Letter to the Editor

Mite allergen–specific IgE is detectable in bronchial secretions of patients with nonatopic asthma and correlates with mucosal expression of periostin

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 cells[1] 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.[3]

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^3 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]

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 report[10] the presence of local IgE was not confirmed in patients with nonatopic asthma by using a microarray analysis (ImmunoCAP 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-treated[4]) 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

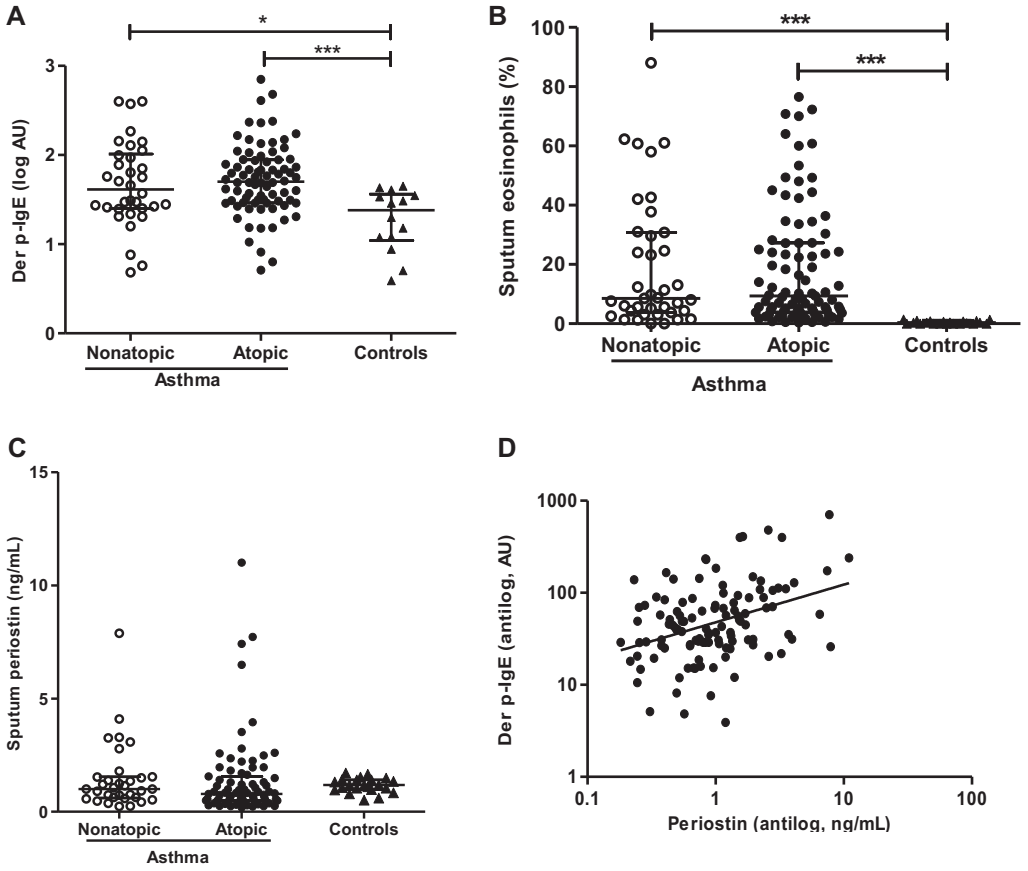

FIG 1. **A,** *D pteronyssinus*–specific IgE antibodies in sputum from patients with nonatopic (n = 34) or atopic (n = 77) asthma and sputum from nonatopic control subjects (n = 14). *Lines* depict medians and ranges. **B,** Sputum eosinophils (percentage of total leukocytes) from patients with nonatopic 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 ***$P < .001$.

