CHAPTER 22

Vitamin D in immune regulation and diabetes mellitus

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Contents

Definition of words and terms

***Autoimmunity*:** An aberrant systemic immune response of an organism against its own healthy cells and tissues, that manifests in various autoimmune diseases.

***Calcidiol (25-hydroxyvitamin D)*:** A precursor for calcitriol and the major circulating metabolite of vitamin D_3. This prohormone is synthesized in the liver from cholecalciferol by its hydroxylation in the 25 position by the enzyme cholecalciferol-25-hydroxylase.

***Calcitriol (1,25-dihydroxycholecalciferol)*:** The hormonally active form of vitamin D, which is formed in the kidney tissue from 25OHD by the renal 25-hydroxyvitamin D_3-1-(alpha)-hydroxylase.

***Cholecalciferol (vitamin D_3)*:** A steroid hormone and one of the most common forms of the vitamin D group, which is synthesized in the skin of animals from 7-dehydrocholesterol under the influence of UV-radiation or may be absorbed from dietary sources in the intestine.

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***Cytokines*:** A broad category of small secreted proteins that are involved in interactions and communications between cells during immune responses and promote the recruitment of specific cells toward the sites of inflammation. Cytokines are produced by a broad range of cell populations, including immune cells (macrophages, B and T lymphocytes, mast cells), endothelial cells, fibroblasts, stromal cells, Schwann cells, etc.

***Diabetes mellitus*:** A group of chronic, metabolic, polyethiologic, and endocrine-related disorders caused by inherited or acquired deficiency in insulin production (type 1 diabetes, insulin-dependent) by the pancreas, or by the ineffectiveness of the insulin produced, when cells fail to respond to insulin properly (type 2 diabetes, noninsulin-dependent).

***Vitamin D receptor*:** A member of the nuclear hormone receptor superfamily that acts as a ligand-inducible transcription factor. Intracellular vitamin D receptor mediates the action of 1,25-dihydroxycholecalciferol and regulates the expression of a wide variety of genes.

***Immune response*:** A versatile set of adaptive processes to form specifically reactive cells and proteins directed against antigenes. It occurs through consecutive interactions between antigen-presenting cell and various types of T and B cells.

22.1 Introduction

Studies that began with the identification of calcitriol and vitamin D receptor (VDR) in the early 1970s and have continued to the present day have significantly expanded our knowledge of the biological role of vitamin D (VD) in living organisms. The unfading interest concerning the metabolism and properties of VD can be explained by two main reasons: (1) the world trend toward VD deficiency among the population, which is confirmed to be associated with a variety of common human pathologies; and (2) this interest is fueled by new ideas about the pleiotropic extraskeletal effects (para- and autocrine) of VD's hormonally active form (Christakos et al., 2016). Multiple lines of evidence suggest that VD can be involved in inflammatory response in norm and immune-regulated disorders (e.g., infectious diseases, cancers, autoimmunity, and diabetes). This chapter summarizes the data on the immunomodulatory function of calcitriol and analyzes the association of the VD−auto/paracrine system disturbances with chronic inflammation and diabetes mellitus.

22.2 Vitamin D synthesis and activation

Vitamin D (VD) (calciferol) belongs to the group of secosteroids that can be absorbed from dietary sources in the form of cholecalciferol (VD3) or ergocalciferol (VD2), although it is mainly synthesized in the lower layers of the epidermis from 7-dehydrocholesterol (7DHC) in response to the action of ultraviolet B radiation, (Fig. 22.1) (Hoseinzadeh et al., 2018). 7DHC forms a thermodynamically unstable previtamin D3 followed by

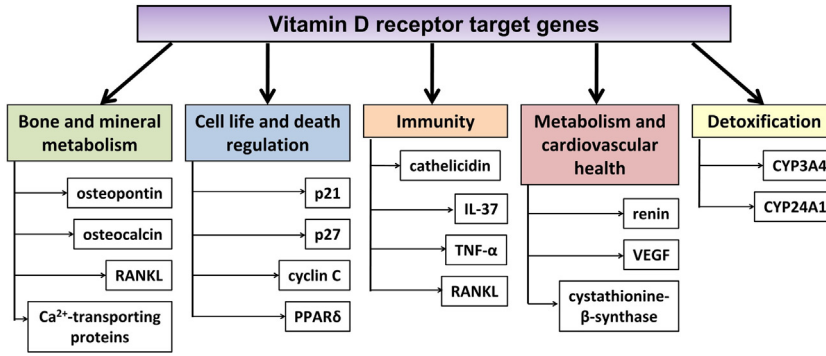

Figure 22.1 Vitamin D receptor target genes.

