

Mite allergen-specific IgE is detectable in bronchial secretions of patients with non-atopic asthma and correlates with mucosal expression of periostin

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To the Editor:

Chronic airway inflammation in asthmatic patients is classically biased toward T_H2-type immunity, with IL-4 and IL-13 as signature cytokines initiating B-cell class-switching to IgE. Immunoglobulin class-switching classically occurs in the lymphoid germinal centers, with B cells migrating in the tissue through the bloodstream. Demonstration of class-switching toward IgE by mucosal resident B cells has been provided in patients with allergic asthma and rhinitis. It is increasingly recognized that this local IgE synthesis can occur independently of systemic IgE sensitization, which is referred to as "atopy" and evidenced by positive serum IgE measurements, skin prick test responses, or both to at least 1 common aeroallergen. Both the precise origin of IgE-positive B cells¹ and the detailed specificities of local IgE remain elusive, but studies in allergic rhinitis and conjunctivitis reported that part of this local IgE might bind to aeroallergens, such as pollens.² Our group also reported that functional IgE antibodies to house dust mite (*Dermatophagoides pteronyssinus*) allergens are detected in sputum from patients with nonatopic asthma.³ Similarly, *D pteronyssinus*-specific IgE was reported to be functional in nasal polyp tissues from nonatopic donors.⁴ The clinical relevance of local specific IgE has been shown by the demonstration that anti-IgE is effective in nasal polyposis and asthma independent of atopy.⁵

In the present study we sought to evaluate sputum IgE antibodies to *D pteronyssinus* in a larger series of asthmatic patients and to correlate this local response to T_H2/eosinophilic inflammation and notably to periostin, which was recently identified as a surrogate serum marker of airway eosinophilia in asthmatic patients. We hypothesized that the local production of allergen-specific IgE antibodies is promoted by IL-13 both in patients with allergic and those with nonatopic forms of asthma.

Study participants (n = 149), including asthmatic patients and healthy control subjects, were assessed for the presence of *D pteronyssinus*-specific IgE in induced sputum. Asthmatic patients were extensively characterized for lung function, blood and sputum eosinophil counts, and serum IgE levels (see Table E1 in this article's Online Repository at www.jacionline.org) and consented to participate in the study approved by the local ethics committee (reference 2005/181). Induced sputum was obtained for each participant by means of inhalation of saline solution (4.5%; detailed methods for processing are provided in the Methods section in this article's Online Repository at www.jacionline.org). Sputum supernatants were assayed for *D pteronyssinus*-specific IgE by using ELISA, as previously described.

Supernatants were also assessed for periostin. The assumption that periostin could be detected in the sputum is based on the *in vitro* observation that human primary bronchoepithelial cells cultured markedly upregulate periostin gene expression on IL-13 stimulation.⁶ We also observed that production of periostin occurs in air-liquid interface bronchoepithelial cultures in the basolateral and apical compartments, with the effect of IL-13 being significant only at the apical pole (Fig 2).

It was first confirmed that *D pteronyssinus*-specific IgE is detectable in sputum at higher levels in asthmatic patients when compared with control subjects irrespective of atopy/systemic IgE sensitization ($P < .05$ and $P < .001$, patients with nonatopic and atopic asthma vs control subjects,

