

RESEARCH

Open Access

Predominance of Th2 polarization by Vitamin D through a STAT6-dependent mechanism

Scott Sloka, Claudia Silva, Jianxiong Wang and V Wee Yong*

Abstract

Background: Vitamin D has several reported immunomodulatory properties including the reduced generation of pro-inflammatory CD4+ T helper 1 (Th1) cells and the increase in levels of the anti-inflammatory Th2 subset. Less clear has been the impact of vitamin D on the pro-inflammatory Th17 subset, and whether and how vitamin D may preferentially drive the polarization of one of the T helper subsets.

Methods: Using human peripheral blood-derived mononuclear cells and mouse splenocytes and lymph node cells in culture, we examined whether and how vitamin D preferentially skews T cells towards the Th1, Th2 or Th17 subsets. Mice afflicted with the multiple sclerosis-like condition, experimental autoimmune encephalomyelitis (EAE), were examined in vivo for the relevance of the tissue culture-derived results.

Results: We report that the biologically active form of vitamin D, 1,25-dihydroxyvitamin D₃ {1,25(OH)₂D₃}, consistently generates human and murine Th2 cells in culture, frequently leaving unchanged the levels of Th1/Th17 cytokines. As a result, the ratio of Th2 to Th1 and Th17 is increased by 1,25(OH)₂D₃. The upregulation of Th2 to Th1 or Th17 subsets by 1,25(OH)₂D₃ is enabled by an increase of the GATA-3 transcription factor, which itself is promoted upstream by an elevation of the STAT6 transcription factor. In mice, the alleviation of EAE severity by 1,25(OH)₂D₃ is accompanied by elevation of levels of GATA-3 and STAT6. Significantly, the efficacy of 1,25(OH)₂D₃ in ameliorating EAE is completely lost in mice genetically deficient for STAT6, which was accompanied by the inability of 1,25(OH)₂D₃ to raise GATA-3 in STAT6 null lymphocytes.

Conclusions: These results of vitamin D promoting a Th2 shift through upstream GATA-3 and STAT6 transcription factors shed mechanistic understanding on the utility of vitamin D in MS.

Background

Multiple sclerosis (MS) is an inflammatory and neurodegenerative disorder with widespread demyelination and axonal loss within the central nervous system (CNS). The underlying etiology remains undefined although both environmental and genetic factors play a role [1,2], resulting in the over-activation of various immune subsets that accumulate in the CNS to produce injury. Familial inheritance, cigarette smoking, viral infection, and ultraviolet (UV) light exposure may all contribute to the risk of MS [1,3].

Vitamin D deficiency has previously [4] and recently been suggested as another contributing factor in the pathogenesis of MS [5-7]. Several studies have reported an inverse association of sunlight exposure, available UV radiation and MS prevalence [5,8,9], implicating vitamin

D since UV B radiation (280 to 315 nm) converts 7-dehydrocholesterol to previtamin D₃ in the epidermal and dermal layers in humans; previtamin D₃ is then converted by a thermal process to vitamin D₃ [10]. Due to the changing angle of declination of the sun, vitamin D insufficiency is common in the winter months in latitudes north of 42 °N latitude [11]. Therefore, vitamin D is of interest as the biological correlate of available UV radiation, although it has been proposed that other factors could also be involved [12].

In humans, vitamin D₃ undergoes hydroxylation in the liver to produce 25-hydroxyvitamin D₃ {25(OH)D₃}, the main circulating form of vitamin D. 25(OH)D₃ can be further hydroxylated in the liver to 24,25-dihydroxyvitamin D₃, or in the kidney to the immunologically active form of vitamin D, 1,25 dihydroxyvitamin D₃ {1,25(OH)₂D₃} [10,13]. Many publications {reviewed in [13-15]} have reported extensively on the immunomodulatory

* Correspondence: vyong@ucalgary.ca

Hotchkiss Brain Institute and the Department of Clinical Neurosciences
University of Calgary, Calgary, Alberta, Canada

properties of 1,25(OH)₂D₃. In particular, 1,25(OH)₂D₃ decreases T cell proliferation, increases the activity and frequency of regulatory T cells, alters the production of specific antibody isotypes, reduces activity of dendritic cells or makes them tolerogenic, and affects tissue-specific lymphocyte homing.

Naïve CD4-positive T helper (Th) cells can differentiate into either pro-inflammatory Th1 and Th17 subsets, or into Th2 subset with anti-inflammatory or regulatory activity [16,17]. Vitamin D has been found to elevate Th2 cytokines [18,19] and to reduce Th1 cytokine levels [20,21]; however, others have also found vitamin D to inhibit Th1 levels without affecting Th2 deviation [22], or to reduce EAE disease severity without altering Th1 or Th2 levels [23]. These results emphasize that there needs to be clarity on the activity and mechanism of vitamin D in CD4 Th1/Th2 differentiation. The literature on vitamin D and Th17 cells is still emerging, and vitamin D has been reported to reduce the level of Th17 cytokines in human studies [24], and to decrease Th17 cells in mice with colitis [25] or EAE [26].

Given the uncertain nature of the impact of vitamin D on T cell subsets, and the recent analysis in genetically altered or chimeric mice of the requirement of T cell expression of vitamin D receptors for amelioration of EAE [27], we have addressed the relationship between vitamin D and Th subsets, focusing on whether 1,25(OH)₂D₃ acts predominantly through altering one of the Th subsets, and of the attendant mechanisms. We first analysed human and mouse T cells in culture, and then extended to EAE studies. We elucidated a central role for STAT6 in regulating the vitamin D-polarization of Th2 cells to alleviate disease activity.

Methods

Isolation of T Cells

Human peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy adult volunteers by Ficoll-Hypaque centrifugation [28]. The PBMCs were washed once with phosphate-buffered saline (PBS) and suspended in serum-free AIM-V medium (Invitrogen Life Technologies, Burlington, Ontario). To activate T cells in the PBMC populations, 96 well round-bottomed plates were coated with 10 or 1000 ng/mL of purified mouse anti-human CD3 (BD Pharmingen, Franklin Lakes, NJ) for a period of 3 h. From previous experiments (data not shown), the coating at 1000 ng/mL of anti-CD3 gives maximal activation of T cells measured by proliferation assays. Since the *in vivo* environment in humans is unlikely to lead to the maximal activation of T cells, a submaximal level of activation with 10 ng/ml anti-CD3 was also used in most experiments as both a comparison to maximal activation and to better reflect physiology. This submaximal level of activation may also permit the more sensitive

measurement of experimental changes that affect T cell activation.

