Vitamin D Supplementation and Regulatory T Cells in Apparently Healthy Subjects: Vitamin D Treatment for Autoimmune Diseases?

Barbara Prietl Msc[1]*, Stefan Pilz MD[1]*, Michael Wolf[1], Andreas Tomaschitz MD[1], Barbara Obermayer-Pietsch MD[1], Winfried Graninger MD[2] and Thomas R. Pieber MD[1,3]

[1]Division of Endocrinology and Nuclear Medicine, and [2]Division of Rheumatology and Immunology, Department of Internal Medicine, Medical University of Graz, and [3]Institute of Medical Technologies and Health Management, Joanneum Research, Graz, Austria

ABSTRACT: **Background:** Epidemiological data show significant associations of vitamin D deficiency and autoimmune diseases. Vitamin D may prevent autoimmunity by stimulating naturally occurring regulatory T cells.

Objectives: To elucidate whether vitamin D supplementation increases Tregs frequency (%Tregs) within circulating CD4+ T cells.

Methods: We performed an uncontrolled vitamin D supplementation trial among 50 apparently healthy subjects including supplementation of 140,000 IU at baseline and after 4 weeks (visit 1). The final follow-up visit was performed 8 weeks after the baseline examination (visit 2). Blood was drawn at each study visit to determine 25-hydroxyvitamin D levels and %Tregs. Tregs were characterized as CD4+CD25++ T cells with expression of the transcription factor forkhead box P3 and low or absent expression of CD127.

Results: Forty-six study participants (65% females, mean age ± SD 31 ± 8 years) completed the trial. 25(OH)D levels increased from 23.9 ± 12.9 ng/ml at baseline to 45.9 ± 14.0 ng/ml at visit 1 and 58.0 ± 15.1 ng/ml at visit 2. %Tregs at baseline were 4.8 ± 1.4. Compared to baseline levels we noticed a significant increase of %Tregs at study visit 1 (5.9 ± 1.7, $P < 0.001$) and 2 (5.6 ± 1.6, $P < 0.001$).

Conclusions: Vitamin D supplementation was associated with significantly increased %Tregs in apparently healthy individuals. This immunomodulatory effect of vitamin D might underlie the associations of vitamin D deficiency and autoimmune diseases. Hence, our finding provides a rationale for further studies to investigate vitamin D effects on autoimmunological processes.

KEY WORDS: 25-hydroxyvitamin D, vitamin D, immunology, autoimmunity, regulatory T cells, Tregs

Vitamin D deficiency has been associated with several adverse health consequences including autoimmune diseases [1-3]. This is of particular interest when considering the high prevalence of hypovitaminosis D, which affects almost half the world's population. Reduced sunlight exposure is mainly responsible for this pandemic of vitamin D deficiency because approximately 80–90% of circulating vitamin D originates from ultraviolet B-induced vitamin D production in the skin, whereas dietary vitamin D intake plays only a minor role. Vitamin D from both sources is hydroxylated to 25-hydroxyvitamin D in the liver and is then further hydroxylated by 1α-hydroxylase to 1,25-dihydroxyvitamin D (1,25[OH]2D), which is the most active vitamin D metabolite. It was recently discovered that apart from the kidney, various organs and cells – such as those of the immune system – also express 1α-hydroxylase [1,4]. The vitamin D receptor, which regulates about 3% of the human genome, is also expressed in immune cells, suggesting physiologic immunomodulatory effects of the vitamin D endocrine system [1-5].

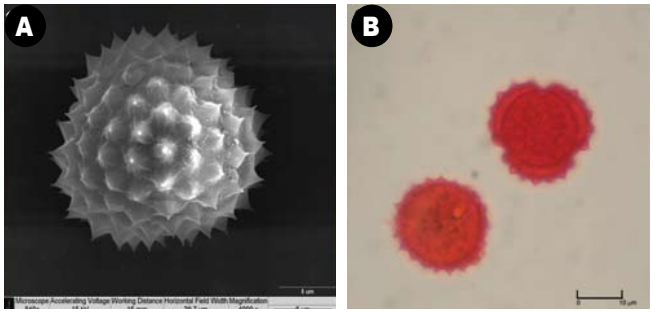
Reduced 25(OH)D levels, which are used to classify the vitamin D status, have been observed in several autoimmune diseases [2,3,6-10]. In addition, hypovitaminosis D as well as low vitamin D intake have been identified as a risk factor for the development of autoimmune diseases, such as type 1 diabetes mellitus or multiple sclerosis [1-3]. In mouse models for autoimmune diseases, vitamin D was shown to prevent type 1 diabetes and experimental autoimmune encephalitis [3]. Accumulating evidence supports the notion that vitamin D could prevent these autoimmune diseases as well as allograft rejection, by increasing the frequency or the effects of naturally occurring regulatory T cells [2,3]. Tregs, which are critical for maintaining immune tolerance, are characterized by high surface expression of CD4 and the interleukin-2 receptor (CD25), low or absent expression of the IL-7 receptor (CD127), and by expression of the transcription factor forkhead box P3 [11-14]. In animal and cell culture studies, tolerogenic dendritic cells are induced by active vitamin D treatment and promote the induction of Tregs, which are suggested to prevent autoimmune diseases due to their immunosuppressive activity [5,15-21]. In human renal transplant recipients, 1,25(OH)2D treatment over 6

