is how the 20-epi-vitamin D_3 analogues compare to existing immunosuppressive agents with selective actions on activated T-cells. The most prominent of these are CyA and FK 506. Both compounds inhibit the transcription of T-cell activation genes and the production of IL-2 [51, 52]. Although the 20-epi-analogues inhibit T-cell activation *in vitro* at concentrations much lower than those needed for CyA, complete inhibition of cell proliferation is never achieved. Maximum inhibition of proliferation reaches only 60–80%, depending on cell type and activating stimulus [53]. CyA, in contrast, is able to cause complete inhibition. This may indicate that not all the stimulated T-lymphocytes possess the $1\alpha,25(OH)_2D_3$ receptor, but may also suggest a more fundamental difference in the mechanism of action. In this respect, a recent report shows that decreased levels of IL-2 and γ-IFN/TNF-β production in human T-lymphocytes incubated with

* Veyron P, Pamphile R, Binderup L and Touraine JL, Immunological properties of 1,25 vitamin D_3 analogues: A strong effect on the prolongation of mouse skin allograft survival. Abstract, *Eighth Workshop on Vitamin D, Paris, July 1991.*

$1\alpha,25(OH)_2D_3$ are not accompanied by any changes in transcriptional rates, but that the stability of their mRNA is decreased [54]. If similar post-transcriptional effects are operative in cells exposed to the 20-epi-analogues, this may provide an additional clue to their mechanism of action and also suggest a useful synergistic effect in combination with other immunosuppressive agents such as CyA.

Lastly, the possible effects of the 20-epi-analogues on the regulation of intracellular calcium levels have to be considered as well. $1\alpha,25(OH)_2D_3$ has been shown to modulate cytosolic free calcium levels by mechanisms considered to be of a non-genomic nature. Rapid increases in intracellular levels are presumably caused by stimulation of calcium influx and/or release of calcium from intracellular stores [55, 56]. In some studies, increased membrane phosphoinositide turnover is observed, leading to activation of protein kinase C, a known regulator of cell proliferation and differentiation [57]. These findings suggest a possible link between the rapid non-genomic membrane-associated effects and the receptor-mediated effects on transcriptional and translational events of $1\alpha,25(OH)_2D_3$. Recently, the mechanism(s) of action of CyA and FK 506 has been linked to signal transduction pathways associated with a rise in intracellular calcium levels [58]. Current investigations with the 20-epi-vitamin D_3 analogues will determine whether these analogues exhibit a similar or increased ability to interact with calcium-mediated signal transduction and whether these effects are pertinent to the immunosuppressive activities of these analogues *in vitro* and *in vivo*.

THERAPEUTIC PROSPECTS OF VITAMIN D ANALOGUES

For more than a decade, vitamin D analogues and metabolites have been used in the treatment of various bone diseases, notably in patients with renal osteodystrophy and in vitamin D-resistant rickets. The discovery of the cell-regulating effects of $1\alpha,25(OH)_2D_3$ on proliferation and differentiation has led to the hope that this compound or its analogues may be useful in the treatment of hyperproliferative disorders such as cancer and psoriasis. A first step in this direction was taken in 1985, when a small study in patients with non-Hodgkin's lymphoma showed that treatment with alfacalcidol [$1\alpha(OH)D_3$] was able to induce tumour regression [59]. A positive correlation was established between the presence and the amount of the $1\alpha,25(OH)_2D_3$ receptor in tumour tissue and the response to treatment. Since then, a number of studies have investigated the therapeutic value of combining $1\alpha(OH)D_3$ with other inducers of cell differentiation and with cytostatics [60]. The usefulness of the new vitamin D analogues in the treatment of cancer is next to be assessed.

With the development of calcipotriol, the use of vitamin D metabolites and analogues in the topical treatment of psoriasis has been firmly established [13]. Future studies will determine whether systemic treatment of psoriasis with vitamin D analogues will offer an equally effective and safe therapy.

In addition, the synthesis of new vitamin D analogues that, at very low concentrations, are able to inhibit T-lymphocyte activation elicited by cytokines or alloantigens, may give rise to a new class of immunosuppressive compounds. These analogues may be of therapeutic interest in the prevention of graft rejection and in the treatment of autoimmune diseases.