respectively; Fig 1, A), with 76% of patients with allergic asthma being sensitized to *D pteronyssinus* (serum *D pteronyssinus*-specific IgE >0.35 kU/L; see Table E1). Sputum eosinophil counts were increased in both patients with atopic and those with nonatopic asthma as either absolute numbers or proportion of leukocytes ($P < .001$, nonatopic and atopic asthma vs control subjects), with no difference between the 2 asthmatic groups (Fig 1, B). Blood and sputum eosinophil counts were significantly correlated ($r = 0.59$, $P < .001$). Only a few patients from our series presented with blood eosinophil counts of 400/mm or greater and sputum eosinophil counts of 3% or greater ($n = 9$ [6.8%]), a profile that was previously associated with poor control of the disease.⁷ In sputum, despite levels being increased only in some asthmatic patients without reaching a significant between-group difference (Fig 1, C), periostin levels significantly correlated with *D pteronyssinus*-specific IgE levels ($r = 0.37$, $P < .0001$; Fig 1, D) but not eosinophil counts ($r = 0.06$; see Video E1 in this article's Online Repository at www.jacionline.org). No significant correlation was found between sputum periostin and sputum total IgE levels ($r = 0.08$, $P = .43$) or other asthma-related parameters. The absence of correlation between periostin levels and sputum eosinophil counts could be related to the large dispersion of the latter in our study, contrasting with a recent report focused on severe eosinophilic asthma.⁸

FIG 1. **A.** *D pteronyssinus*-specific IgE antibodies in sputum from patients with nonatopic ($n = 34$) or atopic ($n = 77$) asthma and sputum from nonatopic control subjects ($n = 14$). *Lines* depict medians and ranges. **B.** Sputum eosinophils (percentage of total leukocytes) from patients with nonatopic or atopic asthma versus nonatopic control subjects. *Lines* depict medians and ranges. Data were obtained from 39 patients with intrinsic asthma, 89 patients with atopic asthma, and 21 control subjects. **C.** Periostin levels as assessed in DPBS sputum samples in patients with nonatopic ($n = 33$) and atopic ($n = 79$) asthma versus nonatopic control subjects ($n = 21$). *Lines* depict medians (interquartile ranges). **D.** Correlation between sputum levels of periostin and *D pteronyssinus*-specific IgE. Data were obtained for 114 of 149 subjects ($r = 0.37$, $P < .0001$), with 35 samples being lower than the detection limit (data not shown) for *D pteronyssinus*-specific IgE or periostin (0.75 ng/mL). * $P < .05$ and *** $P < .001$.

