Published in final edited form as:

Ann Allergy Asthma Immunol. 2018 October ; 121(4): 497–498.e1. doi:10.1016/j.anai.2018.06.004.

Vitamin D insufficiency, T_H2 cytokines, and allergy markers in Puerto Rican children with asthma

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Keywords

Vitamin D; cytokines; children; asthma; Puerto Rico

T_H2 cytokines, including IL-4, IL-5 and IL-13, are central to the development of atopic asthma, driving eosinophilia, bronchial hyperreactivity, and mucus hypersecretion¹. In recent years, treatment of severe atopic asthma has been expanded to include anti-IL-5 antibodies. In parallel, a growing body of evidence suggests that vitamin D may be beneficial in asthma management², perhaps through immune regulation³.

In the U.S., Puerto Rican (PR) children share a disproportionate burden of asthma. We have reported that vitamin D insufficiency (a plasma 25(OH)D <30 ng/mL) is associated with severe asthma exacerbations in these children^{4,5}. Here we first examined the relation between IL-5 or IL-13 and asthma outcomes, including lung function and allergy markers in PR children. On the basis of our previous findings, we then aimed to test whether vitamin D insufficiency modifies any estimated effect of T_H2 cytokines on asthma outcomes.

A total of 678 children aged 6–14 years were recruited in San Juan (PR), using a multistage probability sampling design⁴. The study was approved by the institutional review boards of the University of Puerto Rico and the University of Pittsburgh, and parental consent and participant's assent were obtained. The study protocol included questionnaires, spirometry, and blood sample collection. Participants with data on spirometry, plasma cytokines (IL-5 and IL-13) and allergy markers were included in this analysis (n=523). Asthma was defined

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Conflict of Interest

Dr. Celedón has received research materials from Merck and GSK (inhaled steroids) and Pharmavite (vitamin D and placebo tablets), to provide medications at no cost to participants in two NIH-funded studies that are unrelated to the current work. Dr. Han and Dr. Forno declare no conflicts of interest.

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as physician-diagnosed asthma and 1 episode of wheeze in the previous year. Control subjects had neither physician-diagnosed asthma nor wheeze in the previous year. Atopy was defined as 1 positive (>0.35 kU/L) IgE to any of five common allergens (Der p 1, Bla g 2, Fel d 1, Can f 1, Mus m 1), as measured by the UniCAP 100 (Pharmacia & Upjohn, Kalamazoo, MI). Plasma IL-5 and IL-13 were measured using Bio-Plex Pro Human cytokine IL-5 and IL-13 sets on the BioPlex HTF system (Bio-Rad, Hercules, CA), as per the manufacturer's instructions. We included random duplicates within and between plates, and levels are presented as pg/ml. Undetectable cytokine levels were assigned a constant (half the lowest limit of detection), and IL-5 and IL-13 levels were then log₁₀-transformed for analysis. Plasma vitamin D was measured using Waters high-performance liquid chromatography / mass spectrophotometry (Water Corp. Milford, MA), and vitamin D insufficiency defined as a plasma 25(OH)D <30 ng/ml.

Bivariate analyses were performed using two-sample *t* tests or chi-square tests. Linear regression was used for the multivariable analysis of IL-5 or IL-13 and the outcomes of interest. The analysis of IL-5 or IL-13 and lung function measures (FEV₁, FVC, and FEV₁/FVC) or allergy markers (total IgE and eosinophil count) was first conducted in all participants, and then separately by subgroups (asthma cases vs. controls, atopic asthma vs. non-atopic asthma). We decided *a priori* to conduct analyses stratified by vitamin D insufficiency. All multivariable models were adjusted for age, sex, household income ($<$ \$15,000 or $\geq$ \$15,000/year), body mass index (BMI) z-score, and second-hand smoke (SHS) exposure; those for lung function were additionally adjusted for height and height squared. All analyses conducted in children with asthma were also adjusted for use of inhaled steroids in the previous six months.

Compared with children without asthma ($n=262$), those with asthma ($n=261$) were more likely to be atopic and currently exposed to SHS; and to have: parental history of asthma, higher BMI, higher total IgE and eosinophils, and lower FEV₁ and FEV₁/FVC (data not shown). In a multivariable analysis (eTable 1), each log₁₀-unit increment in IL-5 or IL-13 was significantly associated with 181–202 ml lower FEV₁, 4.1%–5.4% lower FEV₁/FVC, higher total IgE, and higher eosinophil count. Similar results were observed in the subgroup analysis of cases, controls, and subjects with atopic asthma, but not in that of non-atopic asthma (eTable 1).