Key facts of vitamin D receptor target genes

- Vitamin D receptor (VDR) is a member of the nuclear hormone receptor superfamily, and acts as a ligand-inducible transcription factor.
- Intracellular VDR mediates the action of 1,25-dihydroxycholecalciferol (1,25 $(OH)_2D$) and regulates the expression of a wide variety of genes.
- 1,25$(OH)_2D$ directly binds to the VDR. This complex recruits the RXR and interacts with the specific DNA sequences (vitamin D response elements, −VDREs) for activation of vitamin D target genes.
- The highest VDR expression is found in metabolic tissues, such as the intestine, kidney, skin, and the thyroid gland, yet moderate VDR expression occurs in nearly all tissues.
- VDR modulates a variety of biological processes, including calcium and phosphorus homeostasis, cell proliferation, differentiation, migration and apoptosis, angiogenesis, and immune response.
- Mutations in the VDR gene are associated with type 2 vitamin D-resistant rickets.
- Some of the differences between individuals may be related to genetic variations of VDR (polymorphism), affecting VD signaling via VDR.

CYP, Cytochrome P450; *ILs*, interleukins; *PPARδ*, peroxisome proliferator-activated receptor δ; *RANKL*, receptor activator of nuclear factor kappa B ligand; *TNF-α*, tumor necrosis factor-α; *VEGF*, vascular endothelial growth factor.

thermal isomerization to VD3. Vitamin D3 and its metabolites, as lipophilic substances, are transported by the VD-binding protein (VDBP). In the liver, VD3 undergoes hydroxylation by vitamin D 25-hydroxylase (CYP2R1 and CYP27A1 isoforms) to produce 25-hydroxyvitamin D (25OHD3, or calcidiol), the major circulating VD3 metabolite that is largely biologically inactive. Further hydroxylation of 25OHD3 is catalyzed by 1α-hydroxylase (CYP27B1) in the kidney or in extrarenal tissues and leads to the formation of the biologically active 1,25(OH)2D3 (calcitriol). Metabolic inactivation of 25OHD3 and 1,25(OH)2D3 occurs through their hydroxylation by 24-hydroxylase (CYP24A1) (Bikle, 2014).

Two multiligand endocytic receptors, megalin and cubulin, provide the internalization of $1,25(OH)_2D_3$ bound to the VDBP into cells. Intracellularly, the interaction of $1,25(OH)_2D_3$ with the VDR initiates a complex cascade of molecular events culminating in gene transactivation or transrepression (Lu et al., 2018). In general the known VDR-regulated genes can be grouped as shown in Fig. 22.2. $1,25(OH)_2D_3$ synthesized in the kidney mainly fulfils the endocrine function shown in Fig. 22.1.

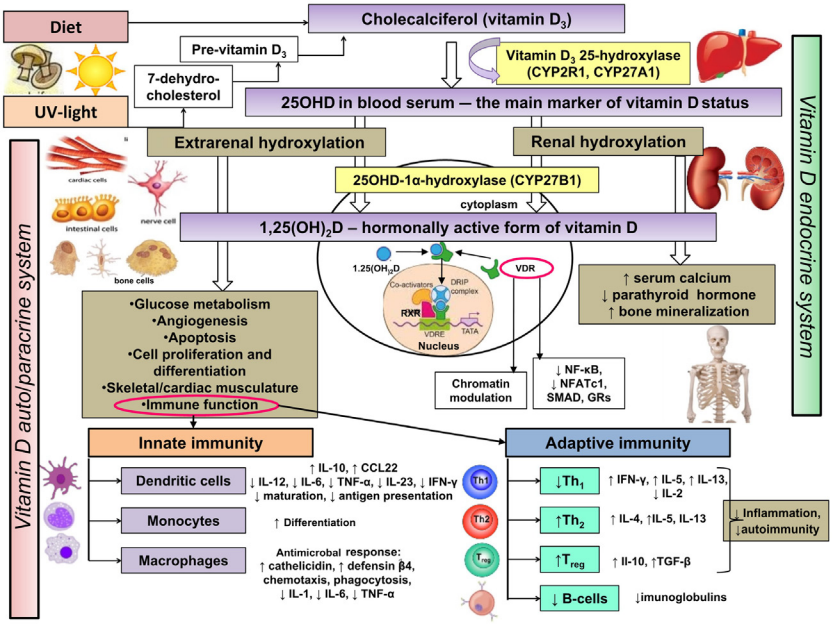

Figure 22.2 Vitamin D–auto/paracrine system in immune regulation
For the manifestation of biological activities VD undergoes two-stage hydroxylation by the enzymes of the cytochrome P450 family. This pathway involves the formation of the main circulating metabolite, calcidiol, produced by CYP2R1 and the hormonally active form, calcitriol, synthesized by CYP27B1. Calcitriol, via VD receptors, modulates transcription of various genes, involved in mineral metabolism and in noncalcemic functions, including innate and adaptive immunity. In brief, vitamin D immunomodulation includes the attenuation of Th1 and stimulation of Th2 cells proliferation. Similarly, vitamin D induces the synthesis, secretion, and release of antiinflammatory cytokines, while inhibiting proinflammatory cytokines. *1,25(OH)$_2$D*, 1,25-Dihydroxycholecalciferol; *25OHD*, 25-hydroxyvitamin D; *CYP*, cytochrome P450; *GRs*, glucocorticoid receptors; *IFN-γ*, interferon-γ; *ILs*, interleukins; *NFATc1*, nuclear factor of activated T cells 1; *NF-κB*, nuclear factor kappa B; *RXR*, retinoid X receptor; *TGF-β*, transforming growth factor β; *Th1*, type 1 T helper; *Th2*, type 2 T helper; *TNF-α*, tumor necrosis factor-α; *Treg*, regulatory T cells; *VDR*, vitamin D receptor; *VDRE*, vitamin D response element.