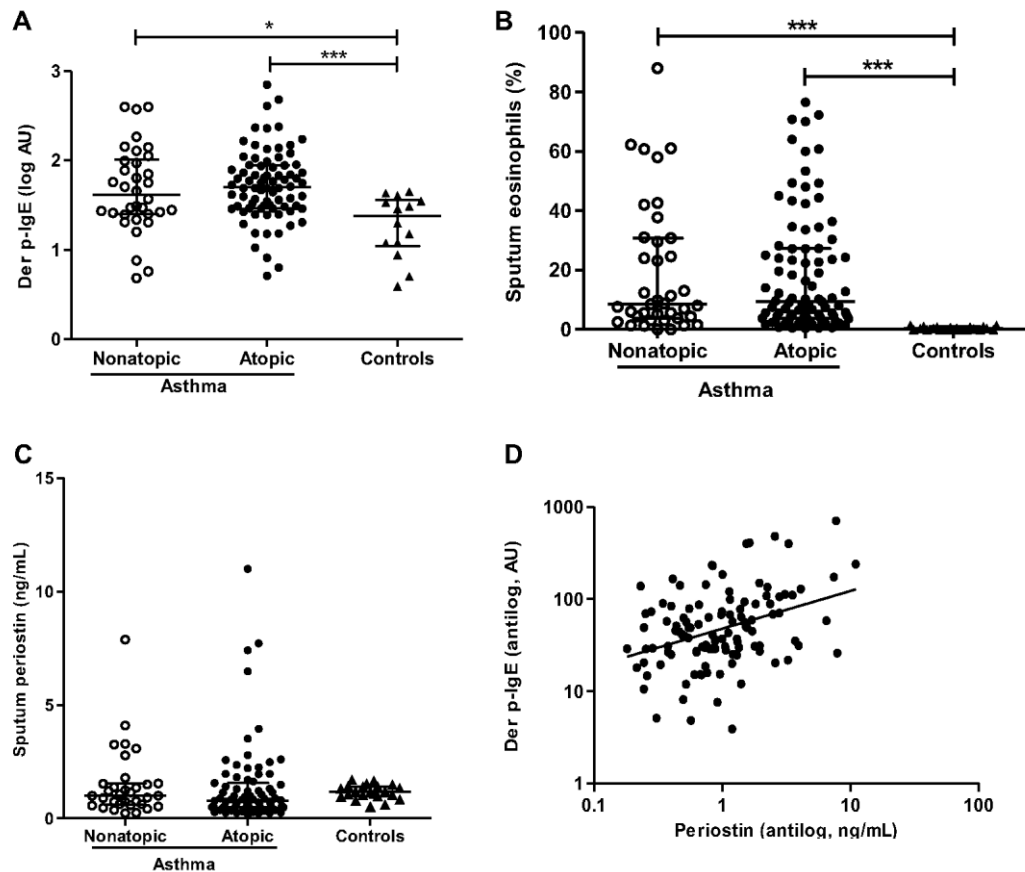
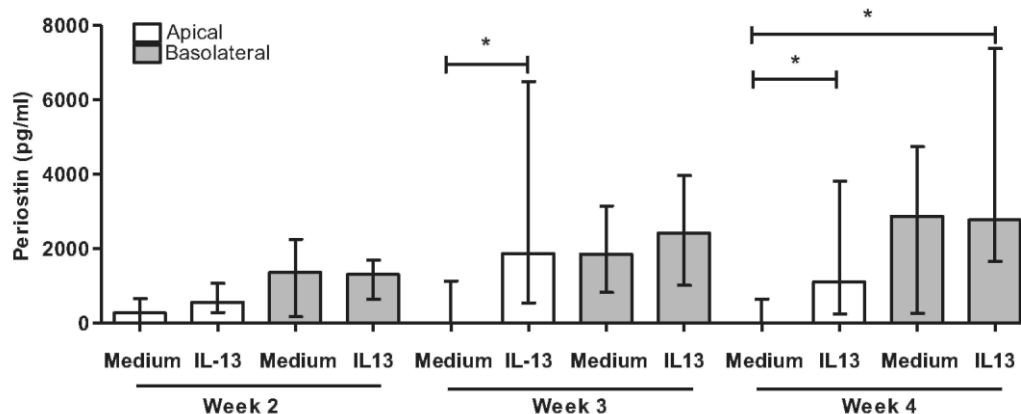


FIG 2. Periostin release (during culture for 48 hours) by human airway epithelial cells (AECs) on recombinant IL-13 stimulation (10 ng/mL every other day) versus control (medium alone) values in the basolateral and apical compartments, as assessed at weeks 2,3, and 4 in air-liquid conditions. Data are from AECs of 14 different donors. Lines depict medians (interquartile ranges). * $P < .05$.



By using immunologic or molecular techniques, several studies demonstrated that local IgE production occurs in the upper and lower airways of patients with nonatopic rhinitis, nasal polyposis, and asthma. In contrast, the specificity of this local IgE was only recently explored.^{2-4,9} In the most recent report¹⁰ the presence of local IgE was not confirmed in patients with nonatopic asthma by using a microarray analysis (Immuno-CAP ISAC; Thermo Scientific, Uppsala, Sweden) of bronchial biopsy homogenates. IgE antibodies to a large panel of allergens, including dust mite allergens, were not detected in these samples. The discrepancy between these results and ours could relate to methodological issues. The sensitivity of the microarray could be lower, as suggested by the detection of specific IgE in only a few patients with allergic asthma (in contrast to positive serum results), probably because of the described loss of total IgE after extraction and the assay characteristics, as the authors recognize. Another possibility relates to the site of sampling because it is uncertain whether tissue-bound IgE levels and secretory (sputum) IgE levels are correlated and could be affected by different local factors, including epithelial transport through the low-affinity receptor for IgE (CD23). Thus allergen-specific IgE responses should be clarified in patients with nonatopic asthma by assessing the different compartments of the bronchial mucosa, ideally by using different techniques, to detect the presence of local IgE antibodies. Of note, this study assayed fluid-phase (and not dithiothreitol-treated⁴) sputum samples, which could have affected the results by neglecting cell-bound IgE. In addition, the functionality and epitope repertoires should also be investigated and compared with those in serum because the clinical relevance of this mucosal response remains in question based on results of *in vivo* allergen challenge. Thus in contrast to patients with rhinitis, in whom one group observed clinical responses in some patients, no clinical reactivity was observed on lung challenge with mite allergen in patients with nonatopic asthma.³

