



Letters

In vitro cytokine production after in vivo desensitization in an allopurinol-induced delayed allergic reaction

We report the case of a 42-year old man who from the age of 30 experienced intermittent episodes of arthritis affecting different joints. Repeated analyses revealed hyperuricemia, with levels over 7 mg/dL. The patient was diagnosed with gout. Three and 5 years previously he presented in the first 24 to 48 hours after the administration of allopurinol 300 mg (Zyloric, Faes Farma, Spain), with lesions of the mucous membranes of the glans penis, red macule initially that subsequently evolved to mucosal edema accompanied by pain, exudation of a serous liquid, and difficulty in urinating, which persisted for 7 to 15 days but resolved completely after the administration of topical and oral corticosteroids. He was subsequently examined in our Allergy Department, and the diagnosis of allopurinol-induced fixed drug eruption was confirmed after the oral administration of 100 mg of allopurinol, which led to recurrence of the lesions on the glands that began 24 hours after administration. The patient was included in a protocol of induction of tolerance: day 1, 25 μ g; day 2, 50 μ g; day 3, 100 μ g; day 4, 200 μ g; day 5, 500 μ g; day 6, 1 mg; day 7, 2 mg; day 8, 5 mg; day 9, 10 mg; day 10, 25 mg; day 11, 50 mg; day 12, 100 mg; day 13, 150 mg; day 14, 200 mg; day 15, 250 mg; and day 16, 300 mg. The induction of tolerance process concluded successfully, and no adverse effects were experienced. Twelve months later, the patient continues to take allopurinol at a dosage of 300 mg/day.

The induction of allopurinol tolerance has been described in 32 patients with cutaneous reaction induced by allopurinol,¹ and was completed in 87.5% of the patients, with a protocol that extended over 28 days up to a dose of 100 mg/day. In other cases of fixed drug eruption by allopurinol,^{2,3} tolerance was achieved at 100 and 300 mg of allopurinol with a protocol for a month and 15 days, respectively, similar to ours.

The production of interleukin (IL) 2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)- α , IFN- γ , and IL-17 cytokines (Human CBA, BD Biosciences, San José, California) was quantified after the in vitro

culture of mononuclear cells isolated from the patient (PBMC) at baseline (RPMI-1640; 2 mM L-glutamine; 10% fetal calf serum [FCS]) and after stimulation with phytohemagglutinin (10 μ g/mL) and with allopurinol (0.5, 0.2, and 0.1 mg/mL) in a culture chamber (Hera cell, Heraeus) for 72 h at 37°C, 5% CO₂, before in vivo induction of tolerance and 1 month afterward. As a control, the same response was analyzed using cells from a healthy subject. The samples were acquired on the flow cytometer FACSCanto II (BD Biosciences) and analyzed by FCAP Array software (BD Bioscience) following the manufacturer's instructions.

We observed no production of IL-2, IL-4, and IL-17 in the cells of the patient or the control. The results obtained from the in vitro production of IL-6, IL-10, TNF- α , and IFN- γ are shown in Table 1.¹ Particularly important is the elevated production of IL-6 and IL10, as well as a weaker production of TNF- α by the patient's cells at baseline, which increased after induction of tolerance. Furthermore, we observed increases in TNF- α and IFN- γ after the in vitro stimulation with allopurinol in the analysis performed after induction of tolerance.

Recently, an increase in IL-6 and TNF- α expression in PBMCs induced by monosodium urate crystals, the cause of gouty arthritis, has been described.⁴ Moreover, the released of extracellular anti-inflammatory cytokines, as IL-10, in synovial fluid involved in spontaneous resolution of gout has been published.⁵ This could explain the elevated baseline of this cytokines in our patient's cells. The inhibition of IL-10 and IL-6 production in allopurinol-stimulated patients' cells could be caused by a shift to Th1 response involved in the hypersensitivity reaction.

After induction of tolerance, a pattern appears that is similar to that observed in Th1 (TNF- α and IFN- γ) responses previously described in delayed-type allergic reactions to drugs,⁶ which should favor the reappearance of the lesions. Conversely, a counterbalancing mechanism comes into play with an increase in IL-10, probably caused by the CD4⁺ CD25⁺ regulatory T cells, which has previ-

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Table 1
Cytokines detected in supernatants of PBMC cultures in the patient before (pre-) and after (post-) in vivo desensitization with allopurinol and in a healthy control, in basal conditions, after phytohemagglutinin (PHA) and allopurinol stimulation

	Cytokines (pg/mL)											
	IL-6			IL-10			TNF- α			IFN- γ		
	Control	Patients		Control	Patients		Control	Patients		Control	Patients	
		Pre-	Post-		Pre-	Post-		Pre-	Post-		Pre-	Post-
Baseline	64.43	1315	3587	0.28	41.69	55.88	0.10	2.02	5.35	0.41	0.08	0.82
PHA (10 μ g/mL)	3,911.88	530	1,999	31.75	11.59	32.66	26.05	4.26	10.89	186.85	12.47	175.63
Allopurinol												
0.5 mg/mL	7.89	1437	3327	0.00	1.78	9.34	0.41	9.45	16.90	0.00	0.48	0.51
0.2 mg/mL	4.54	655	2638	0.03	0.65	11.92	0.39	7.95	17.92	0.18	0.04	3.25
0.1 mg/mL	8.02	634	2453	0.04	2.31	15.81	0.36	5.85	13.79	0.01	0.00	2.91

ously been described in other desensitization processes involving this same drug in cells of the epidermis⁷ and in peripheral blood⁸ as the mechanism responsible for resolution of the skin lesions. After induction of allopurinol tolerance in our case, the suppressing effect of IL-10 on the Th1 response has presumably prevailed and prevented the recurrence of a cutaneous reaction.

