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REVIEW

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Vitamin D and diabetes

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Abstract Vitamin D deficiency predisposes individuals to type 1 and type 2 diabetes, and receptors for its activated form—1α,25-dihydroxyvitamin D_3—have been identified in both beta cells and immune cells. Vitamin D deficiency has been shown to impair insulin synthesis and secretion in humans and in animal models of diabetes, suggesting a role in the development of type 2 diabetes. Furthermore, epidemiological studies suggest a link between vitamin D deficiency in early life and the later onset of type 1 diabetes. In some populations, type 1 diabetes is associated with certain polymorphisms within the vitamin D receptor gene. In studies in nonobese diabetic mice, pharmacological doses of 1α,25-dihydroxyvitamin D_3, or its structural analogues, have been shown to delay the onset of diabetes, mainly through immune modulation. Vitamin D deficiency may, therefore, be involved in the pathogenesis of both forms of diabetes, and a better understanding of the mechanisms involved could lead to the development of preventive strategies.

Keywords $1,25(OH)_2D_3$ · Autoimmunity · Beta cell · Diabetes · NOD mouse · Prevention · Vitamin D · Vitamin D deficiency · Vitamin D receptor polymorphism

Abbreviations $1,25(OH)_2D_3$: 1α,25-dihydroxyvitamin D_3 · BB: BioBreeding · GM-CSF: granulocyte-macrophage-colony-stimulating factor · NF-κB: nuclear factor-κB · NFAT: nuclear factor of activated T cell · NOD: nonobese diabetic · RXR: retinoid X receptor · Th: T helper · VDR: vitamin D receptor · VDRE: vitamin D response element

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Introduction

The discovery of receptors for 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the activated form of vitamin D, in tissues with no direct role in calcium and bone metabolism (e.g. pancreatic beta cells and cells of the immune system) has broadened our view of the physiological role of this molecule [1, 2]. An increased prevalence of type 2 diabetes has been described in vitamin D-deficient individuals [3–5], and insulin synthesis and secretion have been shown to be impaired in beta cells from vitamin D-deficient animals. Glucose tolerance is restored when vitamin D levels return to normal.

The identification of receptors for $1,25(OH)_2D_3$ in cells of the immune system led to experiments in animal models of type 1 diabetes in which the administration of high doses of $1,25(OH)_2D_3$ was shown to prevent type 1 diabetes [3, 6], mainly through immune regulation. It has been demonstrated that $1,25(OH)_2D_3$ is one of the most powerful blockers of dendritic cell differentiation and that it directly blocks IL-12 secretion [7]. Lymphocyte proliferation is inhibited and regulator cell development is enhanced [8].

This review provides an overview of the data available on the role of vitamin D in type 1 and type 2 diabetes, and discusses possible applications of the molecule or its synthetic analogues [9, 10] in clinical disease. The terminology used in many papers to describe vitamin D and its metabolites is confusing, with misnomers leading to misunderstanding and over-interpretation of data. In this review the term vitamin D refers to the product that is in food (vitamins D_2 and D_3) and is synthesised in the skin under the influence of UVB radiation (vitamin D_3), whereas the metabolically active molecule is referred to as $1,25(OH)_2D_3$.

Vitamin D and its metabolism

In the 17th century, Whistler (in 1645) and Glisson (in 1650) described rickets as a bone disease of young children

(for review see [11]), but it was only in 1923 that Goldblatt and Soames identified and characterised vitamin D [12]. Most vertebrates synthesise vitamin D in their skin under the influence of UV light [1, 13]. An 'efficient' sun exposure—exposure of the face and hands to the sun for 2 h/week—is probably sufficient to maintain normal levels. Food supplementation is required during pregnancy and lactation and for newborns and young children (especially in dark-skinned children living in northern countries). Vitamin D can be obtained from dietary sources of vegetable (vitamin D_2, also known as ergocalciferol) or animal origin (vitamin D_3, also known as cholecalciferol). The best food sources are fatty fish or their liver oils; however, small amounts are also found in butter, cream and egg yolk. Human and cows' milk are poor sources of vitamin D. In many parts of the world, especially North America, fluid and dried milk, as well as some margarines, butter and cereals are supplemented with vitamin D. However, the real vitamin D content is frequently quite different from the labelling standard and often insufficient to reach the daily requirements for vitamin D (400–600 IU/day). Skimmed milk, in particular, frequently has no detectable vitamin D.

Vitamin D_3 itself is biologically inert and requires two successive hydroxylations, one in the liver (on C_{25}) and one in the kidney (on the α position of C_1), to form its hormonally active metabolite, $1,25(OH)_2D_3$ (Fig. 1). Liver 25-hydroxylases and kidney 1α-hydroxylase belong to the large family of cytochrome P450-dependent steroid hy-

