Role of Vitamin D in the Pathogenesis and Therapy of Type 1 Diabetes Mellitus

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1. Introduction

This chapter will review the role of vitamin D in the pathogenesis and treatment of type 1 diabetes mellitus.

We will discuss the mechanisms through which vitamin D might affect pancreatic function. We will summarize the results of in-vitro and animal studies and will conclude with a review of the relevant clinical trials.

2. Definition

Type 1 diabetes mellitus is an autoimmune disease in which the pancreas is unable to respond to secretagogue stimulation with appropriate insulin secretion. Hyperglycemia develops when more than 70-90% of the insulin-producing beta cells are destroyed. An autoimmune destructive process, which plays a central role in the development of type 1 diabetes mellitus, is facilitated by the subject's own genetic susceptibility and by non-genetic factors. Non-genetic factors include viral infections, toxic chemicals, and others. Vitamin D deficiency is a non-genetic factor that appears to be associated with an increased risk of developing type 1 diabetes mellitus.

Type 1 diabetes mellitus complications are classified into acute and chronic. The acute complications include life-threatening conditions like severe hypoglycemia or diabetic ketoacidosis (DKA). Chronic diabetic complications can be divided into microvascular complications (retinopathy, neuropathy and nephropathy) and macrovascular complications (cardiovascular, cerebrovascular and peripheral vascular disease). Severe microvascular and macrovascular complications can lead to renal failure (the most common cause of hemodialysis in the US), blindness or lower extremity amputations.

Overall, uncontrolled diabetes mellitus in patients over 50 years of age reduces life expectancy in males and females by 7.5 and 8.2 years respectively (Franco et al.,2007).

3. Epidemiology

In 2010, about 215,000 people younger than 20 years of age had diabetes (type 1 or type 2) in the United States. A 2011 Centers for Disease Control and Prevention (CDC) report estimates that nearly 26 million Americans have diabetes. Diabetes affects 8.3% of

Americans of all ages and 11.3% of adults aged 20 years and older, according to the National Diabetes Fact Sheet for 2011. About 27% of those with diabetes (approximately 7 million Americans) do not know they have the disease. 1 in every 400 children and adolescents has type 1 diabetes.

Type 1 diabetes mellitus continues to be highly prevalent in many countries, with an overall annual increase estimated at 3% (International Diabetes Federation [IDF] 2010). Worldwide, it is more common in males than in females, with a ratio of 1.5.

The 4th edition of the IDF Diabetes Atlas, released in 2009 at the 20th World Diabetes Congress, estimated that in 2010, 285 million people would have diabetes (6.4% of world's adult population). The same forum predicts that by 2030, 438 million people will have diabetes world-wide. Type 1 diabetes in children is estimated at 480,000 patients worldwide in 2010, and the number of newly diagnosed cases per year is 75,800 (IDF 2010).

3.1 Natural history

The natural history of type 1 diabetes is characterized by an autoimmune destruction of the beta cells in the islands of Langerhans in the pancreas. The autoimmune process has cellular and humoral components, leading to the destruction of the beta cells and a decreased insulin secretion. As beta-cell mass declines, insulin secretion decreases until the available insulin no longer is adequate to maintain normal blood glucose levels. After 70-90% of the beta cells are destroyed, hyperglycemia develops and diabetes may be diagnosed.

The natural history of type 1 diabetes has 4 stages: genetic susceptibility, autoimmune process, pre-diabetes and diabetes.

The rate of beta cell destruction is variable. In some patients years will go by before the onset of diabetes, while other patients may never develop beta cell insufficiency, perhaps due to the regaining of tolerance. Most patients with type 1 diabetes mellitus have one or more susceptible human leukocyte antigen (HLA) class II, and over 90% have beta cell autoantibodies present. The appearance of circulating islet cell autoantibodies is the first detectable sign of this immune process.

4. Pathogenesis of type 1 diabetes mellitus

4.1 Genetic component

Genetics has an important role in the etiology of type 1 diabetes. However, extra-genetic components influence the penetrance of diabetes susceptibility genes. If data are obtained at a single point in time, the risk of type 1 diabetes mellitus between monozygotic twins can be as low as 30%, but if the monozygotic twins are followed long-term, the cumulative incidence of diabetes reaches 65% (Redondo et al., 2008). In the same cohort of monozygotic twins, the rate of persistent autoantibody positivity, type 1 diabetes mellitus, or both, reached 78% (Redondo et al., 2008).

To better understand the genetic susceptibility to diabetes, candidate gene studies were conducted in order to identify genes that are associated with autoimmune type 1 diabetes.

