

## The effect of vitamin D in the evolution of experimental autoimmune encephalomyelitis. Effect on B-lymphocytes function.

Leonilda M. B. Santos (PQ), Paula G. Russini (PG), Amanda B. Piffer (IC), Vitor A. Silva (EM), Vanessa C. B. Mariano (EM), Stephani O Alves (EM), Fabiana F. Aquino (EM).

### Abstract

The first evidence that vitamin D3 deficiency can be an environmental risk factor for multiple sclerosis (MS), has come correlation between the spatial distribution of the EM incidence of UV rays and the prevalence of MS. The immunomodulatory effect of vitamin D3 has been demonstrated in experimental autoimmune encephalomyelitis (EAE). EAE is an inflammatory disease of the central nervous system widely accepted as a model for studying MS. The mechanisms by which vitamin D leads to attenuation of EAE are constant source of study. In addition, suppressor B cells can downregulate the immune response in animal models of inflammatory diseases in mice. An evaluation is the implication of treatment with vitamin D3 in response via B cells during the clinical course of EAE.

Key words: *Vitamin D, experimental autoimmune encephalomyelitis, suppressor B cell*

### Introduction

Vitamin D deficiency is associated with an increased risk of multiple sclerosis (MS) and unfavorable MS disease progression. The immunomodulatory effect of vitamin D has been demonstrated in an experimental model of MS, the experimental autoimmune encephalomyelitis (EAE). EAE is an antigen-driven autoimmune model in which immunization against myelin autoantigens elicits strong CD4+ T lymphocyte responses, which initiate its pathology with central nervous system myelin destruction. Furthermore, B-lymphocytes are also involved in both the pathogenesis and on disease control. The effect of vitamin D on B cells function in EAE model deserves additional studies. Thus, the objective of this work is to investigate the effect of vitamin D on both clinical evolution of EAE and on Blymphocytes function.

### Results and Discussion

EAE was induced by immunization with MOG35- 55 peptide emulsified in complete Freund adjuvant. The clinical expression of the disease was graded on a clinical scale 0-5 according to the severity of the disease. Vitamin D3 (cholecalciferol D3 Sigma Aldr. Mo, USA) was diluted in polyethylene glycol and give orally (5µg/Kg/day) during 2 weeks. The control group was fed with vehicle alone (15 animal/group were studied in three independent experiments). The expression of cytokine mRNA was evaluated by quantitative RT-PCR. The subsets of B and T lymphocytes were determined by Flow cytometry (Galios, Coulter, USA). The present study demonstrated the immunomodulatory effect of vitamin D3 in the EAE model. Animals treated with vitamin D3 had a much less severe disease than the control group. The reduction of the severity of the disease was simultaneous with decreased inflammatory response evaluated by the increase of regulatory B- lymphocytes and anti-inflammatory cytokines. These lymphocytes and cytokines act on

the autoreactive T lymphocytes, probably by inhibiting the migration of these cells into the central nervous system, which explains the reduction in disease severity.

### Conclusions

We demonstrated that the oral administration of vitamin D significantly reduced the severity of EAE. The reduction of the severity of the disease was accompanied by the increase number of Blymphocytes, reduction of pro-inflammatory cytokines such as IFN-γ and IL-17 and increase of anti-inflammatory cytokines such as IL-10.

### Acknowledgement

FAPESP, CNPq and CAPES

<sup>1</sup> Nanduri R.; Mahajan S.; Bhagyaraj E.; Sethi K.; Kalra R.; Chandra V. and Gupta P. Transcriptionally Represses Smad7 Signaling and Activates Extracellular Signal-regulated Kinase (ERK) to Inhibit the Differentiation of an Inflammatory T Helper Cell Subset and Suppress Experimental Autoimmune Encephalomyelitis. May 8, 2015 The Journal of Biological Chemistry, 290, 12222-12236.

<sup>2</sup> Sloka S.; Silva C.; Wang J. and Yong V. W. Predominance of Th2 polarization by vitamin D through a STAT6-dependent mechanism. J Neuroinflammation. 2011 May 24;8:56. doi: 10.1186/1742-2094-8-56.

<sup>3</sup> Klinker M. W. and Lundy S. K. Multiple mechanisms of immune suppression by B lymphocytes. Mol Med. 2012 Feb 10;18:123-37. doi: 10.2119/molmed.2011.00333.

<sup>4</sup> Soleimani M.; Jameie S. B.; Mehdizadeh M.; Keradi M.; Masoumpoor M. and Mehrabi S. Vitamin D3 influence the Th1/Th2 ratio in C57BL/6 induced model of experimental autoimmune encephalomyelitis. Iran J Basic Med Sci. 2014 Oct;17(10):785-92.

<sup>5</sup> Joshi S.; Pantalena L. C.; Liu X. K.; Gaffen S.L.; Liu H.; Rohowsky-Kochan C.; Ichihama K.; Yoshimura A.; Steinman L.; Christakos S. and Youssef S. 1,25-dihydroxyvitamin D(3) ameliorates Th17 autoimmunity via transcriptional modulation of interleukin-17A. Mol Cell Biol. 2011 Sep;31(17):3653-69. doi: 10.1128/MCB.05020-11. Epub 2011 Jul 11.

<sup>6</sup> Wang Y.; Marling S. J.; Zhu J. G.; Severson K. S. and DeLuca H. F. Development of experimental autoimmune encephalomyelitis (EAE) in mice requires vitamin D and the vitamin D receptor. Proc Natl Acad Sci U S A. 2012 May 29;109(22):8501-4. doi: 10.1073/pnas.1206054109. Epub 2012 May 16.