

Presence in Sputum of Functional Dust Mite–Specific IgE Antibodies in Intrinsic Asthma

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Rationale: Intrinsic asthma was described by Rackemann as asthma without allergy. Local IgE production has been documented in intrinsic asthma, but antigen specificity of this response remains elusive.

Objectives: We investigated (1) the presence of dust mite–specific IgE in sputum of patients with intrinsic asthma, (2) their clinical/immunological relevance, and (3) their functionality.

Methods: Specific IgE to *Dermatophagoides pteronyssinus* (Der p) and to recombinant major allergens (rDer p1 and rDer p2) were assayed by ELISA in sputum samples from patients with intrinsic versus atopic asthma and control subjects. Whole-lung challenge was performed with Der p for clinical and inflammatory readouts. Functionality of local IgE to trigger effector cells was assessed using basophil activation test (surface expression of CD203c).

Measurements and Main Results: Both total IgE and Der p–specific IgE levels are increased in patients with intrinsic asthma compared with healthy nonatopic patients. However, no immediate asthmatic responses were observed in patients with intrinsic asthma after Der p exposure. These sputum Der p–specific IgE do, however, recognize major allergens Der p1 and Der p2 and are able to trigger activation of blood basophils from atopic donors.

Conclusions: We confirm that IgE production occurs in intrinsic asthma and show that part of this IgE recognizes Der p antigens. However, this IgE reactivity does not translate into clinical responses to Der p exposure, despite specificity to major allergens and functionality to activate effector cells *in vitro*. We postulate that a second signal that promotes IgE-mediated asthmatic responses through FcεRI is lacking in intrinsic asthma.

Keywords: asthma/immunology; cytokines/analysis; IgE/analysis; non-atopic

Intrinsic asthma was first described by Rackemann (1) in 1947 as a form of asthma that is not triggered by environmental exposures. After discovery of IgE (2), intrinsic asthma was defined as asthma without atopy (i.e., without skin reactivity and/or serum-specific IgE to common aeroallergens). Despite this striking difference, intrinsic asthma shares many clinical features with allergic asthma, including airway inflammation, airway hyperreactivity to nonspecific stimuli, and viral-induced exacerbations. More recent immunopathological studies indicated that intrinsic asthma is associated—as its allergic counterpart—with Th2-type airway inflammation, including increased numbers of

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Evidence of a Th2 bias has been demonstrated both in atopic and intrinsic asthma. Furthermore, mucosal production of IgE also occurs in nonatopic rhinitis or asthma. However, antigen specificity and functionality of mucosal IgE remain elusive in these diseases.

What This Study Adds to the Field

We confirm that local IgE production occurs in sputum of patients with intrinsic asthma. We also show that part of this IgE is specific to *Dermatophagoides pteronyssinus* allergens and that this IgE is able to mediate antigen-driven activation of effector cells.

eosinophils and mast cells and increased expression of Th2 cytokines such as IL-4, IL-5, and IL-13 (3–5).

In addition, some patients with intrinsic asthma display increased serum levels of total IgE (> 150 U/ml in ~ 20% of cases) (6, 7), which correlates with airflow limitation and disease severity (6). Moreover, molecular signatures of local IgE synthesis have been observed in bronchial biopsies of patients with intrinsic asthma (7). However, the antigen specificity of this local IgE response remains elusive. Among hypotheses regarding the pathophysiology of intrinsic asthma are microbial superantigens able to activate IgE-producing B cells (8), autoimmunity to IgE receptors (9), or related to molecular mimicry (10), as well as antigen-specific IgE responses to unidentified allergens. Local IgE responses without systemic evidence of sensitization has been suggested in some patients with nonallergic rhinitis (11) and referred to as entopy.

We hypothesized that some patients with intrinsic asthma may display local IgE responses to perennial allergens (house dust mites), despite absence of serological or skin reactivity. The aim of this study was to explore, in a group of patients with intrinsic asthma without at-risk occupational exposure, the presence in airway secretions of IgE antibodies to house dust mite (*Dermatophagoides pteronyssinus*, Der p), the major perennial allergen associated with chronic asthma in our country. The clinical and immunological effects of lung exposure to Der p allergens were evaluated in these patients, as compared with healthy nonatopic subjects and patients with atopic asthma. Functionality of sputum IgE antibodies was finally addressed in a cell-based *in vitro* assay, using basophils as a model of immediate allergic response. Some of the results of this study have been previously reported in the form of an abstract (12).