However, in order to evaluate their therapeutic potential, the following points have to be considered:

The effects of the new analogues on calcium metabolism have to be reduced considerably, while retaining or increasing their cell-regulating activities. To what extent is this possible? Many of the new analogues probably owe their reduced calcemic activity *in vivo* to an increased rate of metabolic clearance. This makes them eminently suitable for topical treatment, but less so for systemic administration. Nor does selectivity seem to reside at the receptor-binding level, as very extensive homology exists between all studied $1\alpha,25(OH)_2D_3$ receptors. The best hope for a selective effect seems to lie at the transcriptional level, as different genes are expressed in different cells in response to $1\alpha,25(OH)_2D_3$.

This leads immediately to the next question of concern. Is it possible to control the many cell-regulating activities of the vitamin D analogues without compromising the functions of normal cells? So far, the clinical experience with pharmacological doses of $1\alpha,25(OH)_2D_3$ has been reassuring. With regard to the analogues, it must be kept in mind that their main advantage is that they are active at much lower concentrations than $1\alpha,25(OH)_2D_3$. It remains to be shown whether the magnitude of their biological effects can be increased *in vivo*, at non-calcemic doses.

In summary, this commentary has attempted to describe some of the new aspects of our knowledge of the immunological properties of $1\alpha,25(OH)_2D_3$, the physiologically active metabolite of vitamin D_3, and its new analogues. These analogues will, in the future, serve as tools to increase our understanding of the role of vitamin D in immunobiology, not only in basal research but also, hopefully, in the therapy of immune-mediated diseases.

REFERENCES

1. DeLuca HF, Kristinger J and Darwish H, The vitamin D system: 1990. *Kidney Int* **38**: S2–S8, 1990.
2. Collins ED and Norman AW, Vitamin D. In: *Handbook of Vitamins* (Ed. Machlin LJ), pp. 59–98. Marcel Dekker, New York, 1990.
3. Minghetti PP and Norman AW, $1,25(OH)_2$-Vitamin D_3 receptors: Gene regulation and genetic circuitry. *FASEB J* **2**: 3043–3053, 1988.
4. Reichel H, Koeffler HP and Norman AW, The role of the vitamin D endocrine system in health and disease. *N Engl J Med* **320**: 980–991, 1989.
5. Abe E, Miyaura C, Sakagami H, Takeda M, Konno K, Yamazaki T, Yoshiki S and Suda T, Differentiation of mouse myeloid leukemia cells induced by $1\alpha,25$-dihydroxyvitamin D_3. *Proc Natl Acad Sci USA* **78**: 4990–4994, 1981.
6. Hosomi J, Hosoi J, Abe E, Suda T and Kuroki T, Regulation of terminal differentiation of cultured mouse epidermal cells by $1\alpha,25$-dihydroxyvitamin D_3. *Endocrinology* **113**: 1950–1957, 1983.
7. Smith EL, Walworth NC and Holick MF, Effect of

