1, 25-dihydroxyvitamin D3 ameliorates experimental autoimmune encephalomyelitis in C57BL/6 mice via polarization of T regulatory and T helper 2 cells and inhibiting T helper 17 subset

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Abstract

Introduction: The experimental autoimmune encephalomyelitis (EAE) is an animal model for human multiple sclerosis. Vitamin D has several reported immunomodulatory properties including the reduced generation of pro-inflammatory CD4+ 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 drives the polarization of one of the T helper subsets.

Materials and Methods: 24 C57BL/6 male mice were divided into 3 groups: 1-Control, 2-EAE without treatment and 3- 1, 25-dihydroxyvitamin D3 (Vitamin D3) treated EAE. EAE was induced in Groups2 and 3 by subcutaneous injection of MOG and CFA and intraperitoneal injection of pertussis toxin. Mice in group3 received intraperitoneal injection of 40 ng/kg vitamin D3 daily for 10 days. Clinical and weight assessments were performed daily. On day 25 animals were sacrificed. The disease was scored and the mice were examined for the disease symptoms and their weight was recorded till the twenty first day, on day 21 they were anesthetized and then sacrificed. Histological study was performed by staining the brain sections using hematoxilin eosin. FoxP3, CD4, IL-4, IL-10, IL17, IL23 and IFN-γ expression were analyzed in splencytes using real-time PCR. The validation of their protein production was measured using flow cytometery, and the secretion of IL-4, IL-10, IL-17, IL-23 and IFN-γ were validated using ELIZA of the cultured splenocyte's supernatant.

Results: Our results showed significant mean weight increase and Clinical score decrease in group3 comparing with group2. Histological studies revealed lower lymphocytic infiltration and demyelination in group3 compared to group2. Splenocytes proliferation showed significant reduction in group3 in comparison to group2. The percentages of spleen Foxp3+ cell population in CD4+ cells reduced in groups3 compared group2. Expression of transcription factor and cytokines related to Treg and Th2 showed significant increase in group3 in comparison to group2, while cytokines related to Th17 showed lower expression in group 3 compared to group 2.

Conclusion: It seems that 1, 25-dihydroxyvitamin D3 may alleviate disease condition in EAE mice through systemically reducing inflammatory immune responses. Moreover it seems that 1, 25-dihydroxyvitamin D3 drives the polarization of Th2 and Treg subsets and suppressesTh17responses. Hence, 1, 25-dihydroxyvitamin D3 is a potential molecule for therapeutic purposes especially in combination therapies.

Key words: *EAE, Vitamin D3, Th1, Th2, TH17, Treg.*