Human PBMCs were plated at a density of one million cells/mL of anti-CD3 coated 96 well plates (200 μL/well, 200,000 cells per well). An additional 10 ng/mL of anti-CD28 (BD Pharmingen) was added as a suspension to all cultures, and cells were left for 3 days at 37°C in a 5% humidified CO₂ incubator. In order to promote measurable levels of IL-17, some anti-CD3/CD28 activated cultures were further exposed to IL-23 (20 ng/mL) and IL-1β (10 ng/mL) [29]. Specified sister cultures were further exposed to either 0.1, 1 or 10 nM of 1,25(OH)₂D₃ (Bio-Mol, Plymouth Meeting, PA). In some experiments, certain PBMC preparations did not receive anti-CD3 or 1,25(OH)₂D₃, and the floating cells collected 3 days thereafter are referred to as unactivated T cells.

Flow cytometry analyses of the floating cells collected after 3 days of the initiation of anti-CD3 treatment indicated that CD3⁺ T cells constituted approximately 90% of the total cell population (data not shown). Of the CD3 cells, 60% were CD4⁺ and 40% were CD8⁺. For the remaining, approximately 8% were CD56⁺ natural killer cells, approximately 2% were CD19⁺ B lymphocytes, and less than 1% were CD14⁺ monocytes. There was no significant difference in the proportion of the various cell subsets between the unactivated, 10 and 1000 ng/mL anti-CD3 activated PBMC populations (data not shown). Since the majority of cells were T cells, henceforth this human culture population is referred to as T cells.

Quantitative Real-Time polymerase chain reaction (qPCR)

For qPCR, T cell RNA was extracted using the RNeasy Mini Kit columns (Qiagen, Mississauga, ON). DNase treatment (M610A) was performed according to the manufacturer's instructions (Promega, Madison, WI). Total RNA extracted was reverse transcribed using Superscript II (Invitrogen). Resulting cDNA was subjected to real-time quantitative PCR using an iCycler (BioRad). Transcripts were quantified by real-time quantitative PCR on the iCycler using RT2 Real Time SYBR Green/Fluorescein PCR Master Mix (SA Biosciences, Frederick, MA). mRNA expression for each gene was calculated using a comparative cycle threshold method, and was normalized to the amount of the reference gene 18S rRNA (expressed as arbitrary units). All PCR primers were purchased from SA Biosciences (18S, PPH05666E; IFN γ , PPH00380B; IL-5, PPH00692A; IL-17, PPH00537B; TBX21/T-bet, PPH00396A/PPM03727A; GATA-3, PPH02143A/PPM05199A; RORC/ROR γ T, PPH05877A/PPM25095A; STAT6, PPH00760B; Notch 1, PPM04747A).

ELISA

Cytokine production by human and mouse PBMCs was assessed after activation of cells for 72 h. Cytokines in

culture supernatants were measured by ELISA according to the manufacturer's protocol. ELISA kits were purchased from Invitrogen. The IL-17 was of the IL-17F form. Data were analysed using a SpectraMax 384 (Molecular Devices Corporation, Sunnyvale, CA) according to the manufacturer's instructions.

Disease induction in mice and EAE analysis

EAE was induced in female C57BL/6 mice (Jackson Laboratories, Bar Harbor, Maine), aged 8-9 weeks, by injecting subcutaneously (s.c.) 50 µg myelin oligodendrocyte glycoprotein (MOG)₃₅₋₅₅ in Complete Freund's Adjuvant (CFA) (Fisher, Michigan USA) supplemented with 4 mg/ml of Mycobacterium tuberculosis on day 0 [30,31]. Intraperitoneal (i.p.) pertussis toxin (0.1 µg/200 µl, List Biological labs, Hornby, ON) was administered on days 0 and 2. 1,25(OH)2D3 (100 ng) was given every other day i.p. in 50 µL of DMSO, while 50 µL of DMSO was used as the vehicle control; this method of administering 1,25(OH)2D3 to mice has been reported by others [18,19,26,32,33]. Treatment was initiated at the time of MOG immunization. In addition to the wildtype mice, STAT6 $-/-$ knockout (KO) mice on the C57BL/6 background (Jackson Laboratories, Bar Harbour, Maine) were utilized. Animals were assessed daily using a 15-point disease score scale [30,31] replacing the more commonly used 5-point scale since the 15-point scale differentiates individual limb disability, rather than grouping both fore- or hind-limbs together. This allows for a more sensitive characterization of disease progression. The 15-point scale is the sum of the disease state for the tail (scored from 0-2) and all 4 limbs (each limb is scored from 0-3). All animals were handled in accordance with the policies outlined by the Canadian Council for Animal Care and the University of Calgary.

Statistical Analysis

Statistical analysis was performed using R version 2.8.1 (The R Foundation for Statistical Computing) and Matlab version 7.7 (The Mathworks, Natick, MA, USA). Statistical differences for cells in culture were addressed using ANOVA with Bonferroni correction for multiple comparisons. Statistical differences between groups of mice in the EAE experiments were evaluated using a nonparametric analysis Mann-Whitney *U* test. An alpha of 0.05 was selected for statistical significance.

Results

1,25(OH)2D3 differentially shifts human T cells in favor of Th2

We measured the changes in cytokine profile of T cells cultured with 1,25(OH)2D3. Supernatants from human T cells stimulated for 3 days with either 10 or 1000 ng/mL of anti-CD3, to reflect low and maximal activation of T cells, were analysed by ELISA for protein content of