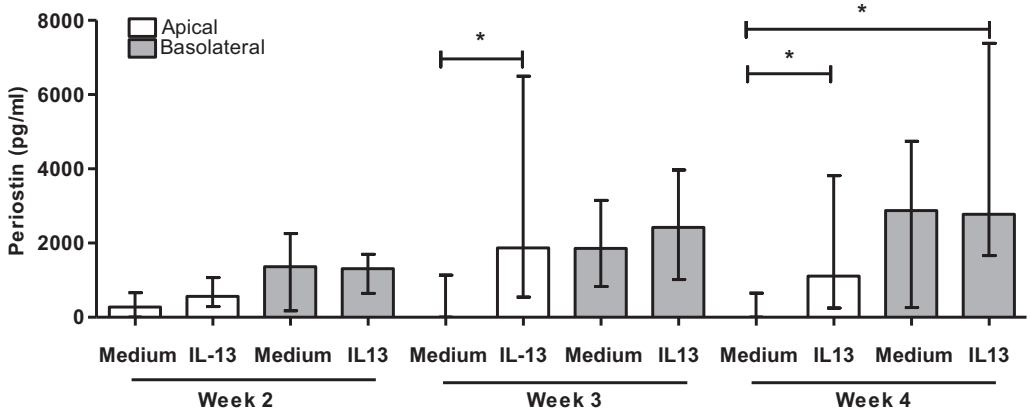

FIG 2. Periostin release (during culture for 48 hours) by human airway epithelial cells (AECs) on recombinant IL-13 stimulation (10 ng/mL every other day) versus control (medium alone) values in the basolateral and apical compartments, as assessed at weeks 2, 3, and 4 in air-liquid conditions. Data are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

in vivo allergen challenge. Thus in contrast to patients with rhinitis, in whom one group observed clinical responses in some patients,[2] 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 al[8] 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.

Jonathan Mouthuy, PhD[a]
Sophie Viart, PhD[a]
Maha Z. Ladjemi, PhD[a]
Bruno Detry, MSc[a]
Monique Henket, MSc[b]
Claus Bachert, MD, PhD[c]
Louis Renaud, MD, PhD[b]
Charles Pilette, MD, PhD[a]

From [a]Université Catholique de Louvain (UCL), Institut de Recherche Expérimentale et Clinique (IREC), Pole de Pneumologie, ORL & Dermatologie, Brussels, Belgium; [b]the Department of Pulmonary Medicine, CHU Sart-Tilman, Liege, IGIGA Research Group, University of Liege, Liege, Belgium; and [c]the Upper Airway Research Laboratory and Department of Otorhinolaryngology, Ghent University, Ghent, Belgium. E-mail: charles.pilette@uclouvain.be.

Disclosure of potential conflict of interest: J. Mouthuy has received research support from Novartis and AstraZeneca and consultancy fees from Novartis. L. Renaud is on the boards for AstraZeneca, GlaxoSmithKline, and Mundipharma; has received consultancy fees from Orion; has received research support from AstraZeneca, GlaxoSmithKline, Novartis, and Chiesi; has received lecture fees from Novartis, Chiesi, and Pfizer; and has received payment for developing educational presentations from Menarini. C. Pilette has received research support from Novartis, AstraZeneca, GlaxoSmithKline, and Chiesi and is on the advisory boards for Novartis, AstraZeneca, Chiesi, and GlaxoSmithKline. The rest of the authors declare that they have no relevant conflicts of interest.

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, López S, Antúnez 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. Omalizumab 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 U S A 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, López-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.

http://dx.doi.org/10.1016/j.jaci.2015.07.018

METHODS

Participants

Asthmatic patients were recruited from outpatient clinics at the Pneumology Department of CHU Liège, Liege, Belgium. Inclusion criteria were a history of asthma (defined according to the Global Initiative for Asthma guidelines) since at least 6 months, with documented nonspecific bronchial hyperreactivity to methacholine, peak expiratory flow variability (>20% diurnal variability), or both and age of between 18 and 70 years. Patients with nonatopic asthma were characterized by the absence of a skin prick test response to a battery of 18 common aeroallergens in our country (cockroach, *D pteronyssinus*, *Dermatophagoides farinae*, cat, dog, tree pollens [birch, alder, hazel, and ash], grass pollens [5-grass mix], *Artemisia* species, plantain, *Aspergillus* species [mix], *Cladosporium* species, *Alternaria* species, *Penicillium* species, latex, and bird feathers) and by negative (<0.35 kU/L) serum specific IgE levels (to *D pteronyssinus*, *D farinae*, cat, dog, *Aspergillus fumigatus*, *Alternaria tenuis*, and *Cladosporium herbarum*). Smoking status was recorded at the time of inclusion. All subjects were stable at the time of inclusion, with no evidence of respiratory tract infection within the past 6 weeks. All subjects provided written informed consent to participate in the study, which was approved by our local ethics committee (reference 2005/181). Patients' and control subjects' characteristics are depicted in Table E1.

