High dose vitamin D treatment regulates the gene expression pattern in T helper cells of type 1 diabetes patients

M. Penna-Martinez[1], H. Hess[1], C. Döring[2], N. Nejatian[1], D. Bogdanou[1], F. Shoghi[1], M-L. Hansmann[2] and K. Badenhoop[1]
[1]Department of Internal Medicine, Division of Endocrinology, [2]Senckenberg Institute of Pathology, University Hospital Frankfurt am Main, Germany

Introduction / Objectives

Dysregulated T helper cells and vitamin D (VD) deficiency are important factors in the pathogenesis of Type 1 diabetes mellitus (T1D)[(1)(2)(3)]. Therefore, we investigated the immune effects of high dose VD treatment on gene expression pattern (GEP) in T helper cells (Th) before and after VD-therapy in patients with T1D.

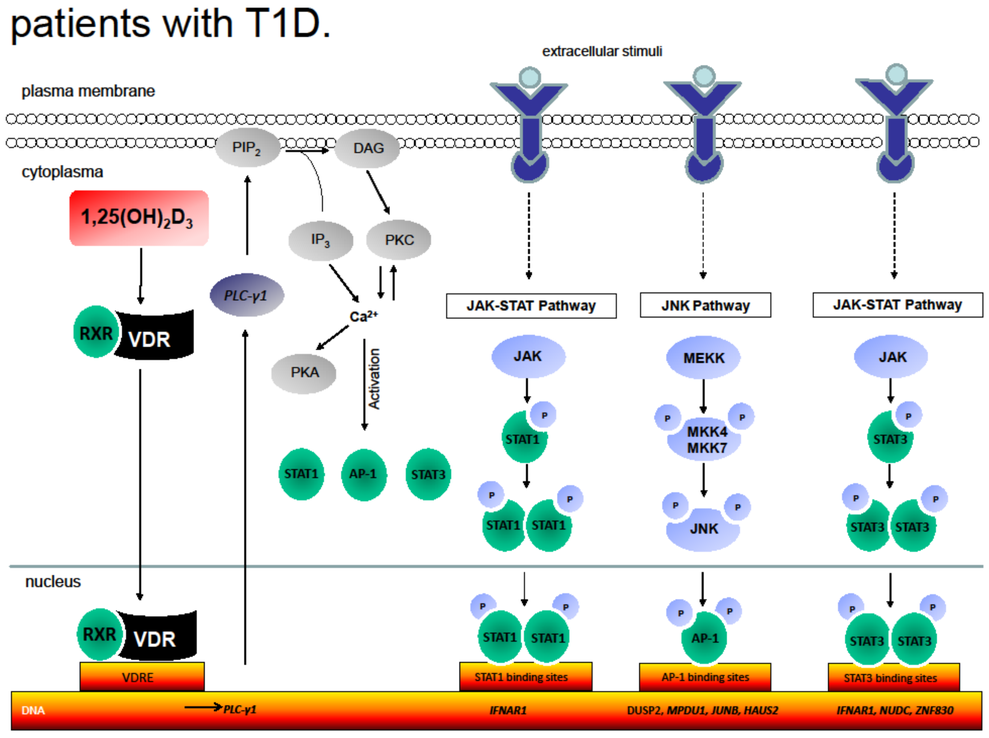

Figure 1: Vitamin D-mediated gene transcription in T helper cells. $1,25(OH)_2D_3$ binds to vitamin D receptor (VDR), which dimerizes with the retinoid X receptor (RXR). The VDR-RXR complex is translocated into the nucleus where it binds to vitamin D response element (VDRE) in VD responsive genes. For example, it induced upregulation of *PLC-γ1* gene expression. Phospholipase C-gamma 1 (PLC-γ1) activates cAMP-dependent protein kinase A (PKA) and protein kinase C (PKC) which increase the intracellular calcium level [(4)]. This could lead to an activation of AP-1, STAT1 and STAT3 necessary transcription factors for variety of genes e.g. *dual specificity phosphatase 2 (DUSP2)*, *mannose-P-dolichol utilization defect 1 (MPDU1)*, *jun B proto-oncogene (JUNB)*, *HAUS augmin-like complex, subunit 2 (HAUS2)*, *interferon receptor 1 (IFNAR1)*, *nuclear distribution protein (NUDC) and zinc finger protein 830 (ZNF830)*. Furthermore