Calcitriol was most commonly reported to stimulate the absorption of phosphate and calcium in the intestine, regulate bone tissue remodeling, suppress the synthesis of parathyroid hormone (PTH), and enhance the expression of fibroblast growth factor 23 (FGF23), which inhibits CYP27B1 activity in the kidney (Fleet, 2017). Apart from the fact that renal synthesis is the main source of circulatory $1,25(OH)_2D_3$, in extrarenal tissues (brain, vessels, skin, large intestine, placenta, prostate, and immune cells, etc.). Calcitriol also regulates local molecular and biochemical processes, showing potential to induce nonclassical auto/paracrine responses (Bikle, 2016).

The level of circulating 25OHD is generally accepted as a biomarker for VD status. 25OHD values lower than 25 nmol/L are related to rickets and osteomalacia, and hereafter are designated as severe deficiency. The emerging data highlighting the association of low VD levels with nonskeletal pathologies raises the question as to the optimal levels necessary for general health and well-being. At present most experts agree that plasma 25OHD levels <50 nmol/L may sustain long-term adverse health consequences and are classified as "deficiency." As the 75 nmol/L of 25OHD is the level above which there is no further stimulation of PTH, it is therefore considered as "sufficiency." Table 22.1 summarizes the criteria proposed by the US Endocrine Society for VD status, intended for normal healthy populations to ensure not only skeletal health (Pramyothin and Holick, 2012).

Table 22.1 Vitamin D status guidelines.

Vitamin D status	25OHD (nmol/L)	25OHD (ng/mL)
Deficiency	<50	<20
Insufficiency	50–75	20–30
Sufficiency	75–100	30–44
Toxicity	>250	>100

Key facts of vitamin D status

- Circulating 25OHD is a robust and reliable marker of vitamin D status.
- Vitamin D deficiency is associated with an increased risk of several diseases such as diabetes, cancer, autoimmune, cardiovascular, and infectious diseases, depression, dementia, and musculoskeletal **decline**.
- Clinical risk factors for vitamin D deficiency are inadequate sun exposure, limited oral intake, impaired intestinal absorption, long-term use of medications (glucocorticoids, antiepileptic drugs) and aging.
- The concept of the personal vitamin D response index (via measuring vitamin D sensitive molecular parameters) can better describe the efficiency of the molecular response to supplementation with vitamin D and should be provided for an optimized vitamin D supplementation.

22.3 Role of vitamin D in immune regulation and inflammatory responses

In retrospect, the finding that $1,25(OH)_2D_3$ could interfere with the formation of interleukins (ILs) was the first discovery that expanded the VD research field far beyond the limits of calcium homeostasis and the regulation of bone metabolism. The ILs are a large group of cytokines that mediate the interactions between immune and inflammatory cells by modulating cell growth, differentiation, and functional activation. Besides being produced in the leukocytes at different stages of their differentiation, further cell types involved in specific organ-related diseases with confirmed inflammatory components (keratinocytes, trophoblasts, adipocytes, and endothelial cells) are capable of synthesizing ILs and VD-hormone was shown to strongly influence the expression of both ILs and their receptors. Further cytokines, such as tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ), as well as growth factors, such as toll-like receptors, C-reactive protein (CRP), and enzymes, which generate inflammation mediators (cyclooxygenase, 5-lipoxygenase) have also been identified as calcitriol targets (Skrobot et al., 2018).

By influencing various cell types related to the immune system (monocytes/macrophages, dendritic, T and B cells), VD exerts the cell-type specific regulation of genes involved in the inflammatory processes shown in Table 22.1 and provides interaction between VD signaling and other signaling cascades that promote inflammation (Bivona et al., 2017). Two main observations underscore the immune modulatory effect of VD: (1) the presence of VDR in immune cells and their ability to produce VD-hormone locally, which acts on immune cells either in an autocrine, paracrine, or intracrine fashion; and (2) regulates multiple pathways of innate and adaptive segments of the immune system.

22.3.1 Vitamin D and innate immunity

Dendritic cells (DCs), which are the most potent antigen-presenting cells (APCs) of the innate immune system that stimulate the lymphocytes of adaptive immunity to remove the invaders through antigen presentation, are major targets of VD (Barragan et al., 2015). Calcitriol, by inhibiting the secretion of the immune-stimulating cytokine IL-12 and enhancing the production of the immunosuppressive cytokine IL-10 in DCs, decreases the antigen presentation and maintains immature phenotype and tolerogenic properties of these cells (Corripio-Miyar et al., 2017).

Accordingly, $1,25(OH)_2D_3$ prevents activation of costimulatory molecules (CD40, CD80, CD83, and CD86) and major histocompatibility complex (MHC) class II protein expression in DCs resulting in their inability to activate alloreactive T cells (Penna and Adorini, 2000; Brosbøl-Ravnborg et al., 2013). The inhibitory action of calcitriol on DCs differentiation and maturation as well as the modulation of their activation and survival account for reduced T helper 1 (Th1) cell response, which thoroughly explains the immunosuppressive activity of $1,25(OH)_2D_3$.