1α,25-dihydroxyvitamin D_3 on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum-free conditions. *J Invest Dermatol* **86**: 709–714, 1986.
8. Bhalla AK, Amento EP, Clemens TL, Holick MF and Krane SM, Specific high-affinity receptors for 1,25-dihydroxyvitamin D_3 in human peripheral blood mononuclear cells: Presence in monocytes and induction in T-lymphocytes following activation. *J Clin Endocrinol Metab* **57**: 1308–1310, 1983.
9. Provvedini DM, Tsoukas CD, Deftos LJ and Manolagas SC, 1,25-Dihydroxyvitamin D_3 receptors on human leucocytes. *Science* **221**: 1181–1183, 1983.
10. Rigby WFC, Stacy T and Fanger MW, Inhibition of T lymphocyte mitogenesis by 1,25-dihydroxyvitamin D_3 (calcitriol). *J Clin Invest* **74**: 1451–1455, 1984.
11. Vieth R, The mechanisms of vitamin D toxicity. *Bone Miner* **11**: 267–272, 1990.
12. Araujo OE, Flowers FP and Brown KD, Vitamin D therapy in psoriasis. *DICP Ann Pharmacother* **25**: 835–839, 1991.
13. Kragballe K, Gjertsen BT, de Hoop D, Karlsmark T, van de Kerkhof PCM, Larkö O, Nieboer C, Roed-Petersen J, Strand A and Tikjøb G, Double-blind, right/left comparison of calcipotriol and betamethasone valerate in treatment of psoriasis vulgaris. *Lancet* **337**: 193–196, 1991.
14. Amento EP, Vitamin D and the immune system. *Steroids* **49**: 55–72, 1987.
15. Rigby WFC, The immunobiology of vitamin D. *Immunol Today* **9**: 54–58, 1988.
16. Manolagas SC, Hustmyer FG and Yu XP, Immunomodulating properties of 1,25-dihydroxyvitamin D_3. *Kidney Int* **38**: S9–S16, 1990.
17. Tsoukas CD, Watry D, Escobar SS, Provvedini DM, Dinarello CA, Hustmyer FG and Manolagas SC, Inhibition of interleukin-1 production by 1,25-dihydroxyvitamin D_3. *J Clin Endocrinol Metab* **69**: 127–133, 1989.
18. Müller K, Heilmann C, Poulsen LK, Barington T and Bendtzen K, The role of monocytes and T cells in 1,25-dihydroxyvitamin D_3 mediated inhibition of B cell function *in vitro*. *Immunopharmacology* **21**: 121–128, 1991.
19. Bhalla AK, Amento EP and Krane SM, Differential effects of 1,25-dihydroxyvitamin D_3 on human lymphocytes and monocyte/macrophages: Inhibition of interleukin-2 and augmentation of interleukin-1 production. *Cell Immunol* **98**: 311–322, 1986.
20. Koren R, Liberman UA, Maron L, Novogrodsky A and Ravid A, 1,25-Dihydroxyvitamin D_3 acts directly on human lymphocytes and interferes with the cellular response to interleukin-2. *Immunopharmacology* **18**: 187–194, 1989.
21. Vanham G, Ceuppens JL and Bouillon R, T Lymphocytes and their CD4 subset are direct targets for the inhibitory effect of calcitriol. *Cell Immunol* **124**: 320–333, 1989.
22. Gupta S, Fass D, Shimizu M and Vayuvegula B, Potentiation of immunosuppressive effects of cyclosporin A by 1α,25-dihydroxyvitamin D_3. *Cell Immunol* **121**: 290–297, 1989.
23. Lemire JM and Archer DC, 1,25-Dihydroxyvitamin D_3 prevents the *in vivo* induction of murine experimental autoimmune encephalomyelitis. *J Clin Invest* **87**: 1103–1107, 1991.
24. Fournier C, Gepner P, Sadouk M'B and Charrèire J, *In vivo* beneficial effects of cyclosporin A and 1,25-dihydroxyvitamin D_3 on the induction of experimental autoimmune thyroiditis. *Clin Immunol Immunopathol* **54**: 53–63, 1990.