Human leukocyte antigen (HLA) associations have been long recognized in many autoimmune diseases. In type 1 diabetes mellitus, the HLA on chromosome 6p21 is well described and is considered to play an important role in more than 50% of the familial cases in Caucasians (Noble et al., 1996). HLA DR4-DQ8 or DR3-DQ2 haplotypes are detected in up to 90% of patients with type 1 diabetes mellitus (Devendra & Eisenbarth, 2003). The combination

of these 2 types, DR4-DQ8/DR3-DQ2, carries the highest risk and type 1 diabetes mellitus occurs at a very early age in this population. First-degree relatives of the patients who carry the highest risk haplotype combination also have a higher risk of developing diabetes mellitus as compared to the relatives of diabetes patients who do not have this haplotype and who develop type 1 diabetes mellitus later in life (Gillespie et al., 2002).

Another HLA haplotype (DR15-DQ6) might have protective properties, and is found in a much larger percentage in the general population (20%) as compared to less than 1% in patients with type 1 diabetes mellitus (Eisenbarth & Gottlieb, 2004).

HLA haplotypes appear to have an association with islet autoantibodies. Glutamic acid decarboxylase (GAD) antibodies are more frequent in patients with HLA DR3-DQ2, whereas insulin auto-antibodies (IAA) and protein tyrosine phosphatase-like protein antibodies (IA-2 antibodies) are more frequent in patients with HLA DR4-DQ8. Patients that do not have these haplotypes are less likely to develop islet autoantibodies (Achenbach et al., 2005).

Another key genetic factor is the insulin gene (INS), with different forms of the promoter region conferring either protection or increased susceptibility to autoimmune diabetes mellitus (Bennett et al., 1995). The insulin gene contributes 10% to the genetic susceptibility in developing autoimmune diabetes (Bell et al., 1984). The risk of developing diabetes depends on the expression of the insulin protein in the thymus which can cause a defective central tolerance to the insulin molecule. The degree of immune tolerance may be reflected by the less common presence of insulin autoantibodies (IAA) in patients or relatives who have the protective INS class I/III or III/III genotypes (Vafiadis et al., 1997).
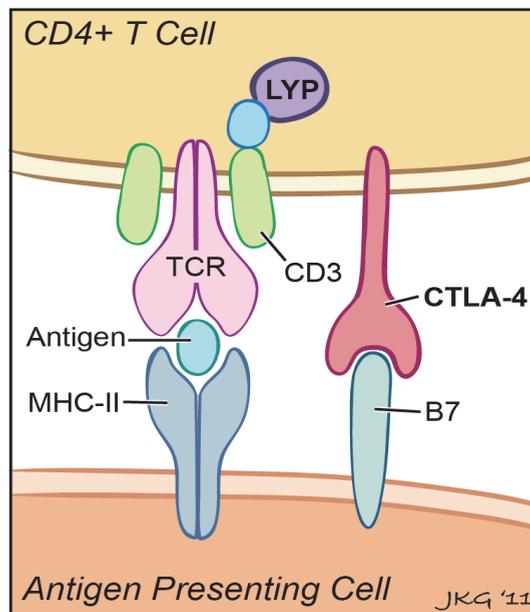

Fig. 1. Antigen Presenting Cell. The activation of the T-cell by various stimuli (antigens), is brought by major histocompatibility complex (MHC-HLA II). This figure shows also, inhibitors of T-cell activation: cytotoxic T lymphocyte antigen 4 (CTLA-4) and lymphoid tyrosine phosphatase (LYP).

T cells are recognized to be a major part of the immune process in diabetes mellitus, and several genes involved in T cell regulation are associated with type 1 diabetes mellitus. Two genes encoding factors that are suppressive to the T cell activation appear to have a close association with autoimmune diabetes: lymphoid tyrosine phosphatase locus (LYP/PTPN22) (Smyth et al., 2004), and cytotoxic T lymphocyte antigen 4 (CTLA-4) (Ueda et al., 2003) (Figure 1), located on chromosome 2q33.

The CTLA-4, which is a T-Lymphocyte receptor, is expressed after T-cell activation (Greenwald et al., 2005). It turns off T-cell responses by inhibiting the production of interleukine-2. CTLA-4 polymorphism in humans has been associated with an increased risk of autoimmune disease, including type 1 diabetes mellitus (Gough et al., 2005).

Another gene linked to an increased risk for type 1 diabetes is the gene for the intercellular adhesion molecule (ICAM-1) (Nejentsev et al., 2003). A recent genome-wide association study described over 40 loci associated with an increased risk for type 1 diabetes (Barrett et al., 2009).

