Vitamin D has a direct immunomodulatory effect on CD8+ T cells of patients with early multiple sclerosis and healthy control subjects* 

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**ABSTRACT:** Little is known on a putative effect of vitamin D on CD8+ T cells. Yet, these cells are involved in the immunopathogenesis of MS. We assessed the cytokine profile of EBV-specific CD8+ T cells of 10 early MS patients and 10 healthy control subjects with or without 1,25(OH)2D3 and found that, with 1,25(OH)2D3, these cells secreted IFN-γ and TNF-α and more IL-5 and TGF-β. CD8+ T cell depletion or even culture with CD8+ T cells only did not abolish the immunomodulatory effect of 1,25(OH)2D3 on CD8+ T cells, suggesting that CD8+ T cells are involved. Methods: PBMC of 10 patients with early MS and 10 healthy controls were stimulated with a pool of EBV immunodominant peptides. Cytokine secretion was assessed with a Cytometric Beads Array (CBA), ELISA and intracellular cytokine staining (ICS). To examine whether vitamin D could directly modulate CD8+ T cell immune responses, we performed a depletion of CD4+ T cells and a sorting of CD8+ T cells. Results: We found that vitamin D-treated PBMC stimulated with the EBV peptide pool adopted an anti-inflammatory profile: significant in IFN-γ and TNF-α 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 either depletion of CD8+ T cells or sorting of CD8+ T cells, we could show that vitamin D can directly modulate CD8+ T cells. Discussion: The cytokine profile of EBV-specific CD8+ T cells was significantly affected by in-vitro addition of 1,25(OH)2D3. Our data suggest that vitamin D confers a direct anti-inflammatory profile to CD8+ T cells. Whether 1,25(OH)2D3 may have an effect on this EBV-specific CD8+ T cell response in vivo warrants further studies. 

**INTRODUCTION:** Lower levels of vitamin D are associated with an increased risk of MS and sunlight exposure and vitamin D supplements have been shown to reduce the risk of MS. These protective effects of vitamin D in MS could be attributable to its immunomodulatory properties. Indeed, the active form of vitamin D, i.e. 1,25(OH)2D3 mediates a shift of antigen-presenting cells (APC) and CD4+ T cells to a less immunoinflammatory profile, 1,25(OH)2D3 inhibits the proliferation of MBP-specific CD4+ T cells, increasing the secretion of IL-10, and decreasing the secretion of IL-6 and IL-17 by those cells (2). No direct effect of vitamin D on CD8+ T cells has been reported so far yet the vitamin D receptor (VDR), a nuclear receptor member of the large steroid receptors family, is expressed in higher amounts in CD8+ than in CD4+ T cells (3). Thus, there are several elements arguing for a significant role of CD8+ T cells in the immunopathogenesis of multiple sclerosis (MS) (4,5). In this study, we examined whether 1,25(OH)2D3 had an immunomodulatory effect on CD8+ T cells in MS patients and healthy control subjects. 

**Vitamin D can directly act on CD8+ T cells and mediate anti-inflammatory responses**

We have shown that CD8+ T cells could respond to vitamin D immunomodulatory effect. Since we aimed at determining whether this CD8+ T cell response to vitamin D is 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 depletion of CD4+ T cells did not abrogate the effect of vitamin D on CD8+ T cells. 

**CONCLUSION:** 
- Cytokine profile of EBV-specific CD8+ T cells was significantly affected in vitro adjunction of vitamin D by decreasing secretion of pro-inflammatory cytokines (IFN-γ and TNF-α) and by increasing secretion of anti-inflammatory cytokines (IL-5 and TGF-β). 
- Vitamin D has a direct effect on CD8+ T cells, at least in vitro, conferring an anti-inflammatory profile. 
- Patients with early MS had a lower secretion of IL-5, underlining the inflammatory state of the disease. Furthermore, addition of vitamin D did not restore the same levels of IL-5 as compared to HC, pointing towards a intrinsic dysregulation of the anti-inflammatory responses. 
- Th1 cells are involved in pro-inflammatory cytokine. However, EBV-specific CD8+ T cells from early MS patients tended to secrete less TNF-α than HC, both at baseline and after addition of vitamin D. Further clarification of the relationship between vitamin D and TNF-α is needed.