25. Chiocchia G, Boissier MC, Pamphile R and Fournier C, Enhancement of skin allograft survival in mice by association of 1-alpha-hydroxyvitamin D_3 to infratherapeutic doses of cyclosporin A. In: *Vitamin D. Gene Regulation, Structure–Function Analysis and Clinical Application* (Eds. Norman AW, Bouillon R and Thomasset M), pp. 514–515. Walter de Gruyter, Berlin, 1991.
26. Müller K, Gram J, Bollerslev J, Diamant M, Barington T, Hansen MB and Bendtzen K, Down-regulation of monocyte functions by treatment of healthy adults with 1α,25-dihydroxyvitamin D_3. *Int J Immunopharmacol* **13**: 525–530, 1991.
27. Komori T, Nakano T, Ohsugi Y and Sugawara Y, The effect of 1α-hydroxyvitamin D_3 on primary antibody formation in mice. *Immunopharmacology* **9**: 141–146, 1985.
28. Wientroub S, Winter CC, Wahl SM and Wahl LM, Effect of vitamin D deficiency on macrophage and lymphocyte function in the rat. *Calcif Tissue Int* **44**: 125–130, 1989.
29. Kitajima I, Maruyama I, Matsubara H, Osame M and Igata A, Immune dysfunction in hypophosphatemic vitamin D-resistant rickets: Immunoregulatory reaction of 1α(OH) vitamin D_3. *Clin Immunol Immunopathol* **53**: 24–31, 1989.
30. Tabata T, Shoji T, Kikunami K, Matsushita Y, Inoue T, Tanaka S, Hino M, Miki T, Nishizawa Y and Morii H, *In vivo* effect of 1α-hydroxyvitamin D_3 on interleukin-2-production in hemodialysis patients. *Nephron* **50**: 295–298, 1988.
31. Binderup L and Bramm E, Effects of a novel vitamin D analogue MC 903 on cell proliferation and differentiation *in vitro* and on calcium metabolism *in vivo*. *Biochem Pharmacol* **37**: 889–895, 1988.
32. Kragballe K and Wildfang IL, Calcipotriol (MC 903), a novel vitamin D_3 analogue, stimulates terminal differentiation and inhibits proliferation of cultured human keratinocytes. *Arch Dermatol Res* **282**: 164–167, 1990.
33. Binderup L, MC 903—a novel vitamin D analogue with potent effects on cell proliferation and cell differentiation. In: *Vitamin D. Molecular, Cellular and Clinical Endocrinology* (Eds. Norman AW, Schaefer K, Grigoleit HG and Herrath D), pp. 300–309. Walter de Gruyter, Berlin, 1988.
34. Bouillon R, Allewaert K, Tan BK, van Baalen H, de Clercq P and Vandewalle M, Biological activity of 1,25-dihydroxy-vitamin D analogs: Influence of relative affinities for DBP and vitamin D receptor. In: *Vitamin D. Gene Regulation, Structure–Function Analysis and Clinical Application* (Eds. Norman AW, Bouillon R and Thomasset M), pp. 131–138. Walter de Gruyter, Berlin, 1991.
35. Sørensen H, Binderup L, Calverley MJ, Hoffmeyer L and Andersen NR, *In vitro* metabolism of Calcipotriol (MC 903), a vitamin D analog. *Biochem Pharmacol* **39**: 391–393, 1990.
36. Kissmeyer A-M and Binderup L, Calcipotriol (MC 903): Pharmacokinetics in rats and biological activities of metabolites. A comparative study with $1,25(OH)_2D_3$. *Biochem Pharmacol* **41**: 1601–1606, 1991.
37. Bower M, Colston KW, Stein RC, Hedley A, Gazet J-C, Ford HT and Coombes RC, Topical calcipotriol treatment in advanced breast cancer. *Lancet* **337**: 701–702, 1991.
38. Müller K, Svenson M and Bendtzen K, 1α,25-Dihydroxyvitamin D_3 and a novel vitamin D analogue MC 903 are potent inhibitors of human interleukin 1 *in vitro*. *Immunol Lett* **17**: 361–366, 1988.
39. de Jong EMGJ and van de Kerkhof PCM, Simultaneous assessment of inflammation and epidermal proliferation in psoriatic plaques during long-term treatment with the vitamin D_3 analogue MC903: Modulations and interrelations. *Br J Dermatol* **124**: 221–229, 1991.