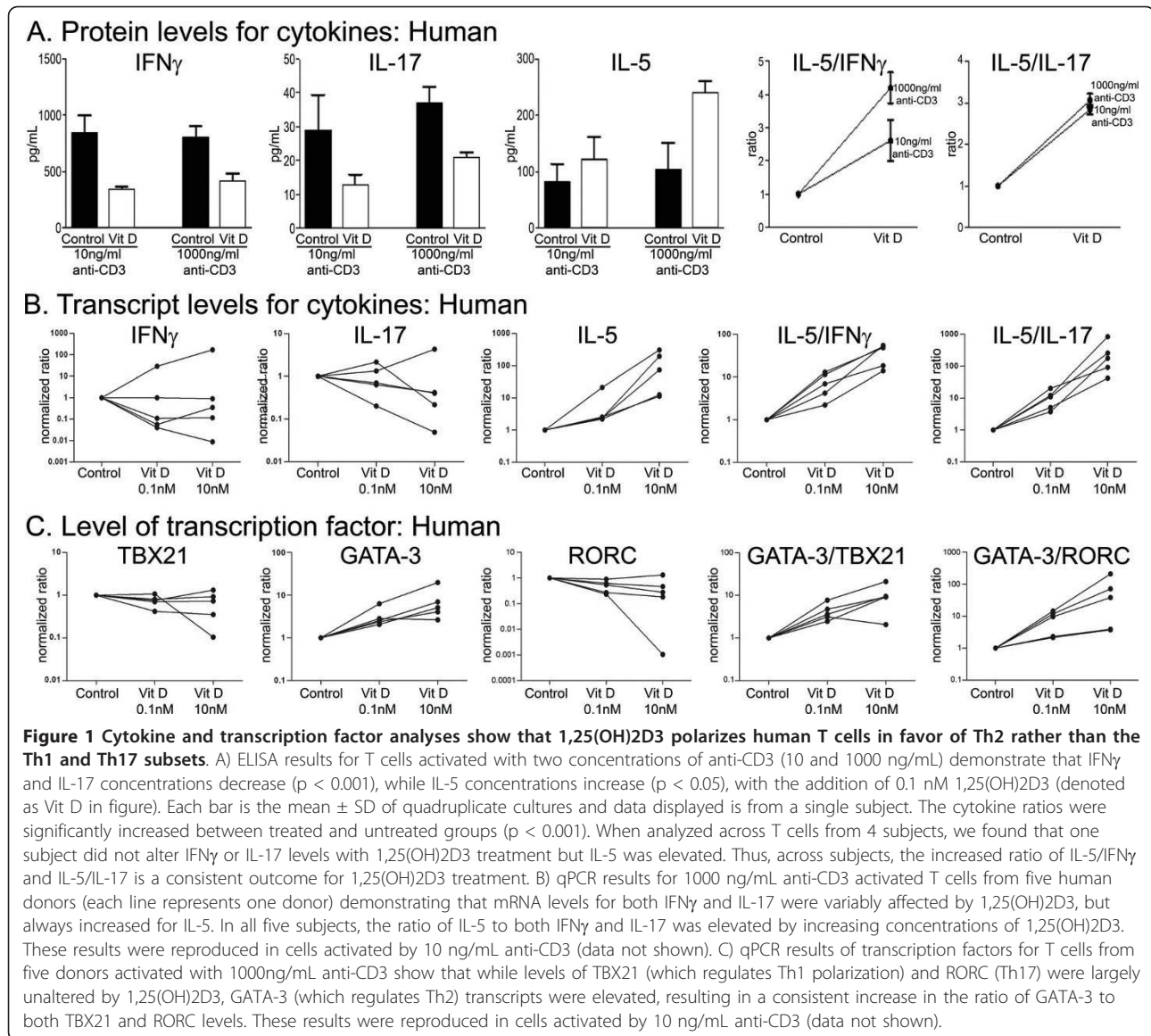
representative cytokines: interferon- γ (IFN γ) for Th1, interleukin (IL)-5 for Th2, and IL-17 for Th17 cells. Figure 1A shows that 1,25(OH)2D3 decreases IFN γ and IL-17 for both levels of anti-CD3 stimulation while increasing IL-5, indicating an elevation of Th2 to Th1/17 cells.

To examine the individual human response to 1,25(OH)2D3 more thoroughly, we next used qPCR of T cell samples cultured from several individuals for 3 days at 10 and 1000 ng/mL (1000ng/mL shown) of anti-CD3 as above. Figure 1B shows that the levels of IFN γ and IL-17 mRNA tended to be inconsistent and to change variably with 1,25(OH)2D3 treatment from one subject to the next; thus, levels of IFN γ and IL-17 mRNA may be increased, decreased, or unaltered for increasing concentrations of 1,25(OH)2D3. In contrast, IL-5 production consistently increased with 1,25(OH)2D3 exposure relative to no treatment, leading to a consistent elevation of the ratio of IL-5 to either IFN γ or IL-17 across T cell samples from the multiple subjects tested (Figure 1B). The mRNA results thus verified the polarization of CD4+ T cell subsets that was suggested by the ELISA assays of cytokines, with 1,25(OH)2D3 treatment consistently favoring Th2 anti-inflammatory over pro-inflammatory Th1 and Th17 subsets.

Specific transcription factors drive the formation of the Th subsets and the mRNA generated above for cytokine analyses were subjected to the levels of TBX21 for Th1, GATA-3 for Th2, and RORC for Th17 subsets. Similar to the above cytokine mRNA results, 1,25(OH)2D3 treatment reproducibly elevated the level of GATA-3 transcription factor mRNA across subjects, while producing variable responses for TBX21 and RORC mRNA levels amongst subjects (Figure 1C). When expressed as a ratio for both levels of anti-CD3 activation, 1,25(OH)2D3 produced an elevation of GATA-3 compared to TBX21 and RORC across all subjects analysed. Thus, the overall effect of 1,25(OH)2D3 on Th polarization could be attributed to its predominance of driving the Th2 subset.

Murine cells respond similarly to human cells

We tested the response of murine T cells to 1,25(OH)2D3 in culture in order to allow subsequent transition to animal models. Mononuclear cells were isolated from mouse spleens and lymph nodes and were cultured for 3 days while being unactivated or stimulated with either 10, 100 or 1000 ng/mL of anti-CD3. We found that mouse IL-17 protein was difficult to detect reliably by ELISAs. As with the human ELISA results, murine T cells produce increasing concentrations of IL-5, but had reduced concentrations of IFN γ (Figure 2A), in response to 1,25(OH)2D3. The ratio of IL-5 to IFN γ protein was thus increased with 1,25(OH)2D3 treatment (Figure 2A).