4.2 Autoimmune process

One of the best animal models for type 1 diabetes mellitus is the nonobese diabetic mouse (NOD). NOD mouse develops type 1 diabetes mellitus spontaneously, over the course of a few months, allowing the investigators to study this process stage by stage. Many reports describe in detail the genetics, the immune process, the influence of the environment and most importantly, the potential therapies to prevent, delay or reverse the destructive process that leads to type 1 diabetes mellitus in this model. Delovitch and Singh (Delovitch & Singh, 1997) reviewed the use of NOD mouse in the studies of type 1 diabetes mellitus. In NOD mice, the first step is the infiltration of the peri-islet regions of the pancreatic islets by dendritic cells (DC) and macrophages, followed by T cells (CD4+ and CD8+). This stage is known as peri-insulitis, occurring around 3-4 weeks of age. It is followed by a slower, progressive T cell destruction of the beta cells (insulitis), by 4-6 months of age (Delovitch & Singh, 1997). Thus, the T cells and the dendritic cells are key players in the immune process leading to type 1 diabetes mellitus.

The dendritic cells (DC) are antigen-presenting cells which originate from the bone marrow. They become active once they capture and process the antigens. After infiltrating the pancreas and undergoing antigenic maturation, DC secrete IL-12 and present the processed antigen (on their surface and in association with the major histocompatibility complex [MHC] class II) to other cells of the immune system (i.e. T cells) (see Fig 1).

T cells are categorized mainly based on their immune actions, achieved via the different cytokines they secrete. Cytokines are classified into two types: type 1 cytokines, which activate the cellular immunity and suppress the humoral immune response, and type 2 cytokines, which activate the humoral immunity and inhibit the cellular immune process (Rabinovitch, 1998).

Th1 cells are preferentially formed from their T cell precursors (T helper 0) under the direct influence of mature DC and IL-12 (Banchereau & Steinman, 1998).

T helper 1 cells (Th1) are involved in cell-mediated immune responses (inflammation, cytotoxicity, delayed hypersensitivity) and produce type 1 cytokines: tumor necrosis factor β (TNFβ), interferon γ (IFNγ), and interleukin 2 (IL-2). T helper 2 cells (Th2) are important in humoral immunity (activate B cells and antibody production, down regulating Th 1 cells) and secrete type 2 cytokines: interleukins 4, 5, 6, 9 and 10 (Rabinovitch, 1998) (Fig. 2).

The Th2 cells are protective for the beta cells. They have an inhibitory effect on the Th1 cells, which are destructive to the pancreatic beta cells. In the NOD mouse, it appears that the immunologic self-tolerance to pancreatic beta cells is lost. The disruption of the equilibrium between Th1 and Th2 cells in the thymus and in the periphery is believed to play a crucial role in the pathogenesis of autoimmune diabetes mellitus (Delovitch & Singh, 1997). Once Th1 cells are produced they will secrete interferon γ (IFN γ) and IL-2, leading to the activation of macrophages and cytotoxic T cells, which are destructive to the pancreatic beta cells (Adorini, 2001). The same Th1 cells will stimulate the IgG2a autoantibodies against the islet beta cells autoantigens (Delovitch & Singh, 1997). Autoimmune diabetes can be transferred from a diabetic NOD mouse to an unaffected mouse via T cells (Bendelac et al., 1987). NOD mice develop a spontaneous loss of T-cell tolerance to glutamic acid decarboxylase antibodies (GAD), leading to autoimmune diabetes (Kaufman et al., 1993). In NOD mice, there is an increased resistance to apoptosis in immunocytes (Leijon et al., 1995, Penha-Goncalves et al., 1995).

Immune responses to several beta-cell proteins have been described (auto-antigens). Exposure to glutamic acid decarboxylase (GAD65 and GAD67) led to an increased T cell proliferation as early as 4 weeks of life in NOD mice, coinciding with the onset of insulitis (Tisch 1993). Some of the other beta-cell antigens elicited an increased immune response after a few more weeks, but there were other beta-cell antigens that did not trigger an immune reaction (for example, amylin) (Tisch 1993). The same study showed that intrathymic injections of GAD65 had a protective effect from autoimmune diabetes in NOD mice (delaying the onset of disease and decreasing the frequency) (Tisch etal., 1993)

GAD65- reactive T cells were proven to have the ability to transfer diabetes to NOD/SCID (severe combined immunodeficiency) mice (Zekzer et al., 1998). To further support the central role of GAD antigen in autoimmune diabetes, the beta-cell-specific suppression of GAD expression in antisense GAD transgenic NOD mice was demonstrated to prevent the production of diabetogenic T cells and the onset of diabetes (Yoon et al., 1999)

In humans, the pancreas becomes infiltrated with mononuclear cells. Autoantibodies to insulin (IAA), glutamic acid decarboxylase (GAD) and insulinoma associated-2 antibody (IA-2) are demonstrated years before the clinical symptoms of diabetes. (Kulmala et al., 1998) T cell responses to several islet cells antigens (insulin, GAD, IA-2) have been reported in IDDM (MacCuish et al., 1975). The presence of autoantibodies alone does not explain the development of diabetes, since it is recognized now that children born to type 1 diabetic mother with high antibody titers transferred through the umbilical cord do not develop diabetes more often than expected. An interesting case was published by Martin et al in 2001, describing a case of type 1 diabetes mellitus occurring in a patient that had a hereditary B-cell defect (Martin et al., 2001).