METHODS

Additional details on methodology are provided in the online supplement.

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Study Participants

Patients with asthma (24 allergic and 29 nonatopic) and 25 healthy nonatopic subjects were recruited. Atopic status was determined on the basis of positive skin prick test to at least one allergen of a battery of 18 common aeroallergens in our country and/or serum-specific IgE greater than 0.35 kU/L (ImmunoCap system; Phadia, Uppsala, Sweden). All enrolled patients with atopic asthma were at least sensitized to *Dermatophagoides pteronyssinus* (Der p). Patients with intrinsic asthma were characterized by absence of skin prick response (to the battery of 18 allergens) and by negative (< 0.35 kU/L) serum-specific IgE (to *Der. pteronyssinus*, *Dermatophagoides farinae*, cat, dog, *Aspergillus fumigatus*, *Alternaria tenuis*, and *Cladosporium herbarum*). Participant characteristics are depicted in Table 1. All subjects gave written informed consent to participate in the study, which was approved by our local ethical committee.

Allergen-Specific Lung Challenge

Twelve patients with intrinsic asthma, 11 patients with allergic asthma, and 6 (nonatopic) control subjects were submitted to a whole-lung challenge to Der p extract (Stallergènes, Antony, France) using a dosimeter (Spira-Elektro2; Hameenlinna, Finland). The starting allergen dose was determined for each allergic patient by 1:100 dilution of the end point of prick-test scale (1, 10, and 100 reactivity index). A starting dilution of 1 reactivity index was used for patients with intrinsic asthma and healthy control subjects. Der p allergen was administered by doubling doses series (2, 4, and 8 inhalations). Symptoms and FEV₁ were assessed 15 minutes after exposure until FEV₁ decreased by at least 20% or more of baseline measure. Subjects remained under observation for 6 hours. Before and 24 hours after the allergen challenge, sputum and blood samples were collected and provocative concentration of histamine causing a 20% fall in FEV₁ (PC₂₀) was determined.

Sputum Sampling and Processing

Induced sputum was obtained by using the method of Pizzichini and colleagues (13), with minor modifications.

Nonspecific Airway Hyperresponsiveness

Airway hyperresponsiveness (AHR) to histamine was assessed using the five-breath dosimeter method according to American Thoracic Society guidelines (14) until an FEV₁ fall 20% or greater from baseline could be detected.

Immunoassays for Sputum Total IgE and Der p-Specific IgE

Total IgE was determined in undiluted sputum dithiothreitol (DTT)-treated samples by sandwich ELISA using monoclonal rat LO-HE17 anti-human IgE (this antibody was a generous gift of H. Bazin and P. Gianello, Université catholique de Louvain, Brussels) as capture Ab and affinity-purified rabbit IgG anti-human IgE (L749 made in our laboratory) as detection Ab.

Specific IgE to Der p and recombinant Der p1 and Der p2 proteins were assayed by ELISA using plates coated with the antigens

(Stallergènes) and using LO-HE17 as detection Ab. Results were expressed in arbitrary units (AU) as referred to values of internal control serum from sensitized subjects, containing IgE to Der p (78.8 kU/L) and recombinant Der p allergens (> 100 kU/L), with 1:2 dilution set as 100 AU. Serum-specific IgE measurements were obtained by using ImmunoCAP system (Phadia).

Specific IgE to cat (*Felis domesticus*; Fel d) and timothy grass pollen (*Phleum pratense*; Phl p) were also determined using the same technique and reference serum containing cat-IgE (45.8 kU/L) or Timothy grass pollen-IgE (> 100 kU/L).

Basophil Activation Test

Basophil activation test was performed following manufacturer's instructions (Allergen Kit; Beckman Coulter, Immunotech, Marseille, France). Preliminary tests were performed to define suboptimal concentrations of Der p extract (final concentration between 4 and 8 ng/ml) allowing to reveal a clear-cut signal when adding sputum from Der p-allergic asthmatics.

Stripping experiments were performed in basophil-enriched peripheral blood mononuclear cells (PBMCs) from atopic donors to remove cell-bound IgE, to confirm that activation was attributable to sputum-specific IgE. Basophil activation was then assessed by gating on CRTh2⁺IgE⁺ PBMCs that display surface expression of CD203c. Data were expressed as percentage of activated CRTh2⁺IgE⁺ cells.

