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Vitamin D3 administration to MS patients leads to increased serum levels of latency activated peptide (LAP) of TGF-beta

Julia Åivo [a,*], Arno Hänninen [b], Jorma Ilonen [c], Merja Soilu-Hänninen [a]

[a] Turku University Hospital, Division of Clinical Neurosciences, University of Turku, Turku, Finland
[b] University of Turku, Department of Clinical Microbiology and Immunology, Turku, Finland
[c] Immunogenetics Laboratory, University of Turku, Turku, Finland

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ABSTRACT

Background: Deficiency of vitamin D is an environmental risk factor for MS. Vitamin D has immunomodulatory effects, including promotion of T-cell differentiation into T-regulatory cells, which produces regulatory cytokines including TGF-β. Increasing serum vitamin D levels have been associated with decreased disease activity in MS patients, but there are only few studies concerning the immunological effects of vitamin D supplementation in MS. In this study we investigated the effect of weekly supplementation of vitamin D3 or placebo on serum levels of multiple cytokines in patients with relapsing remitting MS.
Methods: The study was conducted on the patient cohort of the Finnish Vitamin D study. All patients were using IFN-beta-1b and were randomized to add-on treatment with either cholecalciferol 20,000 IU/week or placebo. Concentrations of LAP (TGF-β), INF-γ, IL-17A, IL-2, IL-10, IL-9, IL-22, IL-6, IL-13, IL-4, IL-5, IL-1β and TNF-α were determined at screening and at 12 months using commercial fluorescent bead immunoassay kits.
Results: LAP (TGF-β) levels increased significantly in the vitamin D treated group from a mean of 47 (SE 11) pg/ml to 55 (SE 14) pg/ml in 12 months (p-value = 0.0249). Placebo treatment had no significant effect on LAP levels. The levels of the other cytokines did not change significantly in either group.
Conclusions: We showed increased serum latency activated peptide (LAP) of TGF-β levels in MS patients treated with vitamin D3. The immune regulatory effects of TGF-beta may play a role in the improved MRI outcomes that we observed earlier in the vitamin D treated group of patients.

1. Introduction

Several lines of evidence have linked poor vitamin D status to increased risk of developing MS (Ascherio et al., 2010). Studies concerning vitamin D3 insufficiency and MS disease activity are more limited and interventional studies have not been powered to meet clinical endpoints. However, it has been shown that patients with MS have lower vitamin D levels during relapse than in remission (Smolders et al., 2008a; Soilu-Hänninen et al., 2008) and lower vitamin D levels are associated with higher relapse risk in MS (Simpson et al., 2010; Runia et al., 2012). A prospective study has suggested an inverse relationship with increasing vitamin D levels and new MRI lesions (Mowry et al., 2012). In a RCT focused on safety and radiological endpoints, we previously showed that vitamin D supplementation at a weekly dose of 20,000 IU significantly decreased gadolinium enhancing lesions on brain MRI compared to placebo (Åivo et al., 2012; Soilu-Hänninen et al., 2012). In a recent Italian study in patients with CIS, low levels of vitamin D were associated with increased MS risk (Martinelli et al., 2014). The strongest evidence so far linking vitamin D to MS activity and progression was a recently published analysis of vitamin D levels as early predictors of MS activity and progression in the BENEFIT study patient cohort (Ascherio et al., 2014).

Immunomodulatory effects of vitamin D are mediated through the vitamin D receptor. $1,25(OH)_2D$, the active metabolite of vitamin D, inhibits proliferation of monocytes into dendritic cells (D'Ambrosio et al., 1998; Adorini et al., 2004) and also inhibits $CD4^+$ T cell proliferation and inflammatory cytokine production in vitro (Correale et al., 2009). In a cross-sectional study vitamin D levels were shown to correlate with the suppressive ability of T-regulatory cells (Smolders et al., 2009).

In healthy volunteers vitamin D supplementation at a dose of 5000–10,000 IU/day was associated with increased IL-10 production by PBMC and reduced frequency of Th17 cells (Allen et al., 2012). In 437 overweight subjects, vitamin D 400,000 or 20,000 IU weekly did not significantly affect serum levels of cytokines IL-2, IL-4, IL-5, IL-10, IL-12, IL-13 IL-17, and IFN-γ, monocyte chemotactic protein or high sensitivity C-reactive protein (Jorde et al., 2010).