4.3 Environmental component

The environment is implicated in the pathogenesis of type 1 diabetes mellitus by many studies.

Environmental factors have an important role in initiating an immune process that ultimately leads to pancreatic beta cell destruction and clinically apparent diabetes mellitus. Many environmental factors have been proposed, including viruses (rubella, mumps or coxsackievirus B4), toxic substances and cytotoxins. Nutritional status and diet have also

been implicated as potential players in type 1 diabetes pathogenesis: vitamin D deficiency, early protein diet exposure or exposure to cow's milk in infancy.

Viruses are among the main culprits studied. Before the eradication of rubella in most countries, congenital rubella was strongly associated with the development of type 1 diabetes mellitus (Menser et al., 1978). A recent meta analysis of observational studies has shown an association between type 1 diabetes and enterovirus infection (Yeung 2011).

While some theories implicate viral infections in the pathogenesis of type 1 diabetes, a recent hypothesis argues that a decreased exposure to microbes may contribute to the current increase in autoimmune disease. This theory is known as "the hygiene hypothesis" (Gale, 2002).

It is a known fact that the incidence of autoimmune diabetes follows a geographical pattern, with many studies reporting an association between type 1 diabetes and vitamin D status. A few large ecological studies describe a pattern of geographical variation, with an increased incidence of type 1 diabetes in the areas located north of the equator. Furthermore, seasons appear to also influence the incidence of type 1 diabetes, with the highest incidence during winter and the lowest during summer. The month of birth during springtime is associated with a higher risk of type 1 diabetes (Kahn et al., 2009), a finding that could be explained by possible low circulating vitamin D levels in both mother and fetus through the winter months of the pregnancy.

In order to develop more information about environmental factors that play a role in the pathogenesis of diabetes, an international initiative (the Environmental Determinants of Diabetes in the Young) will be following thousands of infants with an increased genetic risk from birth until adolescence and will gather data about infectious agents, dietary or other environmental factors.

Typically, the treatment for type 1 diabetes mellitus involves insulin therapy, but in the last few years new therapies have been approved as well (for example, Symlin). For newly diagnosed patients with autoimmune diabetes, combination therapy has been suggested in an attempt to minimize beta cell destruction and prolong pancreatic function. The new therapeutic options include: immunotherapy, vaccines, drugs that influence T cell action, anti-inflammatory drugs (for example, one time use of anti-IL-1R drug), or long-term treatment with B cell components to induce regulatory T cells (oral or nasal insulin, insulin peptide therapy, GAD-Alum or the proinsulin DNA vaccines). Glucagon-like peptide 1-related drugs (GLP-1) could be also considered as a therapeutic option because they promote peritubular pancreatic cell growth (Von Herrath, 2010).

5. Vitamin D

Although initially described as a "vitamin", vitamin D is now recognized to be a hormone, synthesized in the human body and exerting its action on other organs via a nuclear receptor (vitamin D receptor, VDR).

Even though vitamin D can be obtained from the diet in small quantities, the main source of vitamin D is the skin. Under the direct influence of ultra violet B light (UVB light), 7-dehydrocholesterol (DHC) (provitamin D3) is converted into pre-vitamin D3, which is then further converted into cholecalciferol (vitamin D3) via thermal isomerization. Interestingly, if pre-vitamin D3 continues to be exposed to UVB, it will be converted into biologically inactive metabolites (tachysterol and lumisterol), preventing a potential UVB- induced vitamin D intoxication (Holick, 1999) The other source of vitamin D is the diet, which

contains cholecalciferol (vitamin D3), originating from animal sources, and ergocalciferol (vitamin D2), deriving from plants (Holick, 1999).