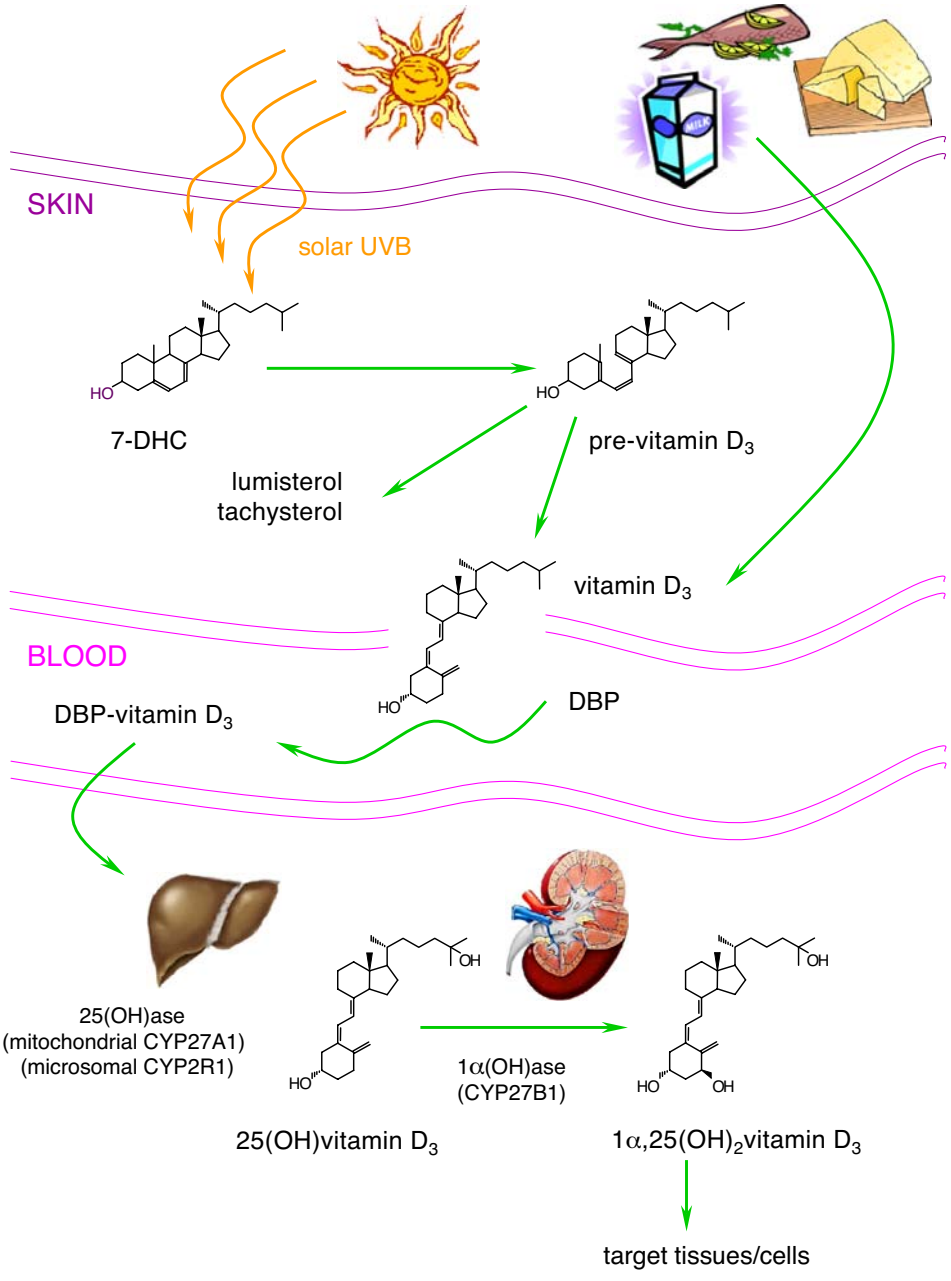

Fig. 1 Synthesis and metabolism of 1α,25-dihydroxyvitamin D_3. Vitamin D can be obtained from food (vitamin D_2 and D_3) or by photobiogenesis in the skin (vitamin D_3). In the blood, all vitamin D metabolites are bound to vitamin D-binding protein (DBP). Vitamin D_3 is converted by two successive hydroxylations in the liver (25-hydroxylases) and kidney (1α-hydroxylase) into its active hormonal form, $1,25(OH)_2D_3$

droxylases [14]. The production of $1,25(OH)_2D_3$ in the kidney is regulated by several factors, particularly by levels of parathyroid hormone, although kidney 1α-hydroxylase is also subject to direct negative feedback inhibition by $1,25(OH)_2D_3$.

The proximal renal tubule is the principal site of 1α-hydroxylation, although high levels of 1α-hydroxylase mRNA have also been found in human keratinocytes [15], dendritic cells [16] and macrophages [17]. This extra-renal production of $1,25(OH)_2D_3$ is regulated in a completely different manner. For example, production in macrophages is resistant to stimulation by parathyroid hormone but may be directly stimulated by immune stimuli such as IFN-γ and lipopolysaccharide [17].

Another hydroxylation enzyme, 24-hydroxylase, initiates the catabolic cascade of 25-hydroxyvitamin D_3 and $1,25(OH)_2D_3$ [18]. In the circulation, all metabolites of vitamin D are bound to a carrier protein known as vitamin D-binding protein [19].