Other differentiated members of the monocytic lineage, that is, macrophages as well as monocytes themselves, have also been investigated, but the effects of $1,25(OH)_2D_3$ in these cells remain controversial. VD suppresses the proliferation and stimulatory properties of monocytes (Brosbøl-Ravnborg et al., 2013). For several members of the interleukin family (e.g., IL-1, IL-6, and IL-8) as well as TNF-α, both positive and negative regulation by calcitriol has been found (Bhalla et al., 1991; Di Rosa et al., 2012). These effects seem to depend on the time of cells' stimulation, the degree of their maturation, the stimulus that is employed, and other factors.

Furthermore, VD affects innate immunity through its direct stimulatory action on the synthesis of cathelicidin and defensin induced by the activation of toll-like receptors. These low molecular weight host defense antimicrobial peptides possess a broad spectrum of activity against bacteria, viruses, and fungi in the immune cells (Sato et al., 2013).

22.3.2 Vitamin D and adaptive immunity

VD modulates adaptive immunity, which is based on an antigen-specific immune response, involving the interaction of T and B cells. These cells, especially in an immunologically active state, can express VDR (Szymczak and Pawliczak, 2016). Calcitriol may act indirectly on lymphocytes through paracrine signaling by APCs or directly through VDR. Early studies indicated that $1,25(OH)_2D_3$ suppresses T lymphocyte proliferation most probably by reducing IL-2 transcription (Chambers et al., 2014). The CD4 + subset of T cells, also referred to as T helper (Th) cells, is known to assist other leukocytes in immune processes, including the maturation of B cells into plasma cells and the activation of cytotoxic T cells and macrophages. CD4 + Th cells recognize peptides presented by MHC II molecules of APCs. They can differentiate into one of several subtypes, such as Th1, Th2, Th17, Th22, and Treg cells, which secrete

various cytokines to facilitate different types of immune responses. Recently, over 100 target genes have been identified in mature CD4 + Th cells with 57 genes being repressed and 45 genes being upregulated by VD-hormone (Mahon et al., 2003).

In vitro studies revealed the ability of $1,25(OH)_2D_3$ to inhibit IFN-γ secretion by CD4 + Th1 cells (Jeffery et al., 2009). Accordingly, VD was indicated to stimulate the formation of Th2 cells by increasing IL-4 synthesis (Mahon et al., 2003). The resulting effect supports a shift of T cell responses from a Th1 type toward Th2 reactions with concomitant suppression of Th17 that promotes the development of tolerogenic phenotype. $1,25(OH)_2D_3$ inhibited the differentiation of Th17 cells through its suppressive effect on IL-17 and RANKL (receptor activator of NF-κB ligand) synthesis (Sun et al., 2018). Previous in vitro and in vivo studies have revealed that the RANKL signals favor the survival of DCs, thereby activating the immune response (Akiyama et al., 2012). In addition, $1,25(OH)_2D_3$ activates Treg cells, which suppress the immune response and mediate immune tolerance by inducing IL-10 formation (Barrat et al., 2002).

Cytotoxic (killer) T cells, also known as CD8 + T cells, destroy virus-infected and tumor cells, and are also implicated in proinflammatory responses and autoimmunity. It was shown that VD reduces the proliferation of CD8 + T cells, which recognize their targets by binding to antigen associated with MHC I molecules present on the surface of all nucleated cells. They express VDR at the highest level compared to other immune cells. VDR-knockout CD8 + T cells exhibit an increased proliferation without antigen stimulation due to an increased production of IL-2 (Chen et al., 2014). Several studies have also demonstrated the regulatory role of VD on the expression of some other cytokines secreted by CD8 + T cells, such as IL-6, IL-12, TNF-α, IL-5, and TGF-β.

22.3.3 Cytokines in regulation of vitamin D-metabolizing enzymes

Renal CYP27B1 is mainly regulated by PTH (produced in response to low calcium status), FGF23 (produced in the bone to inhibit CYP27B1 in response to elevated serum phosphates), and calcitriol itself (Kägi et al., 2018). Regarding the regulation of extrarenal CYP27B1 synthesis, it was established to be tissue-specific and strongly dependent on cytokines. This is the case for INF-γ and TNF-α, for which it has been shown that they stimulate CYP27B1 production in monocytes and macrophages without

PTH involvement. Moreover, CYP27B1 in immune cells is not negatively regulated by $1,25(OH)_2D_3$ and the role for CYP24A1 is also negligible. Importantly, the key limiting factor for efficient calcitriol synthesis in immune cells is the availability of $25OHD_3$ (Overbergh et al., 2006). Similarly as in immune cells, keratinocytes also demonstrated the stimulatory potential of INF-γ and TNF-α on CYP27B1 expression. Calcitriol was not active to directly inhibit this gene. However, calcitriol can restrict its own abundance in the epidermis by inducing the catabolizing enzyme CYP24A1. $1,25(OH)_2D_3$-induced transcription of CYP24A1 is readily implementable due to the presence of multiple VD response elements (VDRE) within its gene promoter (Xie et al., 2002).