40. Oxholm A, Oxholm P and Staberg B, Expression of interleukin-6-like molecules and tumour necrosis factor after topical treatment of psoriasis with a new vitamin D analogue (MC 903). *Acta Derm Venereol (Stockh)* **69**: 385–390, 1989.
41. Abe J, Morikawa M, Miyamoto K, Kaiho S, Fukushima M, Miyaura C, Abe E, Suda T and Nishii Y, Synthetic analogues of vitamin D_3 with an oxygen atom in the side chain skeleton. *FEBS Lett* **226**: 58–62, 1982.
42. Abe J, Takita Y, Nakano T, Miyaura C, Suda T and Nishii Y, A synthetic analogue of vitamin D_3, 22-oxa-1α,25-dihydroxyvitamin D_3 is a potent modulator of *in vivo* immunoregulating activity without inducing hypercalcemia in mice. *Endocrinology* **124**: 2645–2647, 1989.
43. Kobayashi T, Okano T, Tsugawa N, Masuda S, Takeuchi A and Nishii Y, Metabolism of a novel synthetic vitamin D_3 analog, [^{3}H-22-oxa-1,25-dihydroxyvitamin D_3], in rats. *Bone Miner* **10**: S296, 1990.
44. Zhou JY, Norman AW, Lübbert M, Collins ED, Uskokovic MR and Koeffler HP, Novel vitamin D analogues that modulate leukemic cell growth and differentiation with little effect on either intestinal calcium absorption or bone calcium mobilization. *Blood* **74**: 82–93, 1989.
45. Zhou J-Y, Norman AW, Chen D-L, Sun G-w, Uskokovic M and Koeffler HP, 1,25-Dihydroxy-16-ene-23-yne-vitamin D_3 prolongs survival time of leukemic mice. *Proc Natl Acad Sci USA* **87**: 3929–3932, 1990.
46. Ikekawa N, Structures and biological activities of vitamin D metabolites and their analogues. *Med Res Rev* **7**: 333–366, 1987.
47. Hansen K, Calverley MJ and Binderup L, Synthesis and biological activity of 22-oxa vitamin D analogues. In: *Vitamin D. Gene Regulation, Structure–Function Analysis and Clinical Application* (Eds. Norman AW, Bouillon R and Thomasset M), pp. 161–162. Walter de Gruyter, Berlin, 1991.
48. Binderup L, Latini S, Binderup E, Bretting C, Calverley M and Hansen K, 20-Epi-vitamin D_3 analogues: A novel class of potent regulators of cell growth and immune responses. *Biochem Pharmacol* **42**: 1569–1575, 1991.
49. Lillevang ST, Rosenkvist J, Andersen CB, Larsen S, Kemp E and Kristensen T, Single and combined effects of vitamin D analogue KH 1060 and Cyclosporin A on mercuric-chloride-induced autoimmune diseases in the Brown Norway rat. *Clin Exp Immunol*, in press.
50. Inaba M, Okuno S, Inoue A, Nishizawa Y, Morii H and DeLuca HF, DNA binding property of vitamin D_3 receptors associated with 26,26,26,27,27,27-hexafluoro-1,25-dihydroxyvitamin D_3. *Arch Biochem Biophys* **268**: 35–39, 1989.
51. di Padova FE, Pharmacology of cyclosporine (Sandimmune. V. Pharmacological effects on immune function: *In vitro* studies. *Pharmacol Rev* **41**: 373–405, 1989.
52. Thomson AW, FK 506. Profile of an important new immunosuppressant. *Transplant Rev* **4**: 1–13, 1990.
53. Latini S and Binderup L, KH 1060—A new vitamin D analogue with potent effects on cell-mediated immune responses *in vitro*. In: *Vitamin D. Gene Regulation, Structure–Function Analysis and Clinical Application* (Eds. Norman AW, Bouillon R and Thomasset M), pp. 516–517. Walter de Gruyter, Berlin, 1991.
54. Rigby WFC and Hamilton BJ, Regulation of T lymphocyte activation at a posttranscriptional level by 1,25-dihydroxyvitamin D_3. *Proceedings of the Eighth Workshop on Vitamin D, Paris, July 1991*, in press.
55. Citivelli R, Kim YS, Gunsten SL, Fujomori A, Huskey M, Avioli LV and Hruska KA, Nongenomic activation of the calcium message system by vitamin D metabolites in osteoblast-like cells. *Endocrinology* **127**: 2253–2262, 1990.
56. Bittiner B, Bleehen SS and MacNeil S, 1α,25$(OH)_2$ vitamin D_3 increases intracellular calcium in human keratinocytes. *Br J Dermatol* **124**: 230–235, 1991.
57. Wali RK, Baum CL, Sitrin MD and Brasitus TA, 1,25-$(OH)_2$ vitamin D_3 stimulates membrane phosphoinositide turnover, activates protein kinase C, and increases cytosolic calcium in rat colonic epithelium. *J Clin Invest* **85**: 1296–1303, 1990.
58. Sigal NJ, Siekierka JJ and Dumont FJ, Observations on the mechanism of action of FK-506: A pharmacologic probe of lymphocyte signal transduction. *Biochem Pharmacol* **40**: 2201–2208, 1990.
59. Cunningham D, Gilchrist NL, Cowan RA, Forrest GJ, McArdle CS and Soukop M, Alfacalcidol as a modulator of growth of low grade non-Hodgkin's lymphomas. *Br Med J* **291**: 1153–1155, 1985.
60. Helström E, Robert K-H, Gahrton G, Mellstedt H, Lindemalm C, Einhorn S, Björkholm M, Grimfors G, Udén A-M, Samuelsson J, Öst Å, Killander A, Nilsson B, Winqvist I and Olsson I, Therapeutic effects of low-dose cytosine arabinoside, alpha-interferon, 1α-hydroxyvitamin D3 and retinoic acid in acute leukemia and myelodysplastic syndromes. *Eur J Haematol* **40**: 449–459, 1988.