extracellular stimuli via T cell receptor (TCR) induce an intracellular-signal pathway through kinase-phosphorylation which also activates these transcription factors. $1,25(OH)_2D_3$ could modulate these pathways via increase the calcium concentration [(5)(6)(7)].

Patients and Methods

Seven T1D patients with $25(OH)D_3$ levels below 20 ng/ml received three months 4000 IU/d Vigantol oil as part of the RCT VIDDA1. The $25(OH)D_3$ concentration and gene expression within Th cells were measured at baseline (V1) and after three months treatment (V3). T helper cells were isolated from freshly collected EDTA-blood by density gradient centrifugation, subsequently enriched by magnetic sorting and total RNA was isolated. After cDNA synthesis the gene expression profile was investigated using GeneChip Human 1.0 ST (Affymetrix). $25(OH)D_3$ plasma concentrations were measured by radioimmunoassay. VD-therapy effects on the gene expression were evaluated using the differences between V1 and V3 (expressed in fold changes=FC) within each patient by the statistical computing environment R version 3.0.2 [R Development Core Team, 2005].

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Results

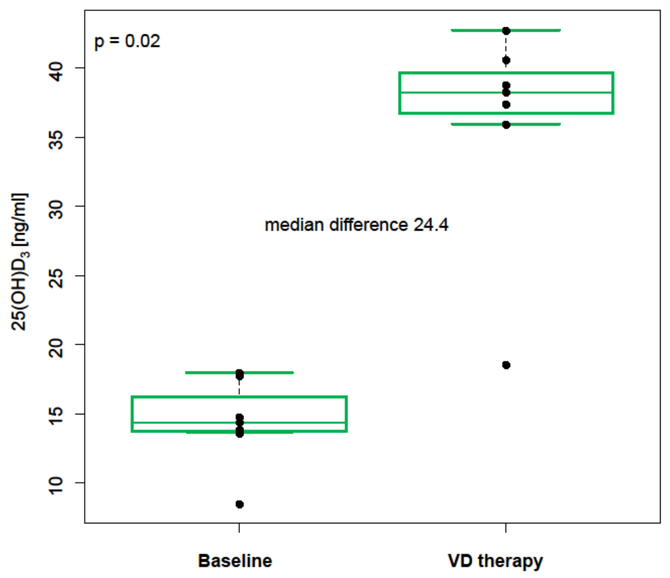

Figure 2: $25(OH)D_3$ level after VD therapy.
The $25(OH)D_3$ concentration increased in median from 14 to 38 ng/ml (median differences 24.4 ng/ml;p = 0.02) after high dose VD.

Table 1: Validation of GEP using real time RT-PCR. The data are expressed as median (interquartile range = IQR). The microarray results were checked in seven randomly selected genes using real time PCR: The upregulation of the genes *JUNB* (p < 0.05) and *DUSP2* (p_{trend} = *0.08*) was confirmed in T1D patients.