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References

- [1] Lochmatter P, Beeler A, Kawataba TT, Gerber BO, Pichler WJ. Drug-specific in vitro release of IL-2, IL-5, IL-13 and IFN- γ in patients with delayed-type drug hypersensitivity. *Allergy*. 2009;64:1269–1278.
- [2] Teraki Y, Shiohara T. Successful desensitization to fixed drug eruption: the presence of CD25+CD4+ T cells in the epidermis of fixed drug eruption lesions may be involved in the induction of desensitization. *Dermatology*. 2004;209:29–32.
- [3] Teraki Y, Shiohara T. IFN- γ -producing effector CD8+ T cells and IL-10-producing regulatory CD4+ T cells in fixed drug eruption. *J Allergy Clin Immunol*. 2003;112:609–615.
- [4] Teraki Y, Kokaji T, Shiohara T. Expansion of IL-10-producing CD4+ and CD8+ T cells in Fixed Drug Eruption. *Dermatology*. 2006;213:83–87.

Relationship of 25-hydroxyvitamin D and asthma control in children

Asthma prevalence and vitamin D deficiency rates have increased in parallel. We sought to address several critical issues in assessing the relationship of vitamin D and asthma.

It is accepted that serum 25-hydroxyvitamin D (25(OH)D) levels less than 10 to 12 ng/mL represent deficiency, levels less than 20 ng/mL represent insufficiency, and levels greater than 30 ng/mL represent sufficiency. A review of existing studies reveals associations between airway diseases and serum 25(OH)D using only a single measurement,^{1–4} and there are no dose-response data on the therapeutic effects of varying vitamin D levels in asthma.

We conducted a 1-year pilot study in children to examine whether increased serum 25(OH)D levels either through seasonal variations (placebo treatment) or with supplementation (vitamin D₃, 1,000 IU/d) result in improved asthma control (assessed by multiple measures, including Asthma Control Test [ACT] scores) in patients with chronic persistent asthma.⁵

Thirty patients, ages 6 to 17 years, with a physician diagnosis of chronic persistent asthma and current daily controller asthma medication were enrolled from Creighton University Medical Center. Patients were randomly assigned to either placebo or 1,000 IU of vitamin D daily. No differences were found in baseline characteristics between the placebo and vitamin D groups. At initial (winter), 6-month (summer) and 1-year (winter) visits, body mass index (BMI; calculated as weight in kilograms divided by height in meters squared), ACT score, spirometry, and serum 25(OH)D levels were measured. Twenty-four patients returned for a 6-month follow-up visit, with 3 patients lost to follow-up from each arm. Twenty patients completed the study, with 2 more patients lost from each arm.

The patient population was mainly nonwhite. Only 1 patient had a sufficient vitamin D level at baseline. The mean ACT score at baseline was 17.8 for both groups.

At the 6-month follow-up, the serum 25(OH)D levels in both the supplemented and placebo groups had increased significantly from baseline but did not differ significantly from each other (Fig 1A). However, only 33% of those in the supplemented group and 41.7% of those in the placebo group were vitamin D sufficient at 6 months (summer). At 1-year follow-up (winter), 50% of those in the supplemented group and 20% of those in the placebo group had sufficient

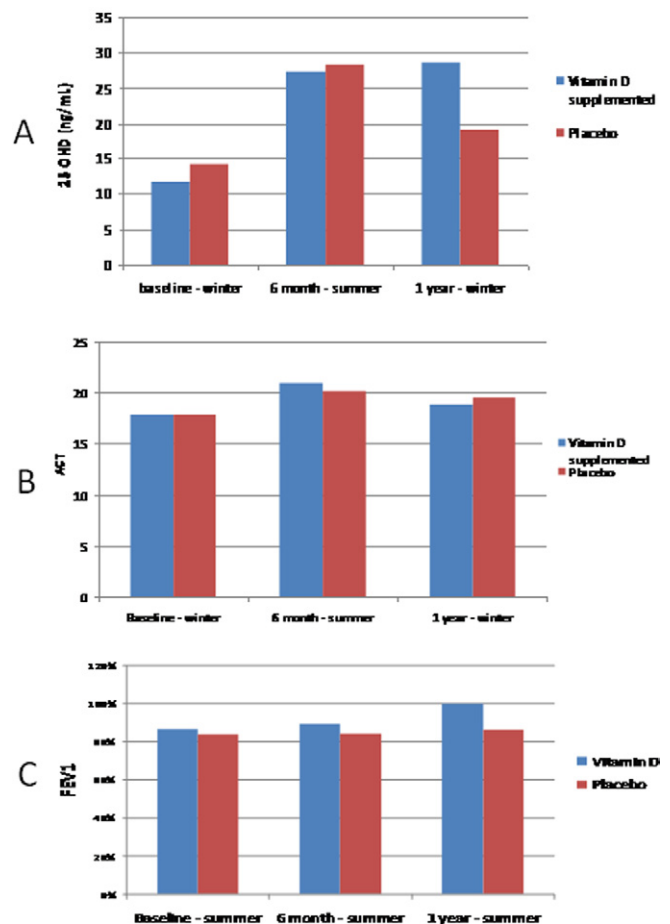


Fig. 1. Effects of season and vitamin D supplementation on serum 25-hydroxyvitamin D levels (A), Asthma Control Test scores (B), and forced expiratory volume in 1 second (C). Patients were enrolled in the winter and treated with either placebo or 1,000 IU/d of vitamin D for 1 year. The listed parameters were assessed at baseline in the winter, 6 months later in the summer, and 12 months later in the next winter. The numbers of patients for each of the 3 periods were 15 in each group at baseline, 12 in each group at 6 months, and 10 in each group at 1 year.

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