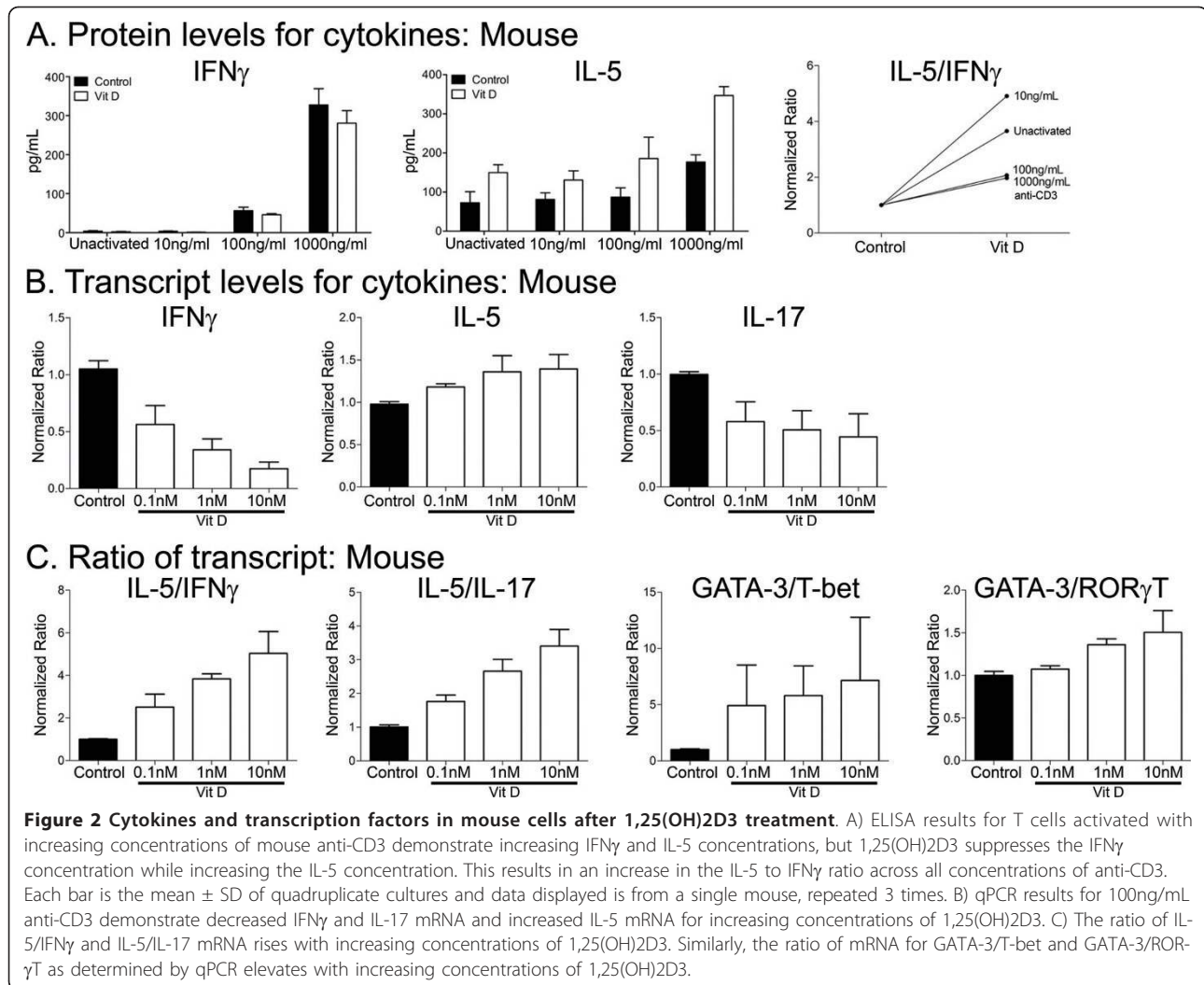
RT-qPCR was next performed on murine T cells stimulated with 1000 ng/mL anti-CD3. IL-5 and GATA-3 transcripts were consistently elevated with increasing 1,25(OH)2D3 concentrations while IFN γ and IL-17 tended to be decreased. The ratios of IL-5 to IFN γ and IL-17 were thus upregulated with increasing concentrations of 1,25(OH)2D3, as were the ratios of GATA-3 to T-bet (the mouse equivalent of TBX21) and ROR γ T (the mouse equivalent of RORC) (Figure 2B), confirming a similar response in mouse and human cells.

STAT6 but not Notch1 is elevated in human PBMCs with increasing 1,25(OH)2D3 concentrations

Since both IL-5 and GATA-3 were consistently elevated with increasing 1,25(OH)2D3 concentrations, we explored upstream factors that affect GATA-3 levels in

human cells. Several factors have been reported to increase GATA-3 expression [34,35], including Notch1 and STAT6.

Human PBMCs stimulated with 10 ng/ml anti-CD3 were treated with 1nM of 1,25(OH)2D3 or left as untreated controls. After 3 days, RNA was harvested and analyzed using RT-qPCR. Figure 3A shows that the ratio of STAT6 to TBX21 transcripts increased with 1,25(OH)2D3 treatment, similar to the ratio of GATA-3 to TBX21, while the ratio of Notch1 to TBX21 remained constant. These results indicate that 1,25(OH)2D3 likely acts through STAT6 to increase GATA-3 levels, with no significant contribution through Notch1. Similar results are noted for cells activated with both 10 and 1000ng/mL of anti-CD3, and in the culture of murine PBMCs (data not shown).



1,25(OH)2D3 treatment of EAE-afflicted mice raises STAT6

We induced C57BL/6 wildtype mice for EAE and examined the amounts of GATA-3 and its potential regulators, STAT6 and Notch1, in the spleen and lymph nodes (Figure 3B) at peak clinical disease. To normalize across samples and for ease of comparisons, we expressed the results as a ratio to T-bet. We compared these data between EAE mice treated with 1,25(OH)2D3 or vehicle.

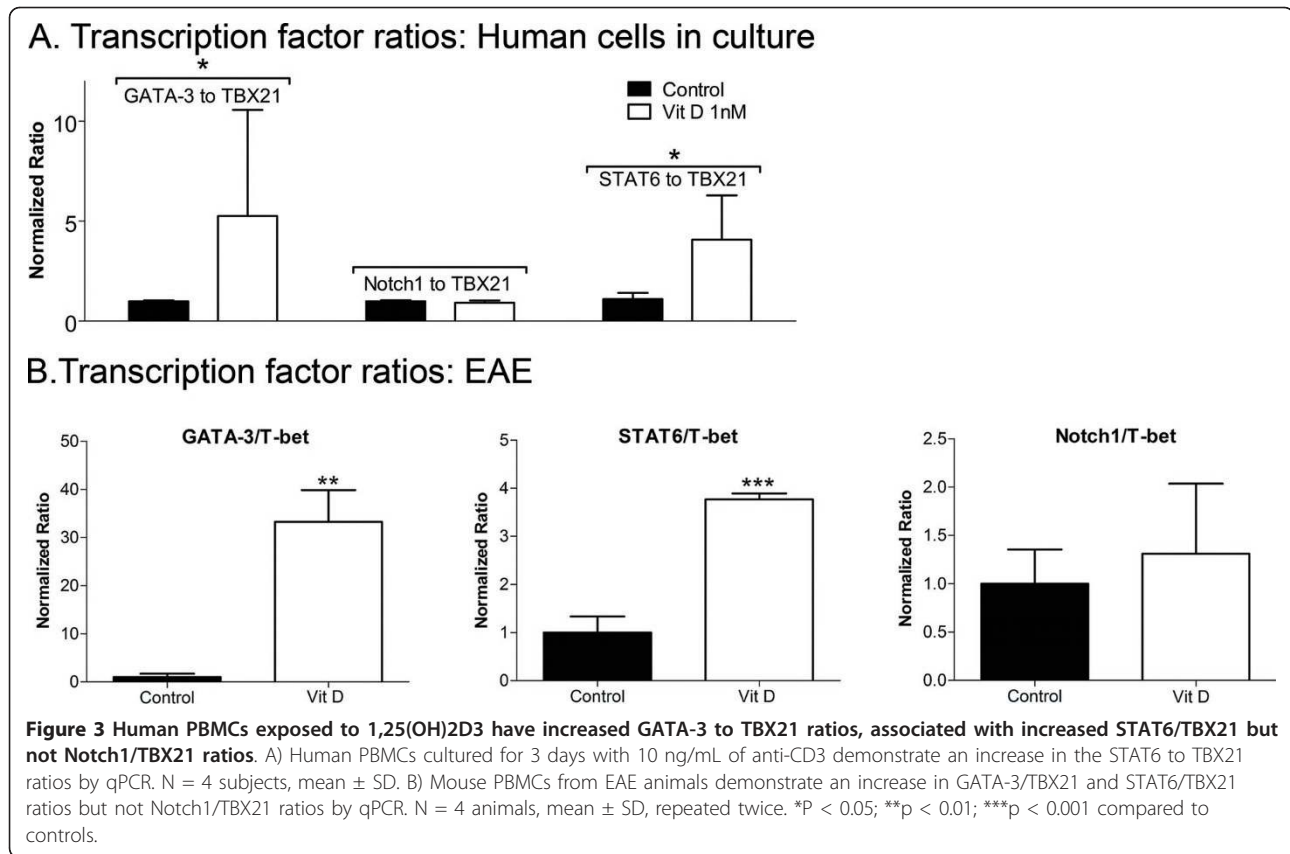
EAE has previously been shown to be largely abrogated with the treatment of 1,25(OH)2D3 [18,19,32,33,36,37]. We have reproduced this finding, and report here that the treatment every other day with 1,25(OH)2D3 essentially prevented C57BL/6 wildtype mice from succumbing to EAE signs (Figure 4A). At termination of the clinical scoring, spleens and lymph nodes were removed and RNA was isolated for qPCR. Figure 3B shows that relative to the vehicle treated EAE mice, the level of transcripts encoding GATA-3 to T-bet was high in 1,25(OH)2D3-