These findings suggest that increased CYP24A1 expression and activity elicited by cytokines, along with insufficient substrate accessibility could be important limiting factors for calcitriol synthesis that might lead to impaired VD signaling in immune and other extrarenal cells and contribute to inflammation. A closer look at whether other proinflammatory cytokines could determine the abnormal outcome of calcitriol action in the immune system, affecting the enzymes that metabolize VD, is warranted. The overall effect of VD on the immune system is summarized in Fig. 22.1.

22.4 Vitamin D and diabetes mellitus

22.4.1 Vitamin D modulates β-cell function

The term "diabetes mellitus" describes multifactorial metabolic disease characterized by hyperglycemia as the result of defects in insulin secretion (type 1 diabetes, T1D) and/or the obstruction of insulin function in target tissues (type 2 diabetes, T2D). T1D is an autoimmune disease caused by the progressive T cell-mediated destruction of insulin-producing β cells in the pancreas. The triggers for the autoimmune attack have not been fully elucidated, but it is now widely accepted that both environmental and genetic factors are important contributors. The discovery of *VDR* and *CYP27B1* coexpression in pancreatic islet cells along with their expression in activated CD4+ and CD8+ T lymphocytes, B cells, granulocytes, and antigen-presenting cells (macrophages and DCs) supports the putative role of an impaired vitamin D pathway in autoimmune diabetes.

Variations at genes within the MHC (in humans, also referred to as the HLA complex) can be the key genetic determinants of T1D

development. The most recognized is the association of T1D with polymorphisms of HLA II genes encoding DQ and DR (3). The DR−DQ haplotypes conferring the highest risk are HLA-DRB1*03 (DR3), typically present in haplotypic association with DQA1*05:01-DQB1*02:01 (DQ2), and HLA-DRB1*04 (DR4) observed in haplotypic association with DQA1*03-DQB1*03:02 (DQ8). It is precisely in conjunction with these variants of HLA II on APCs that the autoantigens are presented to Ths in the thymus and peripheral tissues, particularly in the lymph nodes surrounding the pancreas (Sharp et al., 2018). The most well-known autoantigens include preproinsulin, insulinoma-associated antigen from the family of transmembrane tyrosine protein phosphatases 2, glutamate decarboxylase, and zinc transporter (ZnT8). Upon the action of the stimulus, CD4 + Th1 cells induce pancreatic β cell destruction by stimulating CD8 + killer T cells to attack the Langerhans islets. VD deficiency causes an increased formation of CD4 + Th1 cells and the propagation of proinflammatory cytokines occurs. $1,25(OH)_2D_3$ was indicated to suppress the proliferation of Th1 cells and their ability to produce cytokines. As a consequence of reduced secretion of IL-2 and IFN-γ by CD4 + cells and the stimulation of IL-5 and IL-10 secretion, the immune system switches from the Th1 to tolerogenic Th2 cell response (Alfonso et al, 2009).

In addition to immunomodulating properties, VD seems to play a role in the regulation of insulin secretion from β cells. VD deficiency in rodents negatively affects glucose-stimulated insulin secretion and human epidemiological studies also link poor VD status with T1D and T2D. Preincubation of mouse and human islets with $1,25(OH)_2D_3$ enhances glucose-stimulated insulin secretion and increases glucose-stimulated calcium influx. The R-type voltage-gated calcium channel gene, *Cacna1e* (genetic variability of the major subunit), which contains a conserved VDRE in intron 7, was shown to be highly upregulated by $1,25(OH)_2D_3$ in human and mouse islets (Kjalarsdottir et al., 2018). Evidence from in vitro studies has demonstrated that pancreatic islets, similar to immune cells, express VDR and 25-hydroxyvitamin D-1α-hydroxylase, suggesting that local production of $1,25(OH)_2D_3$ is vital for normal islets functioning. Pancreas can also respond to circulating levels of calcitriol (Wolden-Kirk et al., 2013). However, while an in vitro trial revealed stimulatory action on preproinsulin mRNA in cultured islets isolated from fed rats, there was no effect of calcitriol in vivo. Nevertheless, vitamin D restored insulin secretion in vitamin D-deficient animals (Bourlon et al., 1999).

More recently, dedifferentiation has been identified as one of the mechanisms of β cell failure associated with T2D. In a study on mouse insulinoma cell line cultured in a high-glucose environment, treatment with VD normalized decreased VDR activity and enhanced the expression of essential transcription factors, such as Pdx1 and MafA, subsequently increasing Ins1 and Ins2 expression and protecting β cells against pathological dedifferentiation (Neelankal John et al., 2018).