Sputum sampling and processing

Sputum induction was performed by means of inhalation of either hypertonic or isotonic saline according to postbronchodilator FEV_1 values. Patients with an FEV_1 of greater than 65% of predicted value inhaled 4.5% saline, whereas those with an FEV_1 of less than 65% of predicted value were given 0.9% saline. Saline was inhaled through an ultrasonic nebulizer (Ultra-Neb 2000; DeVilbiss, Somerset, Pa). The cup of the nebulizer was filled with 50 mL of hypertonic/isotonic saline to which we added 1.75 mL of salbutamol solution at 5 mg/mL. FEV_1 was measured at 1, 3, 5, and 10 minutes after the start of the procedure. Inhalation was stopped after 10 minutes or when a decrease in FEV_1 of greater than 20% from baseline had occurred. Subjects were then asked to rinse their mouths with tap water and to cough up sputum into a plastic container. For safety reasons, FEV_1 was measured 10 and 20 minutes after the end of induction in every patient.

Sputum samples were homogenized with 3:1 (vol/wt) Dulbecco PBS (DPBS) Ca^{+}- and Mg^{++}-free solution and centrifuged at 800*g* for 10 minutes at 4°C. Supernatants were stored at −80°C until analysis. The cellular phase was dispersed in 1 mL of DPBS and diluted twice with Sputolysin reagent (Calbiochem, San Diego, Calif). The cell suspension was rocked at room temperature for 20 minutes and centrifuged at 400*g* for 10 minutes at 4°C. After washing with DPBS, the cell pellet was suspended in DPBS for determination of total cell counts and cell viability by using the trypan blue exclusion method with a manual hemocytometer. The differential cell count was performed on cytospin preparations stained with Rapi Diff II (Atom Scientific, Manchester, United Kingdom) by counting 500 nonsquamous cells with a light microscope.[E1]

Sputum fluid-phase measurements

***D pteronyssinus*–specific IgE.** The ELISA technique used to detect IgE in sputum has been extensively detailed.[E2] *D pteronyssinus*–specific antibodies were assayed in PBS supernatants by using ELISA. Microtiter plates were coated with 5 μg/mL *D pteronyssinus* extract (Stallergenes, Antony, France), and 50 μL of undiluted sputum samples was incubated for 1 hour at 37°C. Horseradish peroxidase–conjugated rat monoclonal anti-human IgE (clone LO-HE17, 11 μg/mL) was used as a detection antibody. Extensive controls were used to validate the specificity of our immunoassay by using the clone LO-HE17.[E2]

Periostin. Periostin was assayed in sputum supernatants by using Human Periostin/OSF-2 DuoSet (R&D Systems, Oxon, United Kingdom), according to the manufacturer's instructions. Microtiter plates were coated with 1 μg/mL of the capture antibody diluted in bicarbonate buffer (pH 9.6) and incubated overnight at 4°C. Plates were then blocked with BSA 1% in PBS for 1 hour at 37°C. Standard (12 ng/mL, recombinant periostin) sputum samples (50 μL per well) were then incubated for 1 hour at 37°C. Afterward, biotinylated antibody was added and incubated for 1 hour at 37°C. Streptavidin–poly-HRP (1:10,000, 50 μL per well; BD Biosciences, Erembodegem, Belgium) was then added. The reaction was developed with TMB (Tetramethylbenzidine, 100 μL per well) and stopped after 15 minutes by H_2SO_4. Absorbance was read at 450 nm. Washing of plates was performed 3 times with 0.05% Tween-20 in PBS between each step. Spiking and recovery assays have been run on pooled (10 unselected samples) sputum supernatants to validate the dosing method and reported a mean recovery of 81% of periostin.

