Effect of vitamin D on EBV-specific CD8+ T cells in patients with early multiple sclerosis

Andreas Lysandropoulos[1], Emilie Jaquiéry[2], Anastasia Zekeridou[1], Samantha Jilek Terrasse[2], Mathieu Canales[2], Giuseppe Pantaleo[2], Myriam Schluep[1], Renaud Du Pasquier[1,2]

Services of [1]Neurology and [2]Immunology and Allergy, CHUV, Lausanne;

ABSTRACT:

Introduction: Infection with Epstein-Barr virus (EBV) and a lack in vitamin D are emerging as the two most significant environmental triggers of multiple sclerosis (MS). Since we and others have shown that CD8+ T cells are important immune mediators of the inflammatory response in MS, we examined whether vitamin D directly affects the CD8+ T cell response. We also explored if vitamin D modulates the EBV-specific CD8+ T cell response.

Methods: PBMC of 10 patients with early MS and 10 healthy controls (HC) were stimulated either with a pool of EBV immunodominant peptides or anti-CD3/anti-CD28 beads. Cytokine secretion was assessed with a Cytometric Beads Array (CBA), ELISA and intracellular cytokine staining. To examine whether vitamin D could directly modulate CD8+ T cell immune responses, we depleted CD4+ T cells using a negative selection.

Results: We found that vitamin D-treated PBMC stimulated either with the EBV peptide pool or anti-CD3/anti-CD28 beads adopted an anti-inflammatory profile: significant decrease in IFN-γ and TNF secretion, contrasting with a significant increase in IL-5 and TGF-β secretion. At baseline, but also after vitamin D stimulation, IL-5 was significantly less produced by stimulated CD8+ T cells of early MS than HC. Finally, using depletion of CD4+ T cells, we could show that vitamin D can directly modulate CD8+ T cells.

Discussion: Our data suggest that vitamin D confers an anti-inflammatory profile to CD8+ T cells, without the help of CD4+ T cells. Even if vitamin D has a significant effect on CD8+ T cells of early MS patients, this "rescuing" effect is of smaller magnitude than in HC subjects. Finally, vitamin D does influence the CD8+ T cell response to EBV in early MS patients, suggesting that there is an interplay between these two major environmental factors of MS.

INTRODUCTION:

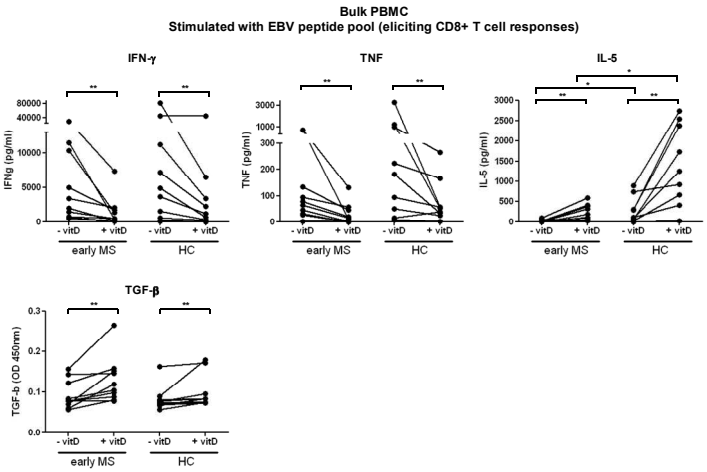
Infection with Epstein-Barr virus (EBV) and a lack in vitamin D are emerging as the two most significant environmental triggers of multiple sclerosis (MS) (1). Indeed, although genetic susceptibility explains the clustering of multiple sclerosis (MS) within families and the sharp decline in risk with increasing genetic distance, it cannot fully explain the geographical variations in MS frequency and the changes in risk that occur with migration, elements that support the presence of concomitant environmental factors. Among those, infection with the Epstein-Barr virus (EBV) and a lack in vitamin D are emerging as the most likely environmental factors. However, if these factors have been independently studied, an estimation of the risk of MS with combination of these two factors has not been performed.

There is evidence that 1,25-(OH)2D3 - the active form of vitamin D – mediates a shift of antigen presenting cells (APC) and CD4+ T cells to a less inflammatory profile (2,3). Although CD8+ T cells do express the vitamin D receptor, a direct effect of 1,25(OH)2D3 on these cells has not been demonstrated until now. Yet, we and others have shown that CD8+ T cells are important immune mediators of the inflammatory response in MS (4,5). In this study, we examined whether vitamin D directly affects the CD8+ T cell response, and more specifically if it modulates the EBV-specific CD8+ T cell response.

Vitamin D decreases production of pro-inflammatory cytokines and increases production of anti-inflammatory cytokines in EBV-specific CD8+ T cells

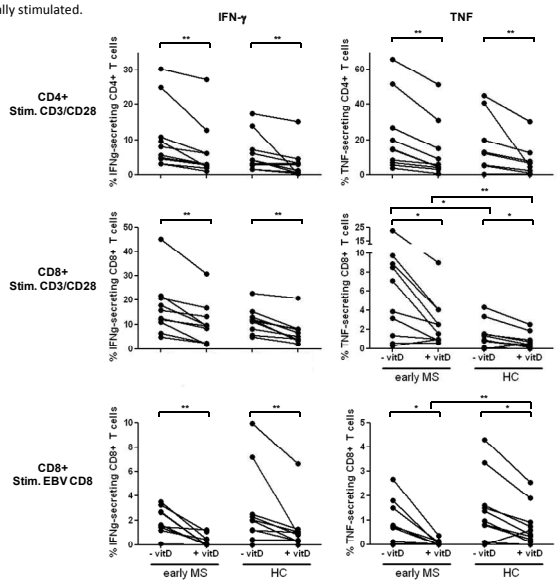
EBV-specific pro-inflammatory cytokines decrease and anti-inflammatory cytokines increase in response to vitamin D exposure. Since vitamin D has been reported to modulate CD4+ T cell responses, we sought to explore whether EBV-specific CD8+ T cell responses were also influenced by the presence of vitamin D. For this, taking advantage of the Cytometric Bead Array (CBA), we analysed the secretion of IFN-γ, TNF, IL-2, IL-4, IL-5 and IL-10 upon EBV-specific stimulation of CD8+ T cells in MS patients and HC. In addition, we assessed the secretion of TGF-β by ELISA.