Molecular action of 1α,25-dihydroxyvitamin D_3

Only the $1,25(OH)_2D_3$ form of vitamin D is metabolically active, and this molecule exerts its effects by activating the nuclear vitamin D receptor (VDR). The VDR is a member of the nuclear receptor super family of ligand-activated transcription factors, which also includes the thyroid hormone receptor, the retinoic acid receptor and the peroxisome proliferator-activated receptor. The structure of the VDR has only very recently been described and the protein is yet to be fully characterised [20]. In humans the gene encoding the VDR is located on chromosome 12cen–q12 and shows extensive polymorphism, including a mononucleotide $(A)_n$ repeat polymorphism in the 3′ untranslated region and four RFLPs: *Fok*I within exon 2, *Bsm*I and *Apa*I within successive introns between exon 7 and exon 9, and *Taq*I within exon 9 (for review see [2]).

The binding of $1,25(OH)_2D_3$ to the VDR leads to the transcription of genes regulated by $1,25(OH)_2D_3$. The

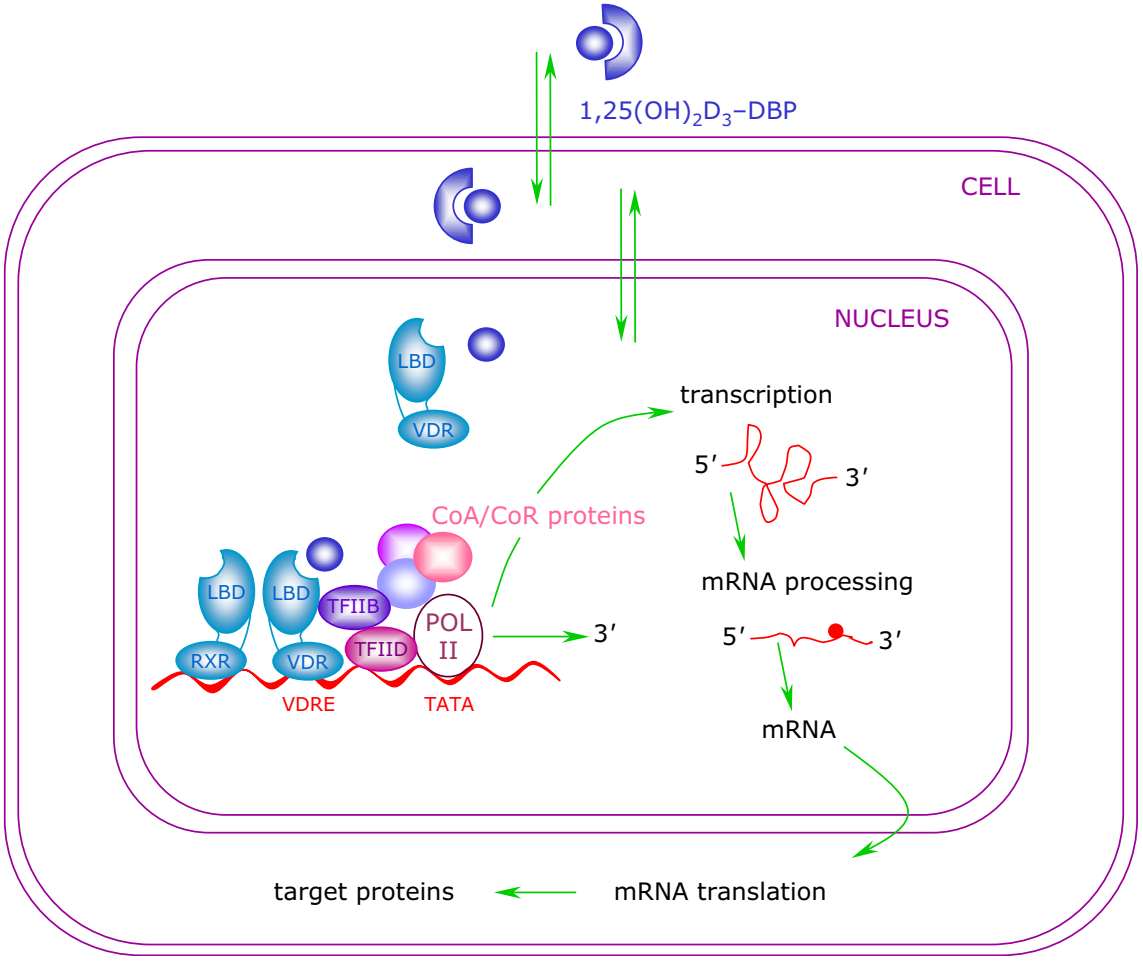

Fig. 2 Genomic actions of 1α,25-dihydroxyvitamin D_3. Molecules of $1,25(OH)_2D_3$ easily penetrate the plasma membrane and exert their genomic effects by activating the VDR. Ligand binding to the VDR induces a conformational change in the receptor and subsequent heterodimerisation with RXR. The RXR–VDR complex binds to the VDRE, which is located within the 5′ flanking region of target genes. Thereafter, co-repressor (CoR) proteins are released from the surface of the VDR, allowing interaction with co-activator (CoA) proteins. These molecules modulate chromatin structure and allow the interaction of the receptor with the RNA polymerase II transcriptional complex (POL II), thus activating transcription of the target gene. *LBD* ligand-binding domain; *TF* transcription factor

mechanism of this transcriptional regulation is very complex and is only just beginning to be unravelled (Fig. 2). The cognate vitamin D response element (VDRE) to which the VDR binds consists of a hexanucleotide direct repeat spaced by three nucleotides (DR3-type VDRE). The VDR usually binds as a heterodimer with the retinoic X receptor (RXR), and the classical effects of $1,25(OH)_2D_3$ are the result of interactions with this nuclear receptor. With respect to its relevance in diabetes, the classical VDRE and other response sites are found within genes encoding proteins with important functions in beta cells and within genes encoding proteins with key roles throughout the immune system, such as cytokines and transcription factors [21–24].