Primary bronchoepithelial cell cultures

Primary airway epithelial cells (AECs) were derived from proximal specimens of lung tissue of 14 patients undergoing lung resection surgery for a solitary tumor. Lung tissue was sampled away from the tumor site to obtain a large sample for primary AEC culture. AECs were then cultured in polarized and air-liquid interface conditions to reconstitute a pseudostratified mucociliary epithelium.[E3] Twelve-well polyester membrane inserts (0.4-μm pore size; Corning, Corning, NY) were coated with 0.2 mg/mL collagen IV (Sigma-Aldrich, St Louis, Mo), and then AECs were seeded at a density of 50,000 cells per well. AECs were cultured in submerged conditions until confluence (approximately 10 days), and cell differentiation was then allowed by removing medium in the apical compartment (ie, the air-liquid interface condition) for 15 to 21 days. AECs were cultured in bronchial epithelial cell basal medium/Dulbecco modified Eagle medium (1:1) medium supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), bovine pituitary extract (52 μg/mL), insulin (5 μg/mL), hydrocortisone (0.5 g/mL), transferrin (10 μg/mL), epinephrine (0.5 μg/mL), epidermal growth factor (0.5 ng/mL), triiodothyronine (3.25 ng/mL), BSA (1.5 μg/mL), and retinoic acid (30 ng/mL; Lonza, Verviers, Belgium). IL-13 (R&D Systems, Abingdon, United Kingdom) was added to basolateral medium (10 ng/mL) every 2 days of air-liquid interface culture for 2, 3, and 4 weeks. The basolateral medium was renewed every other day (600 μL), and 48 hours before each time point sampling, the apical surface was washed with PBS to remove most mucus and secreted factors. The day of collection, the apical surface was sampled with 300 μL of PBS in which periostin content was assessed (in undiluted samples), thus representing its production during 48 hours on resting or IL-13–stimulated conditions. A 2-fold dilution factor has been applied for basolateral concentrations.

Statistics

The Kruskal-Wallis test with the Dunn posttest was applied to periostin levels, IgE (both specific or total) sputum levels, and cellular counts, as well as analysis of AEC-pulsed cultures. Pearson analysis was used for correlation analysis. A *P* value of less than .05 was considered significant.

REFERENCES

E1. Delvaux M, Henket M, Lau L, Kange P, Bartsch P, Djukanovic R, et al. Nebulised salbutamol administered during sputum induction improves bronchoprotection in patients with asthma. Thorax 2004;59:111-5.

E2. 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.

E3. Puchelle E, Zahm JM, Tournier JM, Coraux C. Airway epithelial repair, regeneration, and remodeling after injury in chronic obstructive pulmonary disease. Proc Am Thorac Soc 2006;3:726-33.

TABLE E1. Study participants' characteristics

	Patients with intrinsic asthma (n = 39)	Patients with atopic asthma (n = 89)	Control subjects (n = 21)
Age (y)	54.8 ± 15.5*	47.5 ± 15.9	55.9 ± 9.3
Sex (M/F)	14/25	38/51	5/10
Current smokers or exsmokers	19	35	9
Pack years smoked	24 ± 15	17 ± 18	15 ± 7
ICS therapy	24 (66%)	57 (64%)	—
ICS daily dose (BDP eq)	700 (200-1400)	700 (100-1400)	—
FEV_1 (% predicted)	80 (43-134)†	83 (34-117)†	106 (62-115)
FEV_1/FVC ratio (%)	69.8 (48.3-133.3)	73.5 (41-41)	78 (64-86)
F_{ENO} (ppb)	21.6 (4.5-192)	29.7 (7-347.6)	NA
Blood eosinophils (%)	2.65 (0.1-15.6)	2.9 (0-13.3)	NA
Sputum eosinophils (%)	8.5 (0-88)‡	9.4 (0.4-76.5)	0.4 (0-1.5)
Serum total IgE (kU/L)	139 (4-999)*	161 (2-3183)	NA

Data are presented as medians (ranges), except age and ICS therapy (mean ± SD).

BDP eq, Beclomethasone dipropionate equivalent; *F*, female; *FENO*, fraction of exhaled nitric oxide; *FVC*, forced vital capacity; *ICS*, inhaled corticosteroids; *M*, male; *NA*, not applicable.

*$P < .05$ versus patients with atopic asthma.

†$P < .01$ versus control subjects.

‡$P < .001$ versus control subjects.