Statistics

The Mann-Whitney *U* test was used for unpaired data, and the Wilcoxon test was used for paired analysis. For basophil experiments, data are presented as mean ± SEM and analyzed using unpaired *t* test. Strength of relation between measures was analyzed using Spearman correlation. A *P* value of less than 0.05 was considered as statistically significant. All statistical analyses were performed using GraphPad Prism (version 5.00 for Windows; GraphPad Software, San Diego CA; www.graphpad.com).

RESULTS

Der p-Specific IgE in Sputum from Patients with Intrinsic Asthma

Local production of IgE was first confirmed by assessment of total IgE in sputum from intrinsic and allergic asthma. Sputum total IgE level was significantly increased in patients with intrinsic and allergic asthma as compared with nonatopic control subjects (Figure 1A). In line with this local response, no correlation was found between sputum and serum (total) IgE levels in the overall asthma population ($r = 0.26$, $P = 0.22$) (see Figure E1A in the online supplement).

Sputum Der p-specific IgE were also significantly increased in patients with intrinsic asthma as compared with control subjects ($P = 0.018$). Although overlap was observed between the three groups, median levels of Der p-specific IgE were similar between

TABLE 1. CLINICAL DATA OF STUDY PARTICIPANTS

	Intrinsic Asthma (n = 29)	Allergic Asthma (n = 24)	Control Subjects (n = 25)
Sex, female/male, n	17/12	14/10	17/8
Age, yr	54 (32–73)*†	42 (19–65)†	30 (22–51)
Current smokers/ex-smokers, n	3/10	3/7	7/1
Serum total IgE, kU/L	38.5 (2.0–490.0) (n = 20)	290 (19.0–1,442.0)*‡ (n = 19)	16 (9.0–42.0) (n = 6)
Serum Der p-specific IgE	<0.35 (n = 23)	6.57 (0.1–100)*‡ (n = 20)	<0.35 (n = 6)
Skin Der p response, mm ²	0.0 (0.0–0.0) (n = 29)	55.5 (20–240) (n = 22)	0.0 (n = 25)
FEV ₁ , L	2.59 (1.79–4.21)	2.89 (1.20–5.20)	3.35 (2.42–5.99)*‡
FEV ₁ , % predicted	90.48 (60.73–128.6)	90.2 (40.4–117.4)	104.0 (82.70–128.5)*‡
Inhaled corticoids µg/d	336 (100–1,400) (n = 27)	700 (269–2,100)* (n = 17)	0

Definition of abbreviation: Der p = *Dermatophagoides pteronyssinus*.

Data are presented as median (range) unless otherwise noted. Inhaled corticoids are expressed as µg/d of beclomethasone dipropionate equivalent.

* $P < 0.05$ versus allergic asthma.

† $P < 0.05$ versus control subjects.

‡ $P < 0.05$ versus intrinsic asthma.