22.4.2 Vitamin D—auto/paracrine system in diabetes mellitus

VD insufficiency/deficiency is common in subjects with T1D and T2D; however whether this association is causal remains largely undefined. Regarding the mechanisms of vitamin deficiency in T1D, the interplay of genetic, nutrition, and environmental factors seems to affect the circulating level of the VD status marker, 25OHD. As VD biosynthesis and its signaling are regulated by genes encoding the VDR and enzymes for calciferol activation, their polymorphisms may significantly alter the bioavailability and specific effects of VD metabolites. The increased risk for T1D was shown to be closely related to low 25OHD resulting from VD pathway polymorphisms (e.g., *VDR*, *CYP2R1*, *CYP27B1*, *VDBP*, and *cubulin*). For a comprehensive review please see Penna-Martinez and Badenhoop (2017). Available data, however, indicate variable genetic predispositions to T1D that depend on the ethnic origin of the populations studied.

As VD deficiency was shown to enhance the risk for T1D, it provided the rationale for vitamin D supplementation to manage this disease. The therapeutic benefits from VD treatment on T1D were found in some clinical trials (Grammatiki et al., 2017). Significant positive effects of vitamin D supplementation in the form of alphacalcidole and cholecalciferol on daily insulin dose, fasting, and stimulated C-peptide levels were observed in patients newly diagnosed with T1D, whereas supplementation with calcitriol had no effect (Gregoriou et al., 2017). Intervention trials and meta-analyses demonstrated vitamin D's potential to prevent the development of T1D in infants (Hypponen et al., 2001). Intervention trials also proved the specific requirements of adequate VD doses to achieve VD sufficiency. Treg cells of patients with T1D who received cholecalciferol at a dose of 4000 IU daily for 3 months demonstrated a differential response to VD action according to VDR single nucleotide polymorphisms (Moran-Auth et al., 2015). These data suggest that doses

may need to be personalized to achieve targeted effects due to pharmacogenomic variations in T1D.

Moreover, many human observational studies have associated low VD status with the incidence of metabolic syndrome and T2D. Similar to T1D genetic analysis, several polymorphisms have been described in VDR genes that are capable of altering VDR protein activity and can be ascribed to the development of metabolic syndrome and T2D (Karonova et al., 2018; Han et al., 2017). However, the results of VD intervention trials have been inconsistent. Some observations found that VD supplementation significantly improved the glycemic control and the metabolic parameters in patients with prediabetes and diabetes. Several other meta-analyses showed that VD had no significant effects on fasting glucose, glycated hemoglobin, and insulin resistance in T2D patients.

Only a limited number of experimental trials address the diabetes-associated disturbances in the VD pathway and their prevention by VD supplementation using animal models. In our prior experiments conducted in STZ-induced diabetic male rats, we investigated the role of vitamin D in the regulation of CYP27B1 and VDR expression at transcriptional and translational levels in different tissues of T1D rats (Mazanova et al., 2018). It was shown that T1D caused a decrease in blood 25OHD that correlated with downregulation of CYP27A1 and CYP2R1 expression. VD deficiency was accompanied by elevated synthesis of renal CYP27B1 and VDR. Conversely, CYP27B1 and VDR expression decreased in the liver, bone tissue, and bone marrow. We also revealed a strong increase (interim data) in the expression of the mRNA of the main VD catabolic enzyme CYP24A1 in the liver and kidneys that could be one of the likely mechanisms explaining VD deficiency and the impairment of its signaling in the experimental T1D. Cholecalciferol supplementation at a dose of 1000 IU/per kg of body weight for 30 days was effective in correcting impaired VD−endo/para/autocrine systems in the kidneys and extrarenal tissues of diabetic rats.

22.4.3 Antiinflammatory effect of vitamin D in diabetes mellitus

Some of the previously mentioned mechanisms linking VD to the regulation of the immune response support a role for the VD−endo/para/autocrine system disorders in the pathogenesis of inflammatory and autoimmune diseases, including diabetes mellitus. Accordingly T1D has been associated with VD deficiency and the onset of autoimmunity in

diabetes was established to be preceded by a proinflammatory cytokine profile in serum (Grammatiki et al., 2017). However, the existing observational data are still scarce as to whether there is any causal link between VD deficiency and proinflammatory processes in the manifestation of autoimmune diabetes.

Several clinical investigations have shown that VD either preserved the function of β cells from autoimmune destruction, or made it difficult to reduce the residual β cell function in children and adults with T1D, yet a few trials did not find any role for VD supplementation (Shih et al., 2016). A study of VD effect on Treg cells in TD1 patients revealed an improved suppressive capacity of Treg cells after the treatment as compared with placebo control (Treiber et al., 2015) (Fig. 22.3). Calcitriol has been shown to increase phospho-STAT6, IL-4, and IL-10 levels, as well as arginase activity, and lowers phospho-STAT4, IFN-γ, and IL-17 levels in recent-onset human T1D (Ysmail-Dahlouk et al., 2016). In accordance with these data, calcitriol supplementation decreased serum and urinary levels of inflammatory markers, such as IL-6, TNF-α, and ICAM-1, in diabetic patients (Mao et al., 2014).