treated wildtype mice. As well, levels of STAT6 but not NOTCH1 were elevated by 1,25(OH)2D3 treatment.

STAT6 is required for the therapeutic effects of 1,25(OH)2D3 on EAE

Given that the upregulation of Th2-associated cytokine and transcription factor is paralleled by an increase of STAT6, we addressed whether STAT6 influences the therapeutic effect of 1,25(OH)2D3 in EAE. STAT6 knockout (KO) mice and wildtype controls were induced for EAE, and both succumbed to EAE. While there was a clear separation between the 1,25(OH)2D3-treated animals compared with the control animals in the wildtype groups (Figure 4A), the therapeutic effect of 1,25(OH)2D3 was lost when mice were without STAT6 (Figure 4B). These results emphasize that STAT6 is necessary for 1,25(OH)2D3 to alleviate EAE.

Mice were killed at the conclusion of the EAE clinical scoring above, and their spleens and lymph nodes were



extracted for RNA and analysed by RT-qPCR without further manipulation of tissue. While wildtype mice treated with 1,25(OH)2D3 clearly upregulated GATA-3 transcripts relative to T-bet, STAT6 KO mice did not have this response (Figure 5A), further substantiating the requirement of STAT6 for inducing an elevation of GATA-3 transcripts.

At sacrifice of the mice in Figure 4, lymphocytes were isolated and cultured for 3 days. Figure 5B shows that in wildtype cells from EAE mice previously treated in vivo with vehicle, the ex vivo treatment with 1,25(OH)2D3, with or without MOG restimulation, increased the GATA-3 to T-bet ratio. Significantly, the increase of GATA-3 to T-bet that occurred in wildtype mice did not occur in cells from the STAT6 KO mice (Figure 5C).

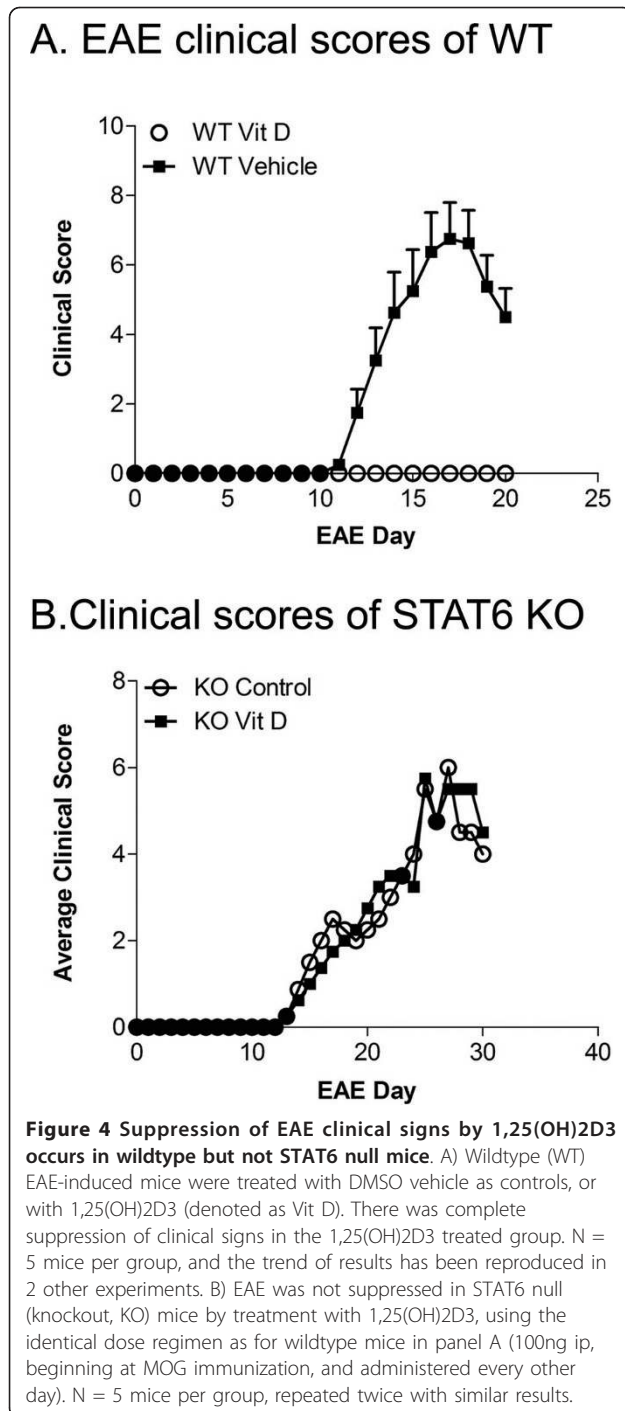
Overall, these results demonstrate that 1,25(OH)2D3 loses its therapeutic efficacy in EAE when STAT6 is absent, and this corresponds with the inability of STAT6 null cells to elevate GATA3 levels in response to 1,25(OH)2D3.