The effects of $1,25(OH)_2D_3$ on tissues other than calcium and bone can only be observed at concentrations (10^{-10} mol/l) that exceed the physiological levels needed for maintenance of calcium and bone homeostasis by a factor of 100–1,000. The fact that cells such as macrophages can secrete $1,25(OH)_2D_3$ suggests that such high concentrations of $1,25(OH)_2D_3$ may be attained locally within specific target areas (e.g. sites of inflammation).

The administration of $1,25(OH)_2D_3$ in humans to exploit its 'non-calcaemic' effects is not realistic, since the doses needed to reach the high levels locally within target organs would lead to severe systemic hypercalcaemia and other (bone) side effects. Structural analogues containing side chain modifications or, more recently, CD-ring modifications have been synthesised that share the 'non-classical' effects of $1,25(OH)_2D_3$ but have lesser effects on calcium and bone [25–27]. At present, more than 2,000 analogues have been patented, and some analogues have already reached the clinic (e.g. calcipotriol for psoriasis). A better understanding of the differential effects of these analogues on various tissues could permit targeted drug design and the development of analogues with an even greater dissociation of calcaemic and non-calcaemic effects.

Vitamin D and type 2 diabetes

Vitamin D deficiency was linked to IGT and type 2 diabetes in humans many years ago [5, 28]. These observations were confirmed in animal models, which demonstrated that pancreatic insulin secretion is inhibited by vitamin D deficiency [29]. Several reports have ascribed an active role to vitamin D in the functional regulation of the endocrine pancreas, particularly the beta cells. Not only are receptors for $1,25(OH)_2D_3$ found in beta cells [30], but the effector part of the vitamin D pathway is also present in the form of vitamin D-dependent calcium-binding protein, also known as calbindin-D_{28k} [31]. The expression of calbindin-D_{28K} has been shown to protect beta cells from cytokine-mediated cell death [32].

Several studies have demonstrated a link between VDR gene polymorphisms and type 2 diabetes, although the findings differ from one population to another. A study in Bangladeshi Asians demonstrated that the *Apa*I RFLP influences insulin secretion in response to glucose [33], while associations between the VDR *Apa*I RFLP and higher fasting plasma glucose levels and glucose intolerance were observed in a community-based study of older adults without known diabetes [34]. More recently, genotyping for *Taq*I, *Apa*I, *Bsm*I and *Fok*I RFLPs revealed that the *Bsm*I RFLP is associated with high fasting glucose levels in young males with low physical activity [35].

Effects of vitamin D and its metabolites on insulin synthesis and secretion

Vitamin D deficiency leads to the impaired secretion of insulin—but not other islet hormones—in both animal models and humans, and induces glucose intolerance [29, 36, 37], while replenishment with vitamin D rectifies the abnormalities [38–40]. This impairment is primarily caused by the direct effect of vitamin D deficiency on the beta cell, but other effects of vitamin D deficiency, such as impaired food intake and hypocalcaemia, may also play a role. Data from VDR knock-out mice are conflicting, with some groups reporting IGT [41] and others reporting no impairment in glucose metabolism [42]. In these studies the genetic background of the mouse in which the VDR knock-out is introduced seems to be of critical importance.

More convincing support for a beneficial effect of $1,25(OH)_2D_3$ on beta cell function has emerged from studies on islets isolated from normal animals in which insulin synthesis and release were provoked by glucose challenge in the presence of high doses of $1,25(OH)_2D_3$ [43].

The mechanism by which $1,25(OH)_2D_3$ might act on insulin secretion is suggested by the significant rise in cytosolic Ca^{2+} levels observed following $1,25(OH)_2D_3$-stimulated secretion of insulin by islet cells. Controversy remains as to whether an influx of external Ca^{2+} via voltage-dependent Ca^{2+} channels is solely responsible for this rise, or whether the mobilisation of Ca^{2+} from intracellular organelles and the activation of release-potentiating systems via protein kinase C and protein kinase A pathways are also involved [44–46].

Interventions with vitamin D and its metabolites in vivo: clinical implications for type 2 diabetes

The restoration of vitamin D reserves in vitamin D-deficient patients has been shown to improve glucose tolerance [47]. The amount of vitamin D administered to these patients with rickets was, however, subject to wide variation (from the daily oral administration of 2,000 IU [28] to a single intramuscular injection of 100,000 IU [3]), making it difficult to draw clear conclusions and establish guidelines. Nonetheless, it is clear that vitamin D deficiency should be treated, and that improved glucose tolerance is a desirable side effect. In contrast, repletion of $1,25(OH)_2D_3$ in the relatively $1,25(OH)_2D_3$-deficient state of uraemia only partially reverses glucose intolerance, probably because insulin resistance is also involved in this condition [48].