It has been previously shown that the treatment of nonobese diabetic (NOD) mice with a vitamin D analog arrests the progression of insulitis,

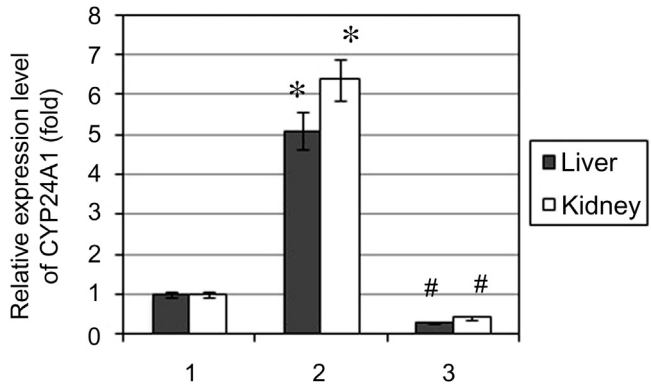

Figure 22.3 mRNA level of CYP24A1 in liver and kidney of diabetic rats and after vitamin D_3 treatment.
Transcript level (mRNA) of CYP24A1 was determined by real-time quantitative polymerase chain reaction (RT-PCR): 1, control group; 2, T1D group; 3, T1D + 100 IU of vitamin D_3 group. Data were normalized to glyceraldehyde 3-phosphate dehydrogenase, pooled from three independent experiments ($n = 6$ rats/group) and calculated using the ΔΔCt method. Results are expressed as mean ± SEM.$^{*}P \leq .05$ versus control group; $^{\#}P \leq .05$ versus diabetes group.

blocks Th1 cell infiltration into the pancreas, and markedly reduces T1D development (Giarratana et al., 2004). Along with the established increase in insulin secretion and suppression of the demise of pancreatic β cells after cholecalciferol administration, animal models also confirmed that the beneficial effect of VD may be due to its antiinflammatory effect. VD decreased interferon γ-positive CD8 + T cells and elevated CD4 + CD25 + FoxP3 + T cells in pancreatic lymph nodes of NOD mice (Takiishi et al., 2014). Moreover, $1,25(OH)_2D_3$ administration to nonobese and nondiabetic mice strongly modulated chemokine and cytokine profiles and prevented diabetes, however, calcitriol elicited unwanted calcemic side effects (Gysemans et al., 2005).

The effect of VD on the T cell segment of the immune system has been the subject of a recent study conducted by our research group on a mouse model of autoimmune diabetes. As is known, the ratio of CD4 + to CD8 + lymphocyte subsets can partially reflect proinflammatory and autoimmune processes in the body (Chen et al., 2014). Consistent with this view, we, in turn, showed that T1D mellitus was accompanied by a shift in the ratio of subsets of peripheral blood lymphocytes as well as spleen lymphocytes toward the prevalence of CD4 + T cells, and these changes were observed under conditions of pronounced deficiency in serum 25OHD (Labudzynskyi et al., 2016a,b). Altered CD4 + /CD8 + ratio can be attributed, at least partially, to an increase in the number of Th1 and Th17 cells, which, unlike Th2, secrete proinflammatory cytokines and are involved in autoimmune reactions (Alfonso et al., 2009). T lymphocytes isolated from the spleen of diabetic animals showed a higher level of inflammatory markers, such as the NF-κB p65 subunit phosphorylated at Serine 311. It was also found that the phytohemagglutinin-induced proliferative activity of the whole fraction of spleen T lymphocyte decreases almost twofold in T1D. Cholecalciferol treatment revealed its significant normalizing effect on the ratio of CD4 + to CD8 + T cells both in the blood and in the spleen. Additionally, VD increased proliferative activity of spleen lymphocytes and diminished the level of activated phospho-p65/NF-kB and its nuclear translocation in spleen T cells.

In line with numerous studies that have found that chronic inflammation is one of the hallmark mechanisms of diabetes-associated complications, we further confirmed the antiinflammatory effects of VD in liver injury related to experimental T1D. Our findings have shown a possible link (Fig. 22.4) between VD deficiency in T1D and increased hepatic levels of IL-6 and osteopontin mRNA (Labudzynskyi et al., 2016a,b), and

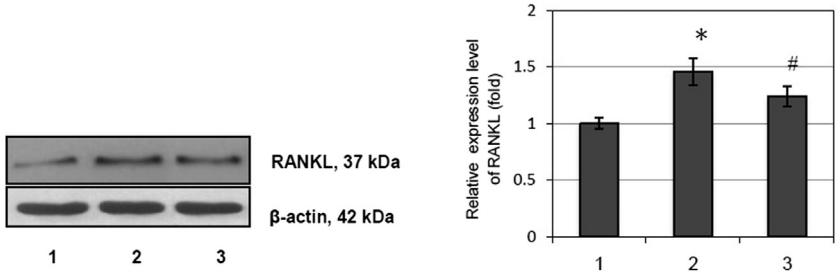

Figure 22.4 Protein level of RANKL in liver tissue of diabetic rats and after vitamin D_3 treatment.
Protein level of RANKL was determined by western-blot analysis: 1, control group; 2, T1D group; 3, T1D + 100 IU of vitamin D_3 group. Representative immunoblots are quantified using β-actin as a loading control. The bar graphs of RANKL are presented as mean ± SEM.$^{*}P \leq .05$ versus control group; $^{\#}P \leq .05$ versus diabetes group.

protein levels of RANKL (unpublished data). Enhanced RANKL content may have either a protective or deteriorating role in inflammation-associated liver injury induced by diabetes. VD supplementation exerted a beneficial effect on inflammatory processes in liver tissue related to T1D.