Regardless of their source, once they enter into the circulation, forms of inactive vitamin D3 or D2 bind to the vitamin D-binding protein (DBP) and are transported to the liver. The inactive vitamin D is activated through a 2-step hydroxylation process via two hydroxylases that belong to the cytochrome P450- dependent steroid hydroxylases (CYP450). In the liver, vitamin D undergoes the first hydroxylation at C-25 via some of the CYP 450 vitamin D 25-hydroxylases, forming calcidiol (25-hydroxyvitamin D) (Prosser & Jones, 2004). This is the major circulating form of vitamin D. At the level of the proximal renal tubule, 25-OH vitamin D is further hydroxylated to calcitriol (1,25 dihydroxyvitamin D, the active form of vitamin D) by the 1α-hydroxylase (1α(OH)ase, CYP27B1) (Prosser & Jones, 2004).

Both calcidiol and calcitriol are inactivated via the 25-hydroxyvitamin D3-24-hydroxylase (CYP24), forming the inactive metabolite 24,25- dihydroxyvitamin D (Holick, 1999).

1α-hydroxylase has been described in many extrarenal tissues: macrophages, monocytes, and placenta, rendering these cells capable of synthesizing 1α-,25(OH)$_2$D$_3$from 25(OH)vitamin D (Weisman et al., 1979, Bhalla et al., 1983, Stoffels et al., 2007, Adams et al., 1983). The activity of 1α-hydroxylase in the immune cells is not under the regulation of parathyroid hormone and 1α-,25(OH)$_2$D$_3$, but rather under immune cytokine regulation. A defect in the up-regulation of 1α-hydroxylase after immune stimulation is described in NOD mouse (Overbergh et al., 2000). Extrarenal distribution of 1α-hydroxylase becomes important in understanding the extra-skeletal effects of vitamin D.

VDR is part of the nuclear receptor super family of ligand-activated transcription factors, which also includes glucocorticoid, thyroid hormone and estrogen receptors. The gene for VDR is located on chromosome 12q12-14, and shows great polymorphism (Haussler et al., 1998). After 1,25 (OH)$_2$D$_3$ binds to VDR, it induces conformational changes that facilitate heterodimerization with the retinoid X receptor and the recruitment of nuclear receptor coactivator proteins, which then act on the chromatin. The specific DNA sequence that is ultimately affected by the vitamin D is known as the vitamin D responsive element (VDRE) (Carlberg & Polly, 1998).

The discovery of the vitamin D receptor (VDR) on the immune cells (Strugnell & DeLuca, 1997), led to the hypothesis that vitamin D could affect the autoimmune processes. However, in VDR deficient mice models, there is no increase in autoimmune diseases (Mathieu et al., 2001)

The protective effects of vitamin D in several autoimmune diseases have been described in animal models (experimental autoimmune encephalomyelitis (Lemire, 1995), murine models of human multiple sclerosis and murine models of rheumatoid arthritis (Cantorna et al., 1996). In other autoimmune diseases, like psoriasis, vitamin D analogues are the mainstay of treatment today.

The extraskeletal effects of 1α-,25(OH)$_2$D$_3$ can usually be observed only at very high concentrations (10^{-10}mol/l), higher than physiological levels needed for calcium balance (concentrations that could probably be achieved in specific target tissues via the macrophages' 1α-hydroxylase) (Mathieu et al., 2005). Thus a risk of hypercalcemia and other side effects of 1α-25(OH)$_2$D$_3$ could occur if it were used for its anti-autoimmune properties. Numerous vitamin D analogs have been developed to exert extraskeletal effects, with less pronounced action on the calcium metabolism. Most of these analogs are used for laboratory

research purposes, but some are part of standard treatment for certain autoimmune diseases (for example, calcipotriol for psoriasis).

There are several theories that attempt to explain the link between Vitamin D and autoimmune diabetes. This relationship appears to be complex, with actions at multiples levels: genetic, autoimmune and also direct action on the pancreatic beta cells.

6. Vitamin D and type 1 diabetes

Animal studies and clinical trials in patients with new onset of type 1 diabetes show that the replacement of vitamin D may arrest the deterioration of pancreatic function and improve C-peptide levels.

There is strong epidemiologic data showing that the population in countries with a high prevalence of type 1 diabetes mellitus is commonly vitamin D deficient. Vitamin D supplementation during pregnancy decreased the risk of the development of type 1 diabetes mellitus for offspring (Fronczak et al., 2003). Supplementation of vitamin D at an early age also decreases the risk for developing type 1 diabetes (Hypponen et al., 2001)

The vitamin D receptor (VDR) has been described on almost every tissue in the human body, including the cells of the immune system, as discussed earlier.

The VDR gene is located on chromosome 12, and has a few allelic variants. It has been reported that some of these allelic variations of the VDR gene are linked to an increased risk of type 1 diabetes mellitus in the German and the Indian Asian population (Pani et al., 2000, Chang et al., 2000). On the other hand, the same association was not found in another population sample (British, Portuguese and Finnish origin) (Guo et al., 2006, Lemos, 2008, Turpeinen, 2002).