Studies on the administration of vitamin D supplements or even higher doses of $1,25(OH)_2D_3$ to vitamin D-sufficient patients with IGT or type 2 diabetes have yielded conflicting results. Some have reported an improvement [49], others no effect [4]. One study even showed a worsening of type 2 diabetes: supplementation in three British Asians with vitamin D deficiency and type 2 diabetes led to increased insulin resistance and the deterioration of glycaemic control [50] (Table 1).

Vitamin D and type 1 diabetes

Several epidemiological studies have described an intriguing correlation between geographical latitude and the incidence of type 1 diabetes, and an inverse correlation between monthly hours of sunshine and the incidence of diabetes. A seasonal pattern of disease onset has also been described for type 1 diabetes [51], once again suggesting an inverse correlation between sunlight and the disease. Vitamin D is an obvious candidate as a mediator of this sunshine effect.

Dietary vitamin D supplementation is often recommended in pregnant women and in children to prevent vitamin D deficiency. Cod liver oil taken during the first year of life reportedly reduced the risk of childhood-onset type 1 diabetes [52], and a multicentre case-control study also showed an association between vitamin D supplementation in infancy and a decreased risk of type 1 diabetes [53]. A further study found that an intake of 2,000 IU of vitamin D during the first year of life diminished the risk of developing type 1 diabetes, and showed that the incidence of childhood diabetes was three times higher in subjects with suspected rickets [54]. More recently, the Diabetes Autoimmunity Study in the Young (DAISY) reported that the presence of islet auto-antibodies in offspring was inversely correlated with maternal dietary vitamin D intake during pregnancy [55] (Table 2).

It remains to be determined whether these observations are the result of supplementation of vitamin D to supraphysiological levels, or are simply the result of the prevention of vitamin D deficiency. Observations in animal models suggest the latter, since regular supplements of vitamin D in neonatal and early life offered no protection against type 1 diabetes in nonobese diabetic (NOD) mice or in BioBreeding (BB) rats [56, 57], whereas the prevalence of diabetes is doubled in NOD mice rendered vitamin D-deficient in early life [58, 59]. The results of genetic studies investigating a possible relationship between VDR polymorphisms and type 1 diabetes are highly confusing: a clear correlation exist in some populations [60–65], whereas no correlation can be found in others [66].

Immunomodulatory effects of 1α,25-dihydroxyvitamin D_3 and its analogues

The identification of VDRs on almost all cells of the immune system, especially antigen-presenting cells (macrophages and dendritic cells) and activated T lymphocytes, prompted the investigation of $1,25(OH)_2D_3$ as a potential immunomodulator (for reviews see [57, 67]). Immune cells —activated macrophages and dendritic cells in particular— contain the enzyme 1α-hydroxylase, which is necessary for the final activating step in the conversion of vitamin D_3 to the metabolically active molecule, and are therefore able to synthesise and secrete $1,25(OH)_2D_3$ [17, 68]. The 1α-hydroxylase present in immune cells is identical to the renal enzyme, but regulation of its expression and activity is different. Whereas the renal enzyme is principally under the control of calcaemic and bone signals (such as para-

Table 1 Vitamin D in the aetiology of type 2 diabetes: published studies in humans

Study parameters	Study design	Type of subjects studied (age at study)	Study results	Reference
Vitamin D intake via supplementation 2,000 IU/day (50 μg) for 1 month	Observational	Vitamin D-deficient women (adulthood)	Improved glucose tolerance and improved beta cell function	[47]
Vitamin D intake via supplementation 2,000 IU/day for 6 months	Case-control	Vitamin D-deficient subjects (adulthood)	Improved insulin secretion	[28]
Vitamin D intake via single i.m. injection 100,000 IU/day	Case-control	Vitamin D-deficient subjects (adulthood)	Improved insulin and C-peptide responses	[3]
$1,25(OH)_2D_3$ treatment 0.5 μg/day for 21 days (or +500 mg Ca^{2+})	Case-control	Uraemic women (adulthood)	Improved first-phase insulin secretion and insulin sensitivity	[48]
$1,25(OH)_2D_3$ treatment 1 μg/day for 4 days	Case-control	Type 2 diabetic women (adulthood)	Improved insulin and C-peptide responses to Sustacal (Mead Johnson, Evansville, IN, USA)	[4]
Hypovitaminosis (<5 ng/ml)	Observational	Type 2 diabetic women (adulthood)	Decreased 25-hydroxyvitamin D_3 levels decreased beta cell function	[99]
Vitamin D intake via supplementation 1,332 IU/day for 1 month	Case-control	Type 2 diabetic women (adulthood)	Improved first-phase insulin secretion	[49]
Vitamin D intake via single i.m. injection 300,000 IU/day	Observational	Type 2 diabetic men and women (adulthood)	Increased insulin resistance	[50]

Table 2 Vitamin D in the aetiology of type 1 diabetes: published studies in humans