We found that CD8+ T cells-specific IFN-γ, TNF (pro-inflammatory), IL-5 and TGF-β (anti-inflammatory) secretion was significantly influenced by vitamin D addition both in MS and HC. IL-2, IL-4 and IL-10 secretion were undetectable in all subjects upon EBV-specific stimulation (data not shown). Again, no difference was seen between the two groups, except for a lower secretion of IL-5 in MS patients.



Production of IFN-γ and TNF in CD4+ and CD8+ T cells following vitamin D exposure is decreased. To ensure that CD8+ T cells could respond to the vitamin D-modulation effect, we resorted to the intra cellular cytokine staining assay to discriminate between CD4+ and CD8+ T cell response after different kinds of stimulation.

Upon antigen-independent (CD3/CD28 beads) or antigen-dependent (EBV peptide pool) T cell stimulation, CD4+ and CD8+ T cells produce IFN-γ and TNF. Production of these cytokines was decreased after exposure to vitamin D in both MS patients and healthy controls (HC), independently of the stimulation. No difference between MS patients and HC was seen, except for antigen non-specific TNF secretion in CD8+ T cells, where it was higher in MS patients with or without vitamin D. Interestingly, this effect was not seen in the previous experiment, where both CD4+ and CD8+ T cells responses were analysed together, probably because CD4+ T cells can contribute to the cytokine secretion even though CD8+ T cells were specifically stimulated.

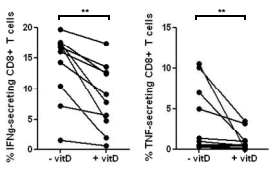


We enrolled 10 MS patients with a first symptom less than one year before the assays and 10 healthy controls (HC). PBMC were isolated and frozen for further use. PBMC were stimulated for one week with either CD3/CD28 beads to elicit both CD4+ and CD8+ T cell responses, or an EBV pool of nonamer peptides, to elicit CD8+ T cells responses , in presence or absence of 100 nM vitamin D. Secretion of cytokines (IFN-γ, TNF, IL-2, IL-4, IL-5 and IL-10) was assessed using the Human Th1/Th2 Cytokine Kit (Cytometric Bead Array). TGF-β secretion was assessed using a commercially available ELISA kit. In addition, production of IFN-γ and TNF by CD4+ and CD8+ T cells was assessed by intracellular cytokine staining with the following antibodies: CD3, CD4, CD8, IFN-γ and TNF. Cells were analysed on a LSRII. MS, multiple sclerosis; HC, healthy controls. *p<0.05, **p<0.01, ***p<0.005 (Mann-Withney ranked test and Wilcoxon paired ranked test).

Vitamin D can directly act on CD8+, without the help of CD4+ T cells

Depletion of CD4+ T cells does not abrogate the effect of vitamin D. In previous experiments, we have shown that CD8+ T cells could respond to vitamin D modulation effect. Here, we aimed at determining whether this CD8+ T cell response to vitamin D was independent from the presence of CD4+ T cells. Thus, we examined the production of pro-inflammatory cytokines by CD8+ T cells upon stimulation, after depletion of CD4+ T cells.

We found that the CD3/CD28-stimulated CD8+ T cells were responsive to vitamin D, independently from CD4+ T cells.



PBMC of 10 subjects (2 early MS and 8 HC) were first CD4+-depleted and then stimulated with CD3/CD28 beads for one week, in the presence or absence of 100nM of vitamin D. Production of IFN-γ and TNF was assessed by intracellular cytokine staining with the following antibodies: CD3, CD4, CD8, IFN-γ and TNF. Cells were analysed on a LSRII. **p<0.01 (Wilcoxon paired ranked test).

CONCLUSION:

- In agreement with previous work (2,3), we have shown that vitamin D has an anti-inflammatory action on T cells, by decreasing secretion of pro-inflammatory cytokines (IFN-γ and TNF) and by increasing secretion of anti-inflammatory cytokines (IL-5 and TGF-β).
- This effect was seen in both specific (EBV) and aspecific (CD3/CD28 beads) antigen stimulation of T cells in the presence of vitamin D.
- Early MS patients had a higher secretion of TNF and lower secretion of IL-5, underlining the inflammatory state of the disease. Furthermore, addition of vitamin D did not restore the same levels of both cytokines as compared to HC, pointing towards a intrinsic dysregulation of anti-inflammatory responses.
- Up to now, the effect of vitamin D has been described only in CD4+ T cells (3). We show here that it can modulate the response of CD8+ T cells and confer them an anti-inflammatory profile, interestingly without the help of CD4+ T cells.
- Finally, since vitamin D can influence the CD8+ T cell responses to EBV in early MS patients, it suggests that there might be an interplay between these two major environmental factors at the onset of MS.

ACKNOWLEDGEMENTS:
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