Discussion

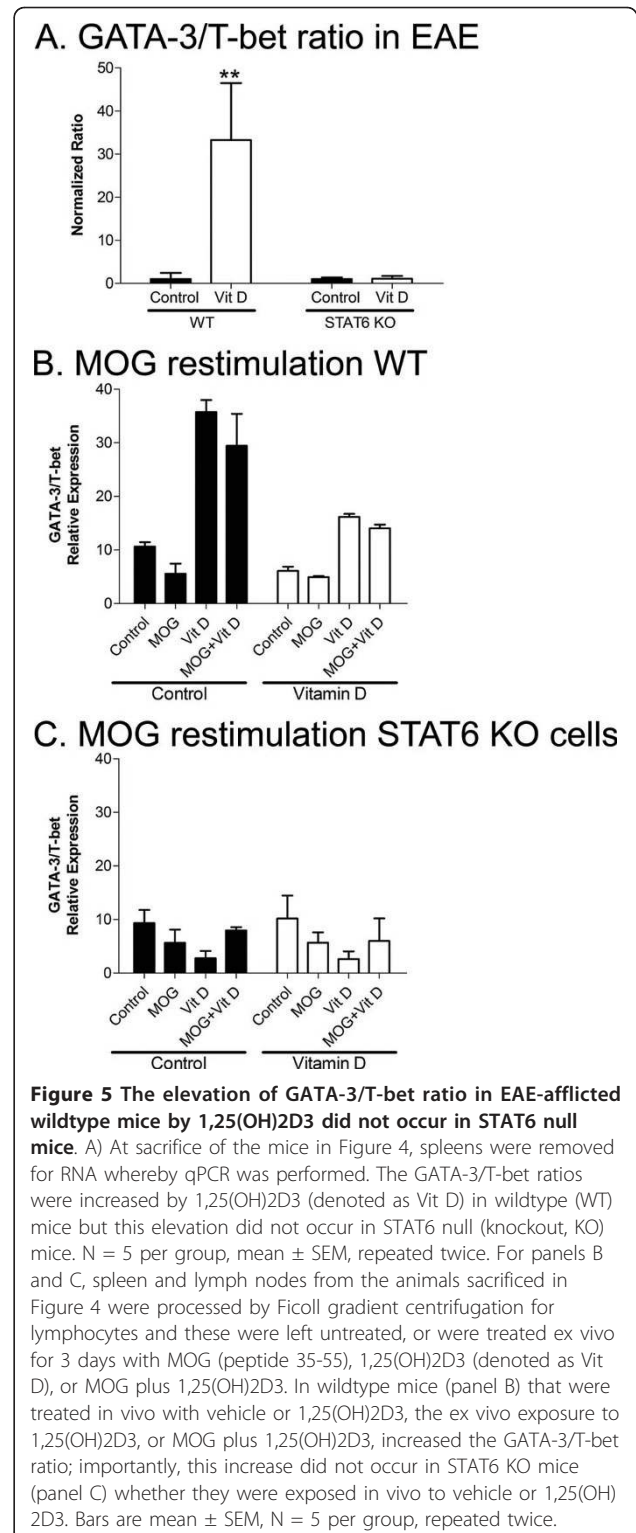
Evidence supporting the involvement of vitamin D in the risk of MS include an inverse correlation between MS prevalence and latitude that has been consistently

observed [8,9,38], strongly suggesting a latitudinally-related environmental contribution to etiology. Available ultraviolet radiation, inversely correlated to latitude, is responsible for the peripheral conversion of 7-dehydro-cholesterol to vitamin D3 in the epidermis. Therefore, vitamin D may be the biological correlate of available ultraviolet radiation conferring disease risk to a population. In MS patients, there is evidence of a seasonality of birth in MS patients (again suggestive of a seasonal environmental factor) [39], oral vitamin D intake appears to be protective [7] and vitamin D levels correlate inversely with disability [40]. In adult [41] or pediatric [42] MS populations, incremental increases in serum 25-hydroxy-vitamin D3 levels are associated with reduced propensity for relapses. Two small trials have suggested that relapse rates may be reduced in patients taking oral vitamin D supplementation [43,44]. More recently, high dose intake (average of 10,000 IU/day) of vitamin D over 1 year in a study of 40 patients, appeared to reduce relapse rate in MS [45].

There is an increasing appreciation that Vitamin D exerts broad regulatory effects on cells of the adaptive and innate immune system. These include reducing antigen presentation through reducing the activity of dendritic cells or promoting their tolerogenic phenotype,



affecting the polarization of monocytoïd cells towards an M2 phenotype that produces anti-inflammatory cytokines (unpublished observations), altering B cell function, decreasing chemokine gradients and reducing tissue-specific homing [13-15,46]. A significant literature in humans also indicates that vitamin D increases the activity of regulatory T cells to prevent the excessive activation of autoreactive T cells [47,48]. These broad



spectrum effects of vitamin D likely contribute to the apparent benefits of vitamin D in MS.

We sought in this manuscript to evaluate the impact of vitamin D on the polarization of CD4+ T helper

subsets. We found a variable and inconsistent response of 1,25(OH)2D3 on generating human Th1 and Th17 subsets, and a predominance in elevating Th2 cells, resulting in the consistent outcome of increased Th2 to Th1 or Th17 ratios. These results were found by measurements of representative cytokines for each subset, and of their transcription factors. Similar results were found for mouse T cells in culture. Extending to studies in vivo, we found that mice treated with 1,25(OH)2D3 had significant generation of Th2 cells in the spleen and lymph nodes detected through GATA-3 upregulation, further emphasizing the predominance of Th2 cells generated through 1,25(OH)2D3 treatment.

In addressing the mechanism by which Th2 cells were generated, our results have highlighted the STAT6 transcription factor upstream of GATA-3, given the correspondence of elevation of STAT6 and GATA-3 in wildtype EAE mice treated with 1,25(OH)2D3, and of the significant loss of effect of 1,25(OH)2D3 in increasing GATA-3 levels in STAT6 null mice. Significantly, the deficiency of STAT6 by using null mice resulted in the inability of 1,25(OH)2D3 to alleviate EAE, and this is linked mechanistically to the failure in STAT6 null mice to elevate GATA-3 levels. Thus, our results have highlighted not only the predominance of generation of Th2 cells by vitamin D, but they have also revealed the intermediary role of 1,25(OH)2D3 in engaging STAT6 to produce Th2 polarization.

Vitamin D has also been reported to lose its efficacy in EAE when mice are deficient in IL-4 [20], IL-10 [49], Rag-1 [50], vitamin D receptor [51] and estrogen receptor signaling [50]. These results are consistent with our results of the lack of efficacy of 1,25(OH)2D3 in STAT6 null mice since IL-4 and IL-10 are cytokines produced by Th2 cells that require STAT6 for genesis, and the Rag-1 mutation leads to defect in the generation of T cells. A link between estrogen and vitamin D is demonstrated by the findings that estrogen regulates the level of the vitamin D receptor [50].

There are limitations to our current study that should be considered. The T cell population following 3 days of anti-CD3/CD28 stimulation of human peripheral blood-derived mononuclear cells constitutes approximately 90% purity, so it is possible that the effect of 1,25(OH)2D3 in generating Th2 predominance is indirect through cells that contaminate the T cell cultures. Moreover, our experiments utilize the known biologically active form of vitamin D, 1,25(OH)2D3, rather than the precursors vitamin D3 or 25-hydroxyvitamin D3. The last is the commonly measured form in humans due to its stability compared to 1,25(OH)2D3, so our results could be tempered by the potential differential rates in humans of converting 25-hydroxyvitamin D3 to 1,25(OH)2D3. As well, we did not determine whether

other vitamin D metabolites, such as 24,25(OH)2D3, influence T cell function; different vitamin D metabolites can be found in MS patients and have been negatively correlated with outcomes of magnetic resonance imaging [52].