Study parameters	Study design	Type of subjects studied (age at study)	Study results	Reference
Vitamin D intake via supplementation for the first year of life	Case-control	Infancy (<5 years)	Reduced risk of childhood-onset type 1 diabetes (OR=0.83)	[53]
		Childhood (5–9 years)	Reduced risk of childhood-onset type 1 diabetes (OR=0.81)	
		Childhood (10–14 years)	Reduced risk of childhood-onset type 1 diabetes (OR=0.47)	
Vitamin D intake via use of cod-liver oil 10 μg for ≥5×/week for the first year of life	Case-control	Childhood (<15 years)	Reduced risk of childhood-onset type 1 diabetes (OR=0.74)	[52]
Vitamin D intake via supplementation ≥2,000 IU/day during the first year of life	Case-control	Infancy till early adulthood (1–31 years)	Reduced risk of childhood-onset type 1 diabetes (OR=0.12)	[54]
Rickets during the first of life	Case-control	Infancy till early adulthood (1–31 years)	Increased risk of childhood-onset type 1 diabetes (OR=2.6)	[54]
Vitamin D intake via food continuous variable IU during pregnancy	Case-control	Infancy (<5 years)	Reduced risk of insulin auto-antibodies in offspring (OR=0.49)	[55]

OR odds ratio

thyroid hormone and $1,25(OH)_2D_3$ itself), the macrophage enzyme is primarily regulated by immune signals, with IFN-γ and Toll-like receptor agonists being powerful stimulators. It is of interest that a defect in the upregulation of 1α-hydroxylase in response to immune stimuli has been reported in NOD mice [17].

A unique feature of $1,25(OH)_2D_3$ as an immunomodulator is that not only does it interact with T cells, but it also —more importantly—targets the central cell in the immune cascade, the antigen-presenting cell (for reviews see [69, 70]) (Fig. 3). In vitro, $1,25(OH)_2D_3$ stimulates the phagocytosis and killing of bacteria by macrophages but suppresses the antigen-presenting capacity of these cells and dendritic cells [71]. It is noteworthy that the expression of MHC-II molecules and adhesion molecules necessary for full T cell stimulation, such as B7.2, is suppressed [72]. Cytokines secreted by antigen-presenting cells for the recruitment and activation of T cells are also directly influenced by $1,25(OH)_2D_3$, and several authors have described the inhibition of a key cytokine in the immune system, IL-12, by $1,25(OH)_2D_3$ and its analogues [7, 73, 74]. This protein, which is produced by macrophages and dendritic cells, is the major determinant of the direction in which the immune system will be activated, since it stimulates the development of CD4 T-helper type 1 (Th-1) cells and inhibits the development of CD4 Th-2 lymphocytes. This inhibition is observed in vitro, but IL-12 suppression and a shift from Th1 to Th2 predominance can also be observed after in vivo administration of $1,25(OH)_2D_3$ or its analogues [75–77]. Inhibition of IL-12 is achieved through a direct interaction between $1,25(OH)_2D_3$ bound to the VDR (as a heterodimer with RXR) and nuclear factor κB (NF-κB), which interferes with the NF-κB-induced transcription of IL-12 [7]. The secretion of other cytokines by macrophages/dendritic cells is also influenced by $1,25(OH)_2D_3$. Prostaglandin E_2, a suppressive cytokine, is stimulated, while the monocyte recruiter granulocyte-macrophage-colony-stimulating factor (GM-CSF) is suppressed. Suppression of GM-CSF is achieved via binding of ligand-bound monomers of the VDR (in the absence of RXR) to a DNA element in the promotor region of the gene encoding GM-CSF [78].

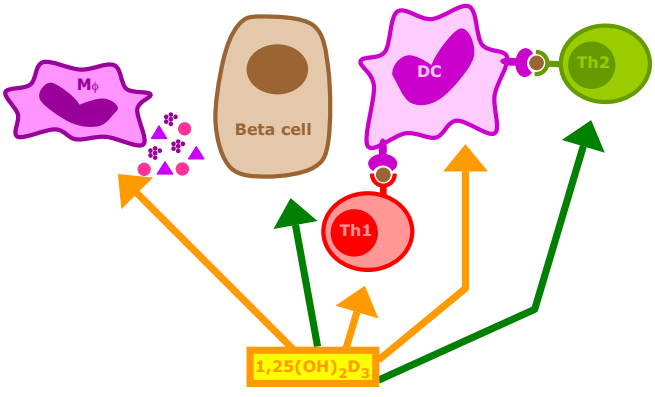

Fig. 3 Mechanisms of action of 1α,25-dihydroxyvitamin D_3 and its analogues in type 1 diabetes (with courtesy to E. van Etten). $1,25(OH)_2D_3$ and its analogues can modulate the immune responses via several mechanisms. They are necessary for normal insulin secretion and biosynthesis and are reported to protect beta cells against cytokines. They downregulate antigen presentation and co-stimulatory molecule expression by antigen-presenting cells, especially dendritic cells (DCs), thus indirectly inhibiting the development of Th lymphocytes along the Th-1 pathway and promoting the induction of Th-2 lymphocytes and/or regulatory T lymphocytes. $1,25(OH)_2D_3$ and its analogues also exert direct effects on T lymphocytes: IL-2 and IFN-γ production by Th-1 lymphocytes is inhibited, whereas the production of the Th2 cytokine IL-4 is directly induced, both shifting the Th balance towards the Th-2 phenotype. *Mϕ* macrophage

Several T cell cytokines are direct targets of 1,25 $(OH)_2D_3$ and its analogues. The secretion of IL-2 is suppressed through the inhibition of nuclear factor of activated T cells (NFAT) complex formation by ligand-bound VDR–RXR heterodimers through binding to the distal NFAT binding site in the human IL-2 promoter [79]. Another key T cell cytokine, IFN-γ, is downregulated by $1,25(OH)_2D_3$ [80]. The promoter region of the gene encoding this protein contains a classical VDRE (DR3 type), and binding of the ligand-bound VDR–RXR heterodimers causes direct suppression of transcription [81].