An interaction between specific VDR polymorphisms and predisposing HLA DRB1 0301 allele was described in North Indian patients (Israni et al., 2009) and is associated with an increased risk of developing type 1 diabetes mellitus.

As discussed earlier, the last step in the activation of vitamin D is facilitated by the key enzyme 1α-hydroxylase, encoded by the CYP27B1 gene on the chromosome 12q13.1-q13.3. Polymorphism in this gene is described as being associated with an increased risk of type 1 diabetes mellitus (Lopez et al., 2004, Bailey et al., 2007). The polymorphism in the CYP27B1 gene could potentially lead to the reduced expression of 1α-hydroxylase, less production of the active 1α,25 (OH) $_2$ D$_3$, and ultimately, to the increased risk of type 1 diabetes.

6.1 Vitamin D and type 1 diabetes: The effects on the immune processes

Vitamin D interacts with most immune cells and affects their cytokine production. Overall, vitamin D has a protective effect on the pancreatic beta cells (Figure 2).

DCs are affected by 1α,25 (OH)$_2$D$_3$ in many ways. DCs mature after they engulf the antigen, increasing the expression of MHC-II molecules on their surface and secreting IL-12. Studies show that vitamin D analogs suppress the expression of MHC-II molecules (Griffin 2000) The cytokine secretion by DC is affected as well: the IL-12 is inhibited (D'Ambrosio 1998), while IL-10 production is increased (Penna 2000). Furthermore, DC apoptosis is promoted by exposure to vitamin D (Penna 2000).

If DC are exposed to 1α,25 (OH)$_2$ D$_3$, they do not mature at a subsequent exposure to an antigen, becoming tolerogenic (Griffin et al., 2001). After being treated with a vitamin D analog, the DC do not simply remain immature, but instead are transformed into

tolerogenic DC with special endocytic properties (Ferreira et al., 2009). Adorini et al published a paper describing how $1\alpha,25(OH)_2 D_3$ can change the dendritic cells into a tolerogenic phenotype which is thought to induce T regulatory cells and inhibit autoimmune diseases, like type 1 diabetes (Adorini, 2003) (Fig 2).

Fig. 2. The immunomodulatory effects of $1\alpha,25(OH)2D3$. At the level of the antigen-presenting cell (such as dendritic cells; DCs), $1\alpha,25(OH)2D3$ inhibits the surface expression of MHC class II-complexed antigen and of co-stimulatory molecules, in addition to production of the cytokine IL-12, thereby indirectly shifting the polarization of T cells from a Th1 towards a Th2 phenotype. In addition, $1\alpha,25(OH)2D3$ has immunomodulatory effects directly at the level of the T cell, by inhibiting the production of the Th1 cytokines IL-2 and IFN-γ and stimulating the production of Th2 cytokines. Moreover, $1\alpha,25(OH)2D3$ favors the induction of regulatory T cells. Both Th2 and Tregs can inhibit Th1 cells through the production of counteracting or inhibitory cytokines. Together, these immunomodulatory effects of $1\alpha,25(OH)2D3$ can lead to the protection of target tissues, such as β cells, in autoimmune diseases and transplantation. CD40L, CD40 ligand; Mf, macrophage; Tc, cytotoxic T cell; TGF- β, transforming growth factor β; Th1,T helper type 1; TNF-α, tumor necrosis factor α; Treg, regulatory T cell. This figure was published in Trends in Endocrinology and Metabolism Vol.16 No.6 August 2005. Vitamin D and type 1 diabetes mellitus: state of art. Chantal Mathieu and Klaus Badenhoop. Copyright @ Elsevier 2005. Used with permission.

Descriptions of the VDR on T lymphocytes lead to the subsequent investigation of vitamin D actions on these immune cells. Interestingly, 1α-hydroxylase is not expressed in the T cells, and vitamin D activated in the macrophages acts on the T cells, suggesting an autocrine action of $1\alpha,25$ dihydroxyvitamin D_3.
Rigby and his team proved that cytokine production by T cells is influenced by vitamin D analogs: IL-2 and IFN γ are inhibited (Rigby et al., 1987), while production of some of the type 2 cytokines (IL-4, 5, and 10) is enhanced (Boonstra et al., 2001)

Inhibition of mitogen-stimulated T-cell cultures by vitamin D has been also reported (Rigby et al., 1984)

On the other hand, suppressor T cells are stimulated by vitamin D, leading to the inhibition of T-cell mediated immunity (Mathieu et al., 1994).

While inhibiting the IL-12 production from the DC, vitamin D is able to shift the differentiation of T naïve cells into Th0 cells and further into Th2 cells (IL-12 is an important cytokine that preferentially promotes the Th1 cell formation from the Th0 cells) (Willheim et al., 1999).