	T1D Gene expression $2^{-[CT\ target - CT\ 18s\ RNA]} \times 10^6$	
	differences V3-V1, median (IQR)	V1 vs V3 p values
HAUS2	0.2 (-0.4 — 1.4)	>0.9
MPDU1	0.9 (-3.0 — 5.3)	>0.8
JUNB	978.1 (122.8 — 1475.8)	**<0.05**
DUSP2	203.5 (-11.1 — 349.7)	**0.08**
IFNAR1	2.9 (-7.9 — 8.9)	>0.9
NUDC	95.3 (-58.2 — 37.1)	>0.8
ZNF830	-8.4 (-12.8 — 14.6)	>0.9

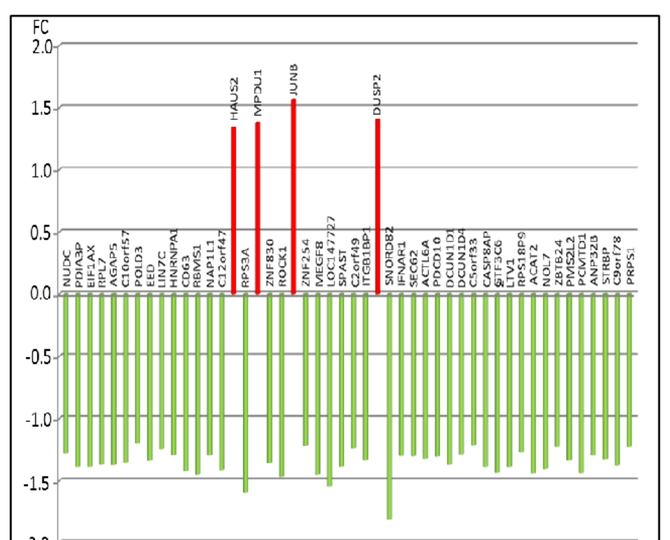

Figure 3: GEP in T1D under VD therapy.
The FC of each gene is shown. Forty-eight annotated genes changed significantly in Th cells from patients with T1D after VD treatment. Important to note, only four genes were upregulated (red columns) whereas forty-four genes were down regulated (green columns).

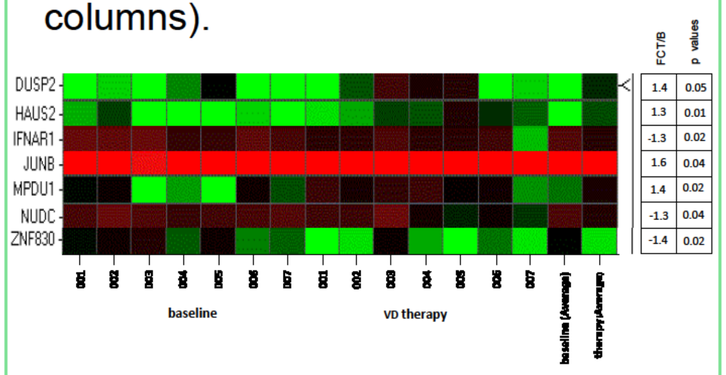

Figure 4: Heatmap of seven selected genes.
Four genes; *DUSP2* (FC: 1.4;p = 0.05), *HAUS2* (FC: 1.3; p = 0.01), *JUNB* (FC: 1.6; p = 0.04) and *MPDU1* (FC: 1.4;p = 0.02) showed a higher expression after VD treatment in T1D patients. In contrast genes which code for *IFNAR1* (FC: -1.3;p = 0.02), *NUDC* (FC: -1.3;p = 0.04)) and *ZNF830* (FC: -1.4; p = 0.02) showed a lower expression.

Conclusions

The elevation of $25(OH)D_3$ induced by Vigantol therapy (4000 IU/day) leads to differential gene expression pattern in Th cells from T1D patients (four genes upregulated/forty-four genes down regulated). The Th cell response to vitamin D results in an upregulated gene set (*DUSP2, HAUS2, JUNB, MPDU1*) and a downregulated gene set (*IFNAR1, NUDC and ZNF830*). Our data as validated by RT-PCR suggest an indirect VD effect particularly on the upregulated gene set (*DUSP2* and *JUNB)* probably via activation of transcription factor such as activator protein 1 (AP-1). These data can help to explain how vitamin D treatment can tip a balance from a pro- to an anti-inflammatory cellular environment.

References

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