Taken together, these observations suggest a physiological role for $1,25(OH)_2D_3$ in the immune system, with a tightly regulated secretion of $1,25(OH)_2D_3$ by macrophages and dendritic cells upon immune stimulation on the one hand and a direct inhibitory effect of the molecule on antigen presentation and T cell proliferation and cytokine secretion on the other hand. These immune effects are typically mediated through the VDR. Therefore, the fact that VDR knock-out mice have no apparent immune abnormalities suggests the redundancy of $1,25(OH)_2D_3$ as a signal in the immune system [42].

Beta cell protection by 1α,25-dihydroxyvitamin D_3 and its analogues

Direct beta cell damage by cytokines and other inflammatory agents is an important step in the pathogenesis of type 1 diabetes. The inhibition of beta cell function (insulin synthesis and insulin secretion) induced by IL-1β or IFN-γ in vitro is prevented by $1,25(OH)_2D_3$ and its analogues, MC903 and KH1060 [82, 83]. In contrast, Mauricio et al observed no effect of $1,25(OH)_2D_3$ on IL-1β-induced beta cell dysfunction [84]. This discrepancy may be due to the fact that an incubation time of 24 h was used in the latter study, whereas incubation periods ranging from 48 to 72 h were used in the studies describing protection. In addition to the alteration of the effects of cytokines on beta cell function, $1,25(OH)_2D_3$ blocks the induction of surface markers by these cytokines [83, 85, 86]. The upregulation of expression of MHC-II molecules and intercellular adhesion molecule-1 on rat islets after exposure to IFN-γ was markedly decreased by co-incubation with $1,25(OH)_2D_3$ or an analogue (ZXY1106) [83]. More recently, Riachy et al demonstrated that $1,25(OH)_2D_3$ might preserve the insulin content of human islets and prevent MHC-I expression, IL-6 production and NO release [85]. Moreover, it was reported that $1,25(OH)_2D_3$ induced and maintained high levels of A20, an anti-apoptotic protein, in rat RINm5F cells and human islets after exposure to inflammatory cytokines [86]. We recently performed a comparative study of different cell systems (whole rat islets, FACS-purified beta cells and INS-1E cells) and did not observe direct protection by $1,25(OH)_2D_3$ against cytokine-induced beta cell death, but demonstrated decreased expression of chemokines by beta cells treated with $1,25(OH)_2D_3$ [87].

In summary, $1,25(OH)_2D_3$ protects beta cells from cytokine-induced beta cell dysfunction but cannot protect them from direct cytokine-induced cell death (Fig. 3).

Effects of 1α,25-dihydroxyvitamin D_3 and its analogues in animal models of type 1 diabetes

The chronic administration of pharmacological doses of $1,25(OH)_2D_3$ reduces the incidence of both insulitis and diabetes in NOD mice [6, 88]. When $1,25(OH)_2D_3$ was administered lifelong from weaning in NOD mice, the incidence of insulitis was reduced from 80% in control mice to 58% in $1,25(OH)_2D_3$-treated mice, and the incidence of diabetes was reduced from 56 to 8%. These findings have been confirmed in other studies [77]. Extensive immunological screening, including FACS analysis of major lymphocyte subsets, evaluation of T cell proliferation and natural killer cell function, did not reveal any major changes in the treated mice compared with the control mice [6]. The reported restoration of deficient suppressor cell function of NOD mice in response to treatment with 1,25 $(OH)_2D_3$ represents a major finding [89]. Adorini et al demonstrated that the regulatory T cell induced by 1,25 $(OH)_2D_3$ or its analogues is most likely a CD4CD25 cell [77]. However, it remains to be determined whether this restoration of suppressor cells is the main mechanism involved in the protection afforded by $1,25(OH)_2D_3$ against diabetes. Protection against insulitis was also observed, suggesting interference with the induction of autoimmunity itself. This is consistent with the observation that 1,25 $(OH)_2D_3$ protects NOD mice against cyclophosphamide-induced diabetes [90].

The molecular basis of protection by $1,25(OH)_2D_3$ seems to involve the reshaping of the immune repertoire, with elimination of effector cells, although the direct protective effects of $1,25(OH)_2D_3$ on the beta cell might also play a role in disease prevention. It has been shown that there is a shift in the production of T cell cytokines from predominantly Th1 (IL-2, IFN-γ) in control mice to Th2 (IL-4, IL-10) in mice treated with $1,25(OH)_2D_3$ or an analogue of the molecule [75]. The shift appeared to be antigen specific and is probably caused by the direct interference of $1,25(OH)_2D_3$ or its analogues by the antigen-presenting dendritic cells. Indeed, $1,25(OH)_2D_3$ induces a reshaping of dendritic cells towards tolerogenic cells [69, 70, 77]. We have also demonstrated that dendritic cells generated in the presence of $1,25(OH)_2D_3$ or an analogue can re-direct already committed T cell clones derived from a type 1 diabetic patient towards non-proliferation [8, 91]. In the NOD mouse, the reshaping of the immune system happens centrally, in the thymus, where treatment with 1,25 $(OH)_2D_3$ restores the sensitivity of T lymphocytes towards apoptosis-inducing signals, thereby enhancing the elimination of autoimmune effector cells [92].