***B. Prietl and S. Pilz contributed equally to this article**

Tregs = regulatory T cells
25(OH)D = 25-hydroxyvitamin D
IL = interleukin

Figure 1. [A] A stand of Ambrosia. **[B]** A flowering branch of *Ambrosia confertifolia*.

Figure 2. Pollen of *A. maritima*. **[A]** By a scanning electron microscope **[B]** Acetolized pollen grains by a light microscope.



patients in the Tel Aviv area for their response to extracts of three species of Ambrosia (the two American species – tall ragweed and short ragweed – bought from Dome/Hollister Stier, and the local one, *Ambrosia maritima*, was extracted and prepared in our laboratory). Twenty-nine of the tested patients showed positive skin-prick test responses to *Ambrosia maritima*, 21 were positive to *Ambrosia trifida* (tall ragweed) and 24 were positive to *Ambrosia artemisiifolia* (short ragweed). Some 21% of the patients who were sensitive to *Ambrosia maritima* responded to the local species only, whereas 5–10% of those patients responded also to the two American species. Forty-three percent of the patients who were sensitized to Ambrosia responded to the pollen extracts of all three species. This irrefutably indicates the similarity of the allergens of the pollen of the different species. However, apart from the common allergens of all three species, some specific allergens seem to exist in the pollen of the local Ambrosia plants and these are identified by local patients.

The invasion of new species of Ambrosia into Israel is still in progress. Plants of *Ambrosia artemisiifolia*, *Ambrosia trifida*, *Ambrosia confertifolia*, Ambrosia grayi and *Ambrosia tenuifolia* were found in Israel [Figure 1]. *Ambrosia confertifolia* has invaded the Hula valley in the eastern Galilee and the central coastal plain on a large scale and has now established dense stands there.

The flowering of *Ambrosia maritima* starts already in July whereas that of *Ambrosia confertifolia* starts only in late August. Pollen production by the coastal plain (Emek Hefer) population of *Ambrosia confertifolia* is extremely high and counts of some 3000 airborne pollen/m3 of air were monitored early in October 2008. The release of the pollen starts early in the morning and peaks at noon. The distance that such pollen traverses depends of course on air turbulence, wind velocity and wind direction. Nevertheless, despite the fact that pollen concentration decreases with time and with distance from the source, it presumably remains far above the threshold concentration for induction of allergy responses (10–20 pollen/m3 air) over a large area of the immediate environment.

The pollen size (projection area) of *Ambrosia confertifolia* seems to be somewhat smaller (~275 μ2) than the pollen of Ambrosia maritima (~330 μ2) [Figure 2]. Nevertheless, it is practically impossible to identify the two species by optical microscopy on the tapes of the common pollen traps.

The recently invading American ragweed species have spread rapidly and is now established in disturbed habitats, along road and railroad sides, mechanically cleared open areas, stream banks, and field margins. The main centers of invasion are the Hula Valley in the Upper Galilee and near the Alexander River in the Sharon plain. Attempts to eradicate the invading species were made only on a very small scale and were discontinued due to damage to agricultural crops that were caused by improper handling of the herbicides. Currently, not one of the regional or government authorities, Ministry of Health, Ministry of Agriculture, Ministry of the Environment or the Nature and National Parks Authority are taking any action aimed at eradicating or at least limiting the spread of this invasive species in Israel. This is a clear case where the impotency of the authorities will result in serious health problems that will cause great suffering to thousands of people and high costs to the public health system. From experience it is known that the time it takes to eradicate a new invasive species is limited. Once it spreads over too large an area it will be out of control. The time to take action is right now if we want to limit future damage to thousands of people caused by this aggressive allergenic invader.

Correspondence:
Dr. Y. Waisel
Dept. of Plant Sciences, Tel Aviv University, Ramat Aviv Israel.
Phone: (972-3) 640-9180, **email:** waisel@post.tau.ac.il

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