Another consideration is that while the concentrations of 1,25(OH)2D3 that we used in culture can be achieved in humans that are properly supplemented with vitamin D, it remains to be determined how much 1,25(OH)2D3 is found in mice injected in this study with intraperitoneal 1,25(OH)2D3. Our injection protocol and dose, however, reproduce those used by other groups [18,19,26,32,33]. Finally, it is of interest to determine whether 1,25(OH)2D3 would be equally efficacious in mice induced for EAE by active immunization with MOG, as in the current study, compared to mice elicited for EAE through the passive transfer of T cells; in these 2 context, different mechanisms are initially engaged to produce an inflammatory insult to the CNS, and their responses to vitamin D could help discern the key mechanisms for vitamin D in ameliorating EAE.

Conclusions

The utility of vitamin D in multiple sclerosis is contributed by the polarization of helper T cells towards those that are of the regulatory, anti-inflammatory Th2 type, even when Th1 and Th17 levels are inconsistently modulated. The efficacy of 1,25(OH)2D3 in generating Th2 cells and in alleviating EAE requires the STAT6 transcription factor that lies upstream of GATA-3, raising the possibility that other approaches to stimulate STAT6 may increase the effectiveness of vitamin D. Thus, our results of the mechanistic understanding of vitamin D activity have relevance to the improvement of therapeutics to ameliorate MS.

List of Abbreviations

1,25(OH)2D3: 1,25-dihydroxyvitamin D3; CFA: Complete Freund's Adjuvant; EAE: Experimental autoimmune encephalomyelitis; MS: multiple sclerosis; MOG: myelin oligodendrocyte glycoprotein; PBMCs: peripheral blood mononuclear cells; PBS: Phosphate buffered saline; Th: T helper cells.

Acknowledgements

This work was supported by an operating grant from the Brain Repair Program of NeuroScience Canada. SS was supported by fellowships from the Alberta Heritage Foundation for Medical Research and the Multiple Sclerosis Society of Canada.

Authors' contributions

SS performed the majority of the experiments of this manuscript, and wrote the first draft of this manuscript. CS and JW provided technical support, and helped with deriving the results of Figures 1 and 2. WVY supervised this project, and edited and completed the writing of the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 21 December 2010 Accepted: 24 May 2011