T2D is characterized by hyperglycemia as a result of a combination of insulin resistance in peripheral tissues and insufficient insulin secretion from pancreatic islet β-cells. There is accumulating evidence that the activation of inflammatory pathways interferes with the metabolism of insulin and impairs its signaling. In turn, insulin resistance results in an enhanced expression of proinflammatory cytokines that cause inflammatory response and low-intensity chronic inflammation, further exacerbating insulin resistance. Meta-analysis of prospective studies have shown that elevated levels of inflammatory cytokines (IL-1β, IL-6, IL-18, and CRP), TNF-α, and low levels of adiponectin strongly correlate with T2D risk (Liu et al., 2016). Only a few clinical trials have been conducted to test the efficacy of VD supplementation on inflammatory markers in T2D, with controversial results. A recent meta-analysis of randomized controlled trials indicates VD's ability to enhance leptin levels with a reducing effect on CRP and TNF-α levels that improved the chronic low-grade inflammation in diabetic patients (Mousa et al., 2018). Nevertheless, in several other studies VD supplementation did not have any positive influence on TNF-α and IL-6 levels, β-cell function, insulin sensitivity, or glycemic control in T2D subjects (Wagner et al., 2016).

T2D animal studies showed that VD administration improved the glycemic index and insulin resistance by decreasing the levels of

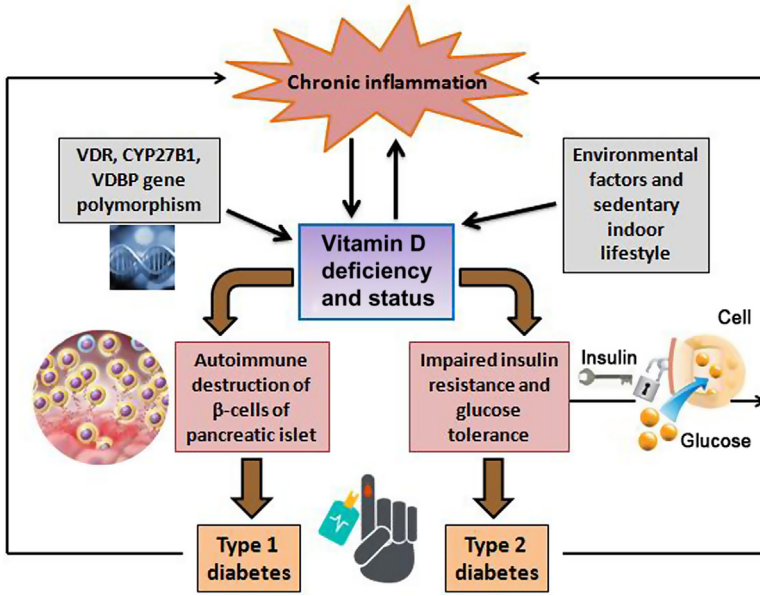

Figure 22.5 Proposed scheme linking vitamin D deficiency to inflammation and diabetes mellitus.

insulin-degrading enzymes and activating the phosphorylation of insulin receptors. The VDR agonist paricalcitol was shown to modulate the markers of pancreatic oxidative stress (catalase, syperoxide dismutase, and glutathione peroxidase) and inflammation (C-peptide, adiponectin, IL-2, and TNF-α) in T2D rats, significantly lowering blood glucose and insulin resistance (Ali et al., 2018) (Fig. 22.5).

22.5 Conclusions

Calcitriol modulates the transcription of various genes, particularly those involved in mineral metabolism and in noncalcemic functions, including immune regulation. Changes in cytochrome activities responsible for the formation of hydroxylated metabolites of cholecalciferol can play a crucial role in disturbing the bioavailability of VD and VDR-mediated cellular signaling linked to the development of widespread chronic diseases. Epidemiological and observational studies have reported an association between low plasma levels of 25OHD and the risk of type 1 and 2 diabetes mellitus. Several studies have shown that inherited variation in VD

genes is associated with diabetes, supporting a genetic etiological role for VD deficiency related to the disease.

VD deficiency may predispose to altered insulin secretion and hyperglycemia either through direct action via VDR activation, or indirectly through augmented inflammatory processes and autoimmunity. VD supplementation prevents the inflammation by downregulating proinflammatory and upregulating antiinflammatory factors. The antiinflammatory effect of VD has been proven to counteract the initiation and progression of diabetes. On the other hand, there is growing evidence that low-intensity chronic inflammation may, in turn, interfere with the normal metabolism of VD, resulting in exacerbated proinflammatory cytokines expression (Fig. 22.5). Some of the current conflicting results regarding insignificant effects of VD supplementation or the lack of an association between VD status and diabetes may be related to a predisposing role for VDR polymorphisms and those of the VD metabolism genes. Future directions may address the relevance of these polymorphisms to immune regulation in diabetes mellitus and other immune-mediated diseases.

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