There are only few studies of the effect of vitamin D supplementation on immune responses in patients with MS. Vitamin D supplementation at a dose of 1000 IU/day significantly increased levels of serum TGF-beta-1 compared with placebo but had no effect on serum levels

* Corresponding author at: Turku University Hospital, Division of Clinical Neurosciences, Kiinamyllynkatu 2-8, PL 20520 Turku, Finland.
E-mail address: julia.aivo@tyks.fi (J. Åivo).

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of TNF-α, IFN-γ and IL-13 (Mahon et al., 2003). Patients treated with vitamin D dose escalated up to 40,000 IU/day had reduction on T cell proliferation compared to controls (Burton et al., 2010). 12 week supplementation with high dose vitamin D3 increased the circulating proportion of IL-10$^+$ CD4-T cells and decreased the ratio of Th1/Th2 cells (Smolders et al., 2010).

In this study we investigated the effect of weekly supplementation with vitamin D3 or matching placebo on the serum levels of LAP (TGF-β), IFN-γ, IL-17A, IL-2, IL-10, IL-9, IL-22, IL-6, IL-13, IL-4, IL-5, IL-1β and TNF-α in MS patients using IFN-beta-1b therapy.

2. Materials and methods

2.1. Patients

This study was conducted on a cohort of patients who participated in the Finnish Vitamin D Study (cholecalciferol as add-on treatment to subcutaneously-administered interferon beta 1-b for the treatment of MS), a double-blind, randomized, parallel group one-year multicenter trial in relapsing remitting MS (RRMS) patients. The design and the results of the study have been reported previously (Åivo et al., 2012; Soilu-Hänninen et al., 2012). All patients gave written informed consent separately to the immunogenetic substudy described in this paper. Ethical approval was obtained from the joint ethics committee of Turku University and Turku University Hospital.

2.2. Cytokine analysis

Serum samples were obtained at screening and at 1, 2, 3, 6, 9 and 12 months. Samples were freshly frozen and kept in −70°. For this immunogenetic study, we used samples taken at screening and at 12 months. Concentrations of IFN-γ, IL-17A, IL-2, IL-10, IL-9, IL-22, IL-6, IL-13, IL-4, IL-5, IL-1β and TNF-α were determined using commercial fluorescent bead immunoassay kit (Human Th1/Th2/Th9/Th17/Th22 13 plex Kit FlowCytomix BMS817FF, eBioscience, USA) and concentrations of LAP (TGF-β) by fluorescent bead immunoassay kit (Human LAP FlowCytomix Simplex Kit, eBioscience, USA). The sensitivities were: LAP 0.69 ng/ml, IFN-γ 1.6 pg/ml, IL-17A 2.5 pg/ml, IL-2 16.4 pg/ml, IL-10 1.9 pg/ml, IL-9 1.5 pg/ml, IL-22 43.3 pg/ml, IL-6 1.2 pg/ml, IL-13 4.5 pg/ml, IL-4 20.8 pg/ml, IL-5 1.6 pg/ml, IL-1β 4.2 pg/ml and TNF-α 1.6 pg/ml. All cytokine analyses were performed at the Department of Clinical Microbiology and Immunology, University of Turku, Turku, Finland.

2.3. HLA-typing

HLA typing of major DR–DQ haplotypes was performed in the Immunogenetics Laboratory of the University of Turku with a PCR-based, lanthanide-labeled hybridization method using time-resolved fluorometry for detection, as described previously (Hermann et al., 2003).

2.4. Statistical analysis

Statistical analysis was done using SAS version 10.0. Changes in cytokine concentrations were evaluated using paired t-testing. p-Values less than 0.05 were considered significant.