The present data show that periostin can be recovered in sputum consistently with the apical release of this protein observed in bronchoepithelial cell cultures at the air-liquid interface on stimulation by the T_H2 cytokine IL-13. Periostin has been reported to be a novel serum biomarker of bronchial eosinophilic inflammation, which might be more reliable than exhaled nitric oxide. The correlation observed in the present study between sputum levels of periostin and *D pteronyssinus*-specific

IgE antibodies, but not eosinophil counts, probably relates to their common triggering mechanism, namely the IL-4/IL-13 cytokine pathway. A recent study by Bobolea et al⁸ reports a significant correlation between sputum periostin levels and eosinophil counts. This discrepancy with our results might be explained by the study population, which focused on patients with severe refractory asthma. In any case, the interest in the sputum periostin level as an additional biomarker of asthma should be considered in further larger studies, notably to clarify the link between sputum levels of periostin and eosinophil counts.

Our data confirm that in asthmatic patients local *D pteronyssinus*-specific IgE antibodies are detectable in sputum irrespective of atopy and show that this bronchial response correlates to the local periostin level as a marker of T_H2 -biased imprinting of the airway epithelium.

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REFERENCES

1. Dullaers M, De Bruyne R, Ramadani F, Gould HJ, Gevaert P, Lambrecht BN. The who, where, and when of IgE in allergic airway disease. *J Allergy Clin Immunol* 2012;129:635-45.
2. Rondon C, Romero JJ, Lopez S, Antunez C, Martín-Casañez E, Torres MJ, et al. Local IgE production and positive nasal provocation test in patients with persistent nonallergic rhinitis. *J Allergy Clin Immunol* 2007;119:899-905.
3. Mouthuy J, Detry B, Sohy C, Pirson F, Pilette C. Presence in sputum of functional dust mite-specific IgE antibodies in intrinsic asthma. *Am J Respir Crit Care Med* 2011;184:206-14.
4. Zhang N, Holtappels G, Gevaert P, Patou J, Dhaliwal B, Gould H, et al. Mucosal tissue polyclonal IgE is functional in response to allergen and SEB. *Allergy* 2011; 66:141-8.
5. Gevaert P, Calus L, Van Zele T, Blomme K, De Ruyck N, Bauters W, et al. Oma-lizumab is effective in allergic and nonallergic patients with nasal polyps and asthma. *J Allergy Clin Immunol* 2013;131:110-6.e1.
6. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci USA* 2007; 104:15858-63.
7. Schleich FN, Louis R. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. *Eur Respir J* 2014;44:97-108.
8. Bobolea I, Barranco P, Del Pozo V, Romero D, Sanz V, Lopez-Carrasco V, et al. Sputum periostin in patients with different severe asthma phenotypes. *Allergy* 2015;70:540-6.
9. Bachert C, Gevaert P, Holtappels G, Johansson SG, van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol* 2001;107:607-14.
10. Pillai P, Fang C, Chan YC, Shamji MH, Harper C, Wu SY, et al. Allergen-specific IgE is not detectable in the bronchial mucosa of nonatopic asthmatic patients. *J Allergy Clin Immunol* 2014;133:1770-2.e11.