In marked contrast to the NOD mouse, no significant difference in the incidence of diabetes was observed be-

Clinical perspectives

The metabolically active form of vitamin D, 1,25 $(OH)_2D_3$, and its analogues have been shown to have effects on the major players involved in the pathogenesis of type 1 and type 2 diabetes. Beta cell function has been shown to be improved by 1,25$(OH)_2D_3$ in vitro and in vivo, and the avoidance of vitamin D deficiency is essential for normal beta cell function. In NOD mice, 1,25$(OH)_2D_3$ protects against insulitis, diabetes and disease recurrence after islet transplantation, primarily through immunomodulatory effects.

The use of 1,25$(OH)_2D_3$ for the prevention or cure of diabetes is limited by its hypercalcaemic and bone remodelling effects, as its protective effects are only observed in response to supra-physiological doses. Future applications of this therapy in human diabetes are conceivable, since the calcaemic and immunomodulatory effects of 1,25$(OH)_2D_3$ can be dissociated by structural analogues of the molecule.

It is likely that these analogues will find a use as beta-cell-protective and -stimulating agents as adjuncts to the current treatment for type 2 diabetes. These agents could play a major role in preventative strategies for type 1 diabetes in humans, because of their profile as both beta cell-protective and immuno-active drugs. More information is required concerning their mechanism of action and long-term safety before they can be routinely administered in humans.

tween control BB rats (33%) and BB rats treated with 1,25 $(OH)_2D_3$ from weaning up to 120 days (24%) [93]. This finding confirms the basic differences in disease pathogenesis in the two available animal models for type 1 diabetes, and indicates that caution is warranted when transferring findings from either of these models to the human situation.

A major obstacle to the administration of 1,25$(OH)_2D_3$ in humans is its important effects on calcium and bone metabolism. However, in view of the therapeutic potential, structural analogues have been designed and synthesised with the aim of achieving a dissociation between calcaemic and immune effects. Several analogues developed in different chemical laboratories have been successfully tested in the NOD mouse [89]. The mechanism of protection against insulitis and diabetes appears to be similar to that of 1,25$(OH)_2D_3$. Effects of the analogues on dendritic cell phenotype, regulator cell induction and beta cell protection have been described [8, 77, 83].

The optimal analogue should provide a combination of beta cell protection, immune modulation and low calcaemic effects. Although several analogues have provided promising results [93], long-term safety data will have to be collated before long-term interventions in individuals at high risk of type 1 diabetes are initiated. A critical question as regards the applicability of analogues in the human situation is whether these 1,25$(OH)_2D_3$ analogues are able to arrest progression to clinically overt diabetes if administered when active beta cell destruction is already present —the situation in pre-diabetic subjects in whom immune intervention is considered [94]. Casteels et al demonstrated that, when combined with a short induction course of a classical immunosuppressant such as cyclosporine A, some of these analogues can arrest the progression of the disease when administered after autoimmunity has started [95]. This approach of combining a short induction course of a classical immunosuppressant with non-hypercalcaemic analogues of 1,25$(OH)_2D_3$ is promising and might open new perspectives for the prevention of autoimmune diabetes in humans.

The capacity of some of the analogues of 1,25$(OH)_2D_3$ to prevent disease recurrence after islet transplantation has been evaluated in spontaneously diabetic NOD mice. The most convincing results were obtained using a combination of KH1060 and cyclosporine A [96]. A similar synergism has been reported between 1,25$(OH)_2D_3$ or an analogue and mycophenolate mofetil [97]. One novel approach involved the combination of analogues of 1,25$(OH)_2D_3$ with other physiological immune modulators, such as recombinant IFN-β [98]. In this study, the combination of recombinant IFN-β and ZXY1106 markedly delayed the recurrence of autoimmune diabetes following islet transplantation [98].

Conclusion

Solid evidence exists that vitamin D deficiency is detrimental to beta cell function, leads to glucose intolerance in animal models and humans, and predisposes to type 2 diabetes. Vitamin D deficiency in early life predisposes NOD mice and humans to the later development of autoimmune diabetes. A major practical conclusion that can be drawn from the studies conducted on vitamin D and diabetes to date is that vitamin D deficiency is undesirable, not only for calcium and bone, but also for glucose metabolism.

Administration of high doses of the activated form of vitamin D, 1,25$(OH)_2D_3$, has immunomodulatory effects that lead to diabetes prevention in animal models of type 1 diabetes. The availability of structural analogues of 1,25 $(OH)_2D_3$ opens new perspectives for the exploitation of these interesting properties in humans. Carefully designed intervention studies using these analogues, either alone or in combination with other immunomodulators, will yield the answer to the question of their clinical potential in diabetes prevention.

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