Published: 24 May 2011

References

- Ebers GC: Environmental factors and multiple sclerosis. *Lancet Neurol* 2008, **7**:268-277.
- Ascherio A, Munger K: Epidemiology of multiple sclerosis: from risk factors to prevention. *Semin Neurol* 2008, **28**:17-28.
- Marrie RA: Environmental risk factors in multiple sclerosis aetiology. *Lancet Neurol* 2004, **3**:709-718.
- Acheson ED, Bachrach CA, Wright FM: Some comments on the relationship of the distribution of multiple sclerosis to latitude, solar radiation, and other variables. *Acta Psychiatr Scand Suppl* 1960, **35**:132-147.
- Simpson S Jr, Taylor B, Blizzard L, Ponsonby AL, Pittas F, Tremlett H, Dwyer T, Gies P, van der Mei I: Higher 25-hydroxyvitamin D is associated with lower relapse risk in multiple sclerosis. *Ann Neurol* 2010, **68**:193-203.
- Van Amerongen BM, Dijkstra CD, Lips P, Polman CH: Multiple sclerosis and vitamin D: an update. *Eur J Clin Nutr* 2004, **58**:1095-1109.
- Ascherio A, Munger KL, Simon KC: Vitamin D and multiple sclerosis. *Lancet Neurol* 2010, **9**:599-612.
- Sloka S, Silva C, Pryse-Phillips W, Patten S, Metz L, Yong VW: A quantitative analysis of suspected environmental causes of MS. *Can J Neurol Sci* 2011, **38**:98-105.
- Beretich BD, Beretich TM: Explaining multiple sclerosis prevalence by ultraviolet exposure: a geospatial analysis. *Mult Scler* 2009, **15**:891-898.
- Webb AR, Holick MF: The role of sunlight in the cutaneous production of vitamin D3. *Annu Rev Nutr* 1988, **8**:375-399.
- Webb AR, Kline L, Holick MF: Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab* 1988, **67**:373-378.
- Becklund BR, Severson KS, Vang SV, DeLuca HF: UV radiation suppresses experimental autoimmune encephalomyelitis independent of vitamin D production. *Proc Natl Acad Sci USA* 2007, **104**:6418-6423.
- Zhang R, Naughton DP: Vitamin D in health and disease: current perspectives. *Nutr J* 2010, **9**:65.
- Moro JR, Iwata M, von Andriano UH: Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat Rev Immunol* 2008, **8**:685-698.
- Pedersen LB, Nashold FE, Spach KM, Hayes CE: 1,25-dihydroxyvitamin D3 reverses experimental autoimmune encephalomyelitis by inhibiting chemokine synthesis and monocyte trafficking. *J Neurosci Res* 2007, **85**:2480-2490.
- Steinman L: A rush to judgment on Th17. *J Exp Med* 2008, **205**:1517-1522.
- Crome SQ, Wang AY, Levings MK: Translational mini-review series on Th17 cells: function and regulation of human T helper 17 cells in health and disease. *Clin Exp Immunol* 2010, **159**:109-119.
- Cantorna MT, Humpal-Winter J, DeLuca HF: In vivo upregulation of interleukin-4 is one mechanism underlying the immunoregulatory effects of 1,25-dihydroxyvitamin D(3). *Arch Biochem Biophys* 2000, **377**:135-138.
- Cantorna MT, Hayes CE, DeLuca HF: 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc Natl Acad Sci USA* 1996, **93**:7861-7864.
- Lemire JM: Immunomodulatory actions of 1,25-dihydroxyvitamin D3. *J Steroid Biochem Mol Biol* 1995, **53**:599-602.
- D'Ambrosio D, Cippitelli M, Cocciolo MG, Mazzeo D, Di Lucia P, Lang R, Sinigaglia F, Panina-Bordignon P: Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvement of NF-kappaB downregulation in transcriptional repression of the p40 gene. *J Clin Invest* 1998, **101**:252-262.
- Mattner F, Smirolto S, Galbati F, Muller M, Di Lucia P, Poliani PL, Martino G, Panina-Bordignon P, Adorini L: Inhibition of Th1 development and treatment of chronic-relapsing experimental allergic encephalomyelitis by a non-hypercalcemic analogue of 1,25-dihydroxyvitamin D(3). *Eur J Immunol* 2000, **30**:498-508.
- Nashold FE, Hoag KA, Goverman J, Hayes CE: Rag-1-dependent cells are necessary for 1,25-dihydroxyvitamin D(3) prevention of experimental autoimmune encephalomyelitis. *J Neuroimmunol* 2001, **119**:16-29.
- Correale J, Ysraelit MC, Galitan MI: Immunomodulatory effects of Vitamin D in multiple sclerosis. *Brain* 2009, **132**:1146-1160.
- Daniel C, Sartory NA, Zahn N, Radeke HH, Stein JM: Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile. *J Pharmacol Exp Ther* 2008, **324**:23-33.
- Chang JH, Cha HR, Lee DS, Seo KY, Kweon MN: 1,25-Dihydroxyvitamin D3 inhibits the differentiation and migration of T(H)17 cells to protect against experimental autoimmune encephalomyelitis. *PLoS One* 2010, **5**:1292-5.
- Mayne CG, Spanier JA, Relland LM, Williams CB, Hayes CE: 1,25-Dihydroxyvitamin D3 acts directly on the T lymphocyte vitamin D receptor to inhibit experimental autoimmune encephalomyelitis. *Eur J Immunol* 2011, **41**:822-832.
- Giuliani F, Goodyer CG, Antel JP, Yong VW: Vulnerability of human neurons to T cell-mediated cytotoxicity. *J Immunol* 2003, **171**:368-379.
- Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F: Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* 2007, **8**:942-949.
- Giuliani F, Metz LM, Wilson T, Fan Y, Bar-Or A, Yong VW: Additive effect of the combination of glatiramer acetate and minocycline in a model of MS. *J Neuroimmunol* 2005, **158**:213-221.
- Goncalves DaSilva A, Yong VW: Matrix metalloproteinase-12 deficiency worsens relapsing-remitting experimental autoimmune encephalomyelitis in association with cytokine and chemokine dysregulation. *Am J Pathol* 2009, **174**:898-909.
- Branisteau DD, Waer M, Sobis H, Marcellis S, Vandeputte M, Bouillon R: Prevention of murine experimental allergic encephalomyelitis: cooperative effects of cyclosporine and 1 alpha, 25-(OH)2D3. *J Neuroimmunol* 1995, **61**:151-160.
- Lemire JM, Archer DC: 1,25-dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. *J Clin Invest* 1991, **87**:1103-1107.
- Zhu J, Guo L, Watson CJ, Hu-Li J, Paul WE: Stat6 is necessary and sufficient for IL-4's role in Th2 differentiation and cell expansion. *J Immunol* 2001, **166**:7276-7281.
- Ouyang W, Lohning M, Gao Z, Assenmacher M, Ranganath S, Radbruch A, Murphy KM: Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity* 2000, **12**:27-37.
- Spach KM, Hayes CE: Vitamin D3 confers protection from autoimmune encephalomyelitis only in female mice. *J Immunol* 2005, **175**:4119-4126.
- Cantorna MT, Hayes CE, DeLuca HF: 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc Natl Acad Sci USA* 1996, **93**:7861-7864.
- Acheson ED, Bachrach CA: The distribution of multiple sclerosis in U. S. veterans by birthplace. *Am J Hyg* 1960, **72**:88-99.
- Willer CJ, Dyment DA, Sadovnick AD, Rothwell PM, Murray TJ, Ebers GC: Timing of birth and risk of multiple sclerosis: population based study. *BMJ* 2005, **330**:120.
- Van der Mei IA, Ponsonby AL, Dwyer T, Blizzard L, Taylor BV, Kilpatrick T, Butzkueven H, McMichael AJ: Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia. *J Neurol* 2007, **254**:581-590.
- Kragt J, van Amerongen B, Killestein J, Dijkstra C, Uitdehaag B, Polman C, Lips P: Higher levels of 25-hydroxyvitamin D are associated with a lower incidence of multiple sclerosis only in women. *Mult Scler* 2009, **15**:9-15.
- Mowry EM, Krupp LB, Milazzo M, Chabas D, Strober JB, Belman AL, McDonald JC, Oksenberg JR, Bacchetti P, Waubant E: Vitamin D status is associated with relapse rate in pediatric-onset multiple sclerosis. *Ann Neurol* 2007, **61**:618-624.
- Goldberg P, Fleming MC, Picard EH: Multiple sclerosis: decreased relapse rate through dietary supplementation with calcium, magnesium and vitamin D. *Med Hypotheses* 1986, **21**:193-200.
- Nordvik I, Myhr KM, Nyland H, Bjerve KS: Effect of dietary advice and n-3 supplementation in newly diagnosed MS patients. *Acta Neurol Scand* 2000, **102**:143-149.
- Burton JM, Kimball S, Vieth R, Bar-Or A, Dosch HM, Cheung R, Gagne D, D'Souza C, Ursell M, O'Connor P: A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis. *Neurology* 2008, **74**:1852-1859.
- Adorini L, Penna G: Control of autoimmune diseases by the vitamin D endocrine system. *Nat Clin Pract Rheumatol* 2008, **4**:404-412.
- Smolders J, Thewissen M, Peelen E, Menheere P, Cohen Tervaert JW, Damoiseaux J, Hupperts R: Vitamin D status is positively correlated with regulatory T cell function in patients with multiple sclerosis. *PLoS One* 2009, **4**:e6635.

48. Royal W, Mia Y, Li H, Naunton K: **Peripheral blood regulatory T cell measurements correlate with serum vitamin D levels in patients with multiple sclerosis.** *J Neuroimmunol* 2009, **213**:135-141.
49. Spach KM, Nashold FE, Dittel BN, Hayes CE: **IL-10 signaling is essential for 1,25-dihydroxyvitamin D3-mediated inhibition of experimental autoimmune encephalomyelitis.** *J Immunol* 2006, **177**:6030-6037.
50. Nashold FE, Spach KM, Spanier JA, Hayes CE: **Estrogen controls vitamin D3-mediated resistance to experimental autoimmune encephalomyelitis by controlling vitamin D3 metabolism and receptor expression.** *J Immunol* 2009, **183**:3672-3681.
51. Meehan TF, DeLuca HF: **The vitamin D receptor is necessary for 1alpha,25-dihydroxyvitamin D(3) to suppress experimental autoimmune encephalomyelitis in mice.** *Arch Biochem Biophys* 2002, **408**:200-204.
52. Weinstock-Guttman B, Zivadinov R, Qu J, Cookfair D, Duan X, Bang E, Bergsland N, Hussein S, Cherneva M, Willis L, Heininen-Brown M, Ramanathan M: **Vitamin D metabolites are associated with clinical and MRI outcomes in multiple sclerosis patients.** *J Neurol Neurosurg Psychiatry* 2011, **82**:189-195.

doi:10.1186/1742-2094-8-56

Cite this article as: Sloka *et al.*: Predominance of Th2 polarization by Vitamin D through a STAT6-dependent mechanism. *Journal of Neuroinflammation* 2011 **8**:56.

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