A recent study reported the direct modulation of CD4+ T cell function by active vitamin D, describing the inhibition of IL-17, IL-21, IFNγ, and the induction of T reg cells expressing CTLA-4 and FoxP3. If the T cells are grown in an environment rich in IL-2 and vitamin D, they express the highest levels of CTLA-4 and FoxP3, and are able to suppress the proliferation of the resting CD4+ T cells (Jeffery et al., 2009).

VDR is normally expressed on the B cells only upon their activation. Chen reported that $1\alpha,25 (OH)_2D_3$ decreased B cell proliferation and immunoglobulin production and induced cell death (Chen et al., 2007).

Vitamin D inhibits the production of inflammatory interleukins: IL-12, IL-2, interferon γ, tumor necrosis factor (TNF)-α, and TNF-β ,while the production of anti-inflammatory cytokines (IL-4, IL-10, TGF-β) is stimulated. This may disrupt the production of Th1 cells, which are destructive for the pancreatic beta cells, with a resultant beneficial effect on the beta cells (Lemire, 1995, van Etten & Mathieu 2005).

6.2 Vitamin D and type 1 diabetes: Direct effects on pancreatic cells

$1\alpha,25 (OH)_2 D_3$ appears to have a direct protective effect against pancreatic beta cell destruction by reducing the expression of MHC class I molecules (Hahn et al, 1997). In addition, vitamin D appears to increase islet cell expression of the A20 protein, which has antiapoptotic function (Riachy et al., 2002) (Fig 2). Vitamin D also decreases the expression of Fas, which is a transmembrane cell surface receptor mediator, involved in pancreatic beta cell apoptosis (Riachy et al., 2006).

7. Animal studies – vitamin D and type 1 diabetes

Insulitis can be inhibited by the administration of high doses of vitamin D in NOD mice (Mathieu et al., 1992), and $1\alpha,25(OH)_2D_3$ can prevent autoimmune diabetes in these animals (Mathieu et al., 1994). In both spontaneously developing and cyclophosphamide induced models of diabetes mellitus, vitamin D protects against autoimmune diabetes in NOD mice through restoration of the deficient suppressor cell function (Mathieu et al., 1995). VDR ligands enhance CD4+CD25+ regulatory T cells; these cells may play a role in protecting against insulitis in NOD mice (Adorini, 2003).

The loss of balance between the Th1 cells and Th2 cells, with the overproduction of the Th1 cells, appears to be central in the autoimmune diabetes pathogenesis. In NOD mice, the exposure to GAD65 leads to T cell proliferation and antibody production (Kaufman et al., 1993), at the same time as insulitis develops. 1,25 dihydroxyvitamin D_3 administration leads to a local immune shift of the balance between the Th1 cells and Th2 cells, favoring the increase in IL-4 production and the decrease in the γ interferon secretion.

Overbergh et al demonstrated that in NOD mice the immune shift between Th1/Th2 cells occurs in the periphery and is not limited to the pancreas (Overbergh et al., 2000). Furthermore, this change in the immune milieu occurs only in the autoantigen–specific immune response (exposure to GAD65, insulin B-chain, heat shock protein 65), and is not observed in the immune response associated with other antigens (ovalbumin, tetanus toxins, etc).

The recurrence of autoimmune diabetes mellitus after islet cell transplant was prevented in NOD mice by treatment with vitamin D analogs in combination with cyclosporine A (Casteels et al., 1998). Further, the administration of a nonhypercalcemic vitamin D analog in combination with an immunosuppressant (cyclosporine A) prevented progression to overt diabetes mellitus, even after the insulitis developed (Casteels et al., 1998). This effect, however, could not be reproduced when the vitamin D analog was administered without the addition of cyclosporine.

The NOD mice have an increased resistance to apoptosis in their immune cells. 1,25-dihydroxyvitamin D3 restores apoptosis in NOD mice in the thymus, leading to the increased destruction of autoimmune effector cells (Casteels et al., 1998).

In the BB rat, another animal model for autoimmune diabetes mellitus, 1,25-dihydroxyvitamin D did not lead to any significant difference in the incidence of diabetes when given from weaning to 120 days (Mathieu et al., 1997). This finding illustrates the issue of potentially different disease mechanisms in various animals and the difficulty of applying research findings from one animal model to another, or to humans.

8. Clinical studies – vitamin D and type 1 diabetes

The data available from human studies is scant and controversial.

A few ecological studies support the theory that vitamin D is a major player in the autoimmune disease pathogenesis, including type 1 diabetes mellitus.