Vitamin D insufficiency was significantly associated with higher IL-5 and IL-13 in children with asthma, particularly atopic asthma (eTable 1). In a multivariable analysis stratified by vitamin D insufficiency, IL-5 was significantly associated with increased total IgE and eosinophils in children with asthma and vitamin D insufficiency, but not in those with asthma and sufficient vitamin D levels (Table 1). Among children with asthma and vitamin D insufficiency, each log₁₀-unit increment in IL-5 was associated with 0.62 and 0.28 log₁₀-unit increments in total IgE and eosinophils, respectively. In these children, each log₁₀-unit increment in IL-13 was significantly associated with a 0.20 log₁₀-unit increment in eosinophils (Table 1).

Vitamin D insufficiency was not significantly associated with lung function in children with or without asthma. Moreover, there was no significant difference in the relation between

IL-5 or IL-13 and FEV₁ or FEV₁/FVC between children with and without vitamin D insufficiency. Vitamin D insufficiency was recently reported to be significantly associated with lower FEV₁, but not with FEV₁/FVC, in a study of 3,001 U.S. children⁶; thus, our negative results for FEV₁ could be explained by limited statistical power.

Our findings indicate that IL-5 and IL-13 are associated with lower lung function and elevated IgE and eosinophils in PR children, particularly among those with atopic asthma. Moreover, vitamin D insufficiency was shown to modify the estimated effect of IL-5 or IL-13 on total IgE and eosinophils, potentially up-regulating T_H2 responses in children with asthma. In mouse models of allergic airway disease, vitamin D treatment has been shown to suppress T_H2 activity and reduce IL-5 and IL-4 production⁷, decreasing serum total IgE and pulmonary eosinophilia⁸. Notably, vitamin D supplementation has been reported to reduce IL-13 responses in patients with cystic fibrosis⁹. Thus, our results further suggest that vitamin D could be an adjunct therapy for children with asthma and high total IgE or eosinophilia.

Our study is cross-sectional, and thus we cannot assess temporal relationships. Moreover, we cannot exclude confounding by unmeasured variables (e.g., air pollution and adherence to medications) and did not examine gene-by-vitamin D interactions. In addition, we had limited statistical power to detect modest effects of IL-5 or IL-13 on total IgE or eosinophils in children with asthma and vitamin D sufficiency. Nonetheless, our results suggest that vitamin D insufficiency has detrimental effects on asthma through T_H2 immune mechanisms. While anti-IL-5 has been shown to be effective in selected subjects with severe asthma¹⁰, ongoing clinical trials will determine whether vitamin D supplementation is a safe and inexpensive strategy to reduce asthma burden during childhood.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Source

This work was supported by grants HL079966, HL117191, and HL119952 from the U.S. National Institutes of Health (NIH), and by The Heinz Endowments. Dr. Forno's contribution was supported by grant HL125666 from the U.S. NIH.

Abbreviations:

25(OH)D	25-hydroxy-vitamin D
BMI	body mass index
FEV₁	forced expiratory volume in one second
FVC	forced vital capacity
IL-5	interleukin-5

IL-13	interleukin-13
SHS	second-hand smoke
T_H2	T helper type 2

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Table 1-

Multivariable analysis of vitamin D insufficiency and plasma levels of IL-5 and IL-13 among children with asthma (n=261)

Exposure	Interleukin-5 (IL-5) ^a	Interleukin-13 (IL-13) ^a
	β (95% confidence interval) ^b	
Vitamin D insufficiency (n=117)		
Total IgE ^a	0.62 (0.12, 1.11)[†]	0.28 (−0.17, 0.73)
Eosinophils ^a	0.28 (0.07, 0.49)[†]	0.20 (0.01, 0.40)[†]
Vitamin D sufficiency (n=144)		
Total IgE ^a	0.24 (−0.15, 0.22)	0.13 (−0.20, 0.47)
Eosinophils ^a	−0.01 (−0.23, 0.21)	0.01 (−0.18, 0.21)

All models were adjusted for age, sex, household income, BMI z-score, and use of inhaled steroids.

^aLog-10 transformed

^bFor example, among children with vitamin D insufficiency, each log-10 unit increment in IL-5 level is associated with an increment of 0.62 log-units (4.17 IU/ml) in total plasma IgE, with a 95% confidence interval of 0.12 log-units (1.32 IU/ml) to 1.11 log-units (12.88 IU/ml).

[†]P<0.05

[‡]P<0.01