



## The Engagement Between Vitamin D and the Immune System: Is Consolidation by a Marriage to Be Expected?

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For several decades it is evident that vitamin D and its metabolites exhibit multiple effects beyond bone metabolism. Multiple association studies have hinted at a role of vitamin D in the pathogenesis of immune mediated inflammatory diseases (IMID). In particular an insufficient vitamin D status, as defined by a low circulating level of 25-OH-D, has been linked to both the prevalence and the severity of these diseases. It has been hypothesized by many researchers in the field, including ourselves, that metabolites of the vitamin D pathway can interfere with maturation of dendritic cells, skew T-cell responses away from pathogenic Th1 and Th17 development, and favor the number and/or function of regulatory T-cells (Treg) [1]. Although vitamin D was first considered merely an immunosuppressive reagent, the concept evolved towards a more modulating role in tolerance and homeostasis [2]. This concept was strengthened by multiple animal models and *in vitro* studies [3]. In the human IMID-setting, however, the situation still remains a controversial issue. Especially because 25-OH-D levels are dependent of many variables, and inflammation may lower 25-OH-D, interpretation of the aforementioned association studies is difficult. A clear-cut causal effect of vitamin D on the immune system can only be derived from well-designed placebo-controlled supplementation studies.

In the recent issue of *EBioMedicine*, the functional genomics analysis of the BEST-D trial was presented and, although the data were thoroughly analyzed from different angles, the results did not reveal any effect of vitamin D supplementation on the whole-blood gene expression [4]. In the BEST-D trial apparently healthy elderly individuals, about 100 individuals per treatment arm, were supplemented with either placebo, 2000 or 4000 IU/day for 12 months. Next to the negative BEST-D trial, there are, however, some substantially smaller supplementation studies in humans that do suggest immune-regulatory effects in line with the concept of immune tolerance and homeostasis. A few examples: first, a pilot study in just 4 healthy individuals supplemented for 15 weeks with 5000–10,000 IU/day reported an increase in IL-10 production by peripheral blood mononuclear cells, probably caused mainly by non T-cells,

and a decreased Th17 response [5]; second, a placebo-controlled supplementation with 140,000 IU/month in 60 healthy individuals revealed after 12 weeks significantly increased numbers, but not function, of Treg [6]; and third, a randomized trial in 40 patients with relapsing-remitting multiple sclerosis (MS) showed a reduction of IL-17 production by Th-cells from patients supplemented with 10,400 IU/day as compared to a regime of only 800 IU/day for 6 months [7]. In contrast, our vitamin D supplementation trial in MS, the SOLARIUM-trial, did not reveal any clear-cut effects on multiple parameters of the adaptive immune system, with a focus on T-cell mediated cytokine responses and regulatory lymphocytes, in the high dose treatment arm, *i.e.*, 14,000 IU/day for 48 weeks ( $n = 30$ ), but did show a decrease in Th2 cells in the placebo group ( $n = 23$ ) [8]. The latter data were interpreted as being supportive that vitamin D could maintain immune homeostasis in the background of ongoing immune deregulation. The examples above clearly show that findings are not consistent and could reflect findings by chance. Indeed, as summarized by Allan et al., much of the evidence is at high risk of bias, with multiple flaws, including analyses of secondary endpoints, and based on small and underpowered studies [9].

The question now arises if the engagement between vitamin D and the immune system will ever result in a solid marriage? Furthermore, how can we explain the discrepancies between on the one hand animal models and *in vitro* studies, and on the other hand human *in vivo* and *ex vivo* studies? In this respect it is important to realize that for human studies most frequently peripheral blood is used, comprising heterogeneous cell-fractions under homeostatic conditions. In addition, the read-out for cytokine responses is extremely diverse and the same holds for the definition of regulatory lymphocytes. A relevant issue is whether these rather a-specific cells and outcomes are the best biomarkers to reveal an effect of vitamin D supplements on relevant immunological mechanisms for selected (pre-morbid) IMID-cohorts. Having this in mind, it is important to realize that the vitamin D receptor (VDR) is primarily expressed in activated lymphocytes, but not in resting lymphocytes. Indeed, the most evident effects of vitamin D, *i.e.*, the bioactive metabolite calcitriol, are observed in combination with lymphocyte activation and antigen-specific read-outs. Assessment of more disease-specific cells and molecules in relevant (pre-morbid) patient cohorts may yield different results. In this respect, it is promising that

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a decrease in circulating anti-EBNA1 antibodies in vitamin D<sub>3</sub>-supplemented MS patients is a very consistent finding [10]. Although we still do not understand the underlying mechanism, we speculate that primary outcomes of future studies should focus on the short time-span of the induction and regulation of the evoked immune response. This approach may shed new light on the sun-shine vitamin.

### Disclosure

The authors declared no conflicts of interest.

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