A study in Northern Europe described the seasonal pattern of disease onset for autoimmune diabetes mellitus (Karvonen et al., 1998). The Diabetes Epidemiology Research International Group reported in 1988 an increased incidence of autoimmune diabetes with lower average yearly temperatures, which, in turn, was strongly associated with increasing latitude distances from the equator. This variation is thought to be due to the decreased exposure of the skin to the UV radiation.

In a very large worldwide study, Mohr et al analyzed the data from the Diabetes Mondial Project Group and found that in children younger than 14 years of age, the incidence rates of type 1 diabetes mellitus were significantly increased at higher latitudes and with low UVB exposure. Incidence rates of type 1 diabetes mellitus approached zero in the region with high UVB irradiance (Mohr et al., 2008).

Several European studies reported a decreased risk of diabetes in infants supplemented with high doses of vitamin D. The EURODIAB substudy 2 study group in seven European centers reported that vitamin D supplementation in infancy decreased the risk of autoimmune diabetes in a fairly consistent manner (Dalquist et al., 1999). Hypponen et al published the results of a birth-cohort study in northern Finland that included all pregnant women who were due to give birth in 1966, and recorded the frequency and the dosing of the vitamin D supplementation in the first year of life, as well as the presence of suspected rickets. 30 years later, the authors found that there was a lower incidence of diabetes mellitus in children who took any dose of vitamin D as compared with children that did not

take any vitamin D supplementation. Even more so, the risk was lower in children that took the highest dose (2000 IU daily) as compared to the lower dose of vitamin D. Children with suspected rickets had a 3 fold increased risk of developing insulin-dependent diabetes mellitus (Hypponen et al., 2001). The risk of developing islet auto-antibodies in the children of mothers that took vitamin D during pregnancy was decreased in the Diabetes Autoimmunity Study in the Young (DAISY) (Fronczak et al., 2003). It is unclear from these studies if the protective effect is due to the supplementation with extra doses of vitamin D or prevention of vitamin D deficiency.

Two new interventional trials have been published in the last 2 years supporting the beneficial effect of vitamin D on the development of autoimmune diabetes.

A pilot study looking at patients with adult-onset latent autoimmune diabetes (LADA) demonstrated that supplementation with 1,25 dihydroxyvitamin D3 for 1 year resulted in beta cell preservation, as assessed by C-peptide levels (Li et al., 2009).

Aljabri et al conducted a prospective study in which patients with vitamin D deficiency were assigned to receive 4000 IU of vitamin D3 daily and had vitamin D 25 (OH) levels and hemoglobin A1c measured at baseline and at 12 weeks. The results revealed that the patients who achieved higher circulating levels of vitamin D 25 (OH) had a lower hemoglobin A1c (Aljabri et al., 2010).

Other studies, however, did not find similar results. A study which examined the effects of supplementation with cod liver oil during the first year of life, found that the infants who were supplemented had a decreased risk of developing childhood-onset type 1 diabetes. However, this decreased risk of type 1 diabetes mellitus was not observed in the infants if the cod liver oil was supplemented during pregnancy or if the vitamin D preparations were supplemented during the first year of the infant's life. Since cod liver oil has a high content of vitamin D along with the long-chain n-3 fatty acids (eicosapentaenoic and docosahexaenoic), it is not clear if these effects are due to the high vitamin D content of the cod liver oil or due to the fatty acids (Stene et al., 2003).

Pittoco et al reported the results of an interventional trial in children with newly diagnosed type 1 diabetes, in which the patients were administered calcitriol or nicotinamide in order to preserve beta-cell function. Even though there was a decrease in the insulin requirements at 3 and 6 months in the calcitriol treated group, at the end of the first year there was no difference between the C-peptide levels or hemoglobin A1c between the two groups (Pitocco et al., 2006).

Bizzarri et al investigated whether supplementation with calcitriol in recent onset autoimmune diabetes has a protective effect on the pancreatic beta cells and found that, at the doses used in the study, calcitriol did not confer protection against the autoimmune destruction of the beta cells (Bizzarri et al., 2010). In Germany, Walter et al supplemented newly diagnosed adult patients with 1,25(OH)2D3 for 18 months. At the end of the study there was no difference in the areas under the curve (AUC) for C-peptide, peak C-peptide, or fasting C-peptide after a mixed meal tolerance test between the treatment and the placebo groups (Walter et al., 2010).

9. Conclusion

In conclusion, the data on the role of vitamin D in the pathogenesis of autoimmune diabetes mellitus is inconclusive. More studies, particularly, interventional trials, with vitamin D or

vitamin D nonhypercalcemic analogs need to be performed before the interaction between autoimmunity, diabetes mellitus and vitamin D is completely understood.

10. Acknowledgements

11. References

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