3. Results

The patient characteristics, 25(OH)D levels and HLA-typing results are shown in Table 1. Serum levels of 25(OH)D increased from a mean of 54 (range 19–82) nmol/l to 109 (range 67–163) nmol/l in the vitamin D3 treatment group at 12 months. In the placebo group mean serum 25(OH)D was 55 (range 16–81) nmol/l at screening and 51 (range 17–94) nmol/l at 12 months. Using vitamin D supplement was not allowed in the study design. There was no difference in the number of smokers between the treatment groups. Groups did not differ significantly by DQB1*0602 status. None of the patients had obtained corticosteroids within 30 days prior to the serum sampling. The percentage of patients reaching a serum level of 100 nmol/l of 25(OH)D was 66% in the vitamin D treated group and 0% in the placebo group at 12 months. There was no hypercalcemia in the vitamin D3 treated patients nor significant difference in any other clinical chemistry parameters between the treatment arms. The percentages of patients with adverse event including upper respiratory tract infections and other mild infections did not differ between the treatment and the placebo arms. The clinical and MRI results of the Finnish Vitamin D Study have been published previously (Åivo et al., 2012; Soilu-Hänninen et al., 2012),

Table 1
Patient characteristics.

	Vitamin D	Placebo
Number of patients	30	29
Sex F/M	18/12	19/10
Age (median, range)	38 (22–53)	35 (24–53)
DQB1*0602 positive/negative	20/10	13/15
BMI (median, range)	24 (18–40)	24 (19–38)
EDSS (median, range)	2.0 (0–4.5)	1.5 (0–4)
Disease duration (years, median, range)	3 (0.5–21)	2 (0.2–15)
ARR (median, 95% CI)	0.5 (0.3–0.6)	0.5 (0.3–0.7)
Vitamin D baseline (mean, range)	54 (19–82)	55 (16–81)
Vitamin D month 12 (nmol/l, mean, range)	109 (67–163)	51 (17–94)

3.1. Serum cytokine levels

Serum levels of LAP (TGF-β) increased significantly in the vitamin D treated group from a mean of 47 (SE 11) pg/ml to 55 (SE 14) pg/ml in 12 months ($p = 0.0249$). In the placebo group, serum levels of LAP increased but this increase did not reach statistical significance ($p = 0.173$) The levels of IFN-γ, IL-17A, IL-2, IL-10, IL-9, IL-22, IL-6, IL-13, IL-4, IL-5, IL-1β and TNF-α did not change statistically significantly in either group (Table 2). There were notable, although statistically non-significant elevations in the serum levels of IFN-gamma ($p = 0.0519$), IL-17A ($p = 0.0666$) and in IL-9 ($p = 0.0679$) in the vitamin D treated patients.

We assessed changes in cytokine levels also in DQB1*0602 positive and negative subgroups within vitamin D3 and placebo treatment arms. The levels of LAP, IFN-γ, IL-17A, IL-2, IL-10, IL-9, IL-22, IL-6, IL-13, IL-4, IL-5, IL-1β and TNF-α did not change statistically significantly in any of these subgroups but there was a trend in LAP in DQB1*0602 positive subgroup of vitamin D3 treated patients, whose LAP levels increased from 45 (SE 15) pg/ml to 55 (SE 19) pg/ml ($p = 0.0512$).

4. Discussion

In addition to its important role in bone mineralization, vitamin D controls the growth and metabolism of many cell types. Vitamin D receptors (VDRs) are nuclear receptors which exist in many cell types, including lymphocytes and dendritic cells (Adams and Hewison, 2008). By binding the VDR in complex with the active vitamin D metabolite 1,25(OH_2)D3 to chromosomal regions regulating gene expression, vitamin D has diverse effects on cellular functions such as proliferation, differentiation, and survival (Jurutka et al., 2001). One important target of vitamin D is nuclear factor kappa B (NFκB), which is inhibited by vitamin D (Adorini et al., 2004). In the immune system, NFκB is the gene target of cytokine and pattern receptor signaling. It promotes the production of proinflammatory cytokines in dendritic cells, and activates T-cell differentiation into effector T-cells (DiDonato et al., 2012). Vitamin D has been shown to inhibit Th17 and Th1 differentiation directly (Cippitelli and Santoni, 1998; Takeuchi et al., 1998; Joshi et al., 2011; Ikeda et al., 2013), and to promote the induction of Foxp3 regulatory (Treg) T cells (Urry et al, 2012). Due to their VDR expression, T cells