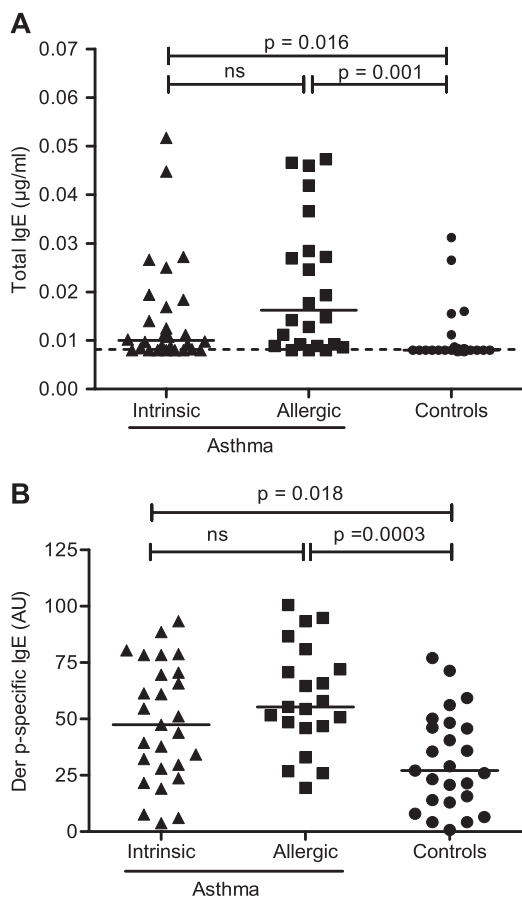


Figure 1. Sputum IgE in intrinsic asthma and specificity to *Dermatophagoides pteronyssinus* (Der p). (A) Total IgE levels in dithiothreitol (DTT)-treated sputum samples from patients with intrinsic asthma ($n = 29$) or allergic asthma ($n = 24$) and from nonatopic control subjects ($n = 25$). Results are expressed in $\mu\text{g/ml}$ as referred to calibration curve of myeloma IgE with a detection limit of $0.008 \mu\text{g/ml}$. Samples below detection limit were considered as not different from it. (B) Der p-specific IgE levels in DTT-treated sputum samples from patients with intrinsic asthma or allergic asthma and from nonatopic control subjects. Horizontal lines depict medians. Data were obtained for $n = 27$ patients with intrinsic asthma, $n = 21$ patients with atopic asthma, and $n = 25$ control subjects. AU = arbitrary units; ns = not significant.

intrinsic and Der p-allergic patients with asthma (Figure 1B). There was no correlation between sputum and serum Der p-specific IgE in patients with allergic asthma ($R = 0.42$, $P = 0.20$) (Figure E1B).

IL-4, a key cytokine for IgE synthesis, was increased in patients with intrinsic asthma but did not reach statistical significance. In contrast, a significant increase was observed in patients with allergic asthma ($P = 0.048$ and $P = 0.006$ compared with patients with intrinsic asthma and control subjects, respectively) (Figure E2). In addition, no relation could be observed between IL-4 levels and local IgE production (total or specific, not shown). No significant effect on total and Der-specific IgE levels was observed when taking into account smoking history (smokers versus never/occasional and less than 10 pack-years) or treatment by inhaled corticosteroids (not shown).

ELISA Specificity and Interferences To Detect Sputum IgE

The clone LO-HE17 was first confirmed specific to human IgE (Figure E3A), and preincubation of serum or sputum with increasing concentrations of Der p abolished the ELISA signal

(Figures E3B and E3C, respectively). In addition, in serum, ELISA for Der p-IgE strongly correlated to Der p ImmunoCAP values ($r = 0.94$, $P < 0.001$).

Absence of interference with DTT or phenylmethylsulfonyl fluoride was also checked (Figure E4). The effect of DTT on Der p-IgE ELISA was assessed in sputum and serum (Figure E4A). A slight decrease in the binding signal was observed when adding DTT (0.05% and 0.1%) in the serum from one patient with allergic asthma, whereas no effect was observed in sputum. A sharp (nonspecific) increase was observed at high concentration (1%) of DTT, both in serum and sputum as well as in PBS control. However, the ELISA signal was not affected in the concentration range used to treat sputum samples, with no effect up to 0.1%, which represents two times the final concentration used. Also, phenylmethylsulfonyl fluoride had no significant effect at 2% (concentration used), whereas high concentrations of 5% or greater induced a decrease of signal in the different specific-IgE ELISA (Figure E4B).

Airway Inflammation in Intrinsic Asthma

Patients with intrinsic asthma, similarly to their allergic counterparts, showed increased sputum eosinophilia compared with healthy subjects ($P = 0.0007$, Figure 2A and Table 2). Also, a mild increase in neutrophils was observed in patients with intrinsic asthma, which was significant when compared with patients with allergic asthma ($P = 0.01$) but not to control subjects, and was associated with a proportional decrease in macrophages.

In line with sputum eosinophilia, sputum IL-5 was significantly increased in patients with intrinsic asthma as compared with control subjects ($P = 0.0017$, Figure 2B). Of note, no significant difference in total protein content could be observed in sputum supernatants from the different groups (not shown).

Clinical Response to Der p Exposure in Intrinsic Asthma

All patients with allergic asthma subjected to Der p challenge ($n = 11$) developed immediate bronchoconstrictive responses to antigen exposure, with a median (range) antigen PD₂₀ of 28.2 ng (0.1–828.4 ng) of Der p1 (Figure 3B). Based on lung function measures (hospital FEV₁ and/or home peak expiratory flow [PEF]), two allergic asthma volunteers also developed late-phase reactions (i.e., > 4 h after the last antigen dose) (Figure E5). No patients with intrinsic asthma ($n = 12$) or healthy control subjects ($n = 6$) developed immediate asthmatic responses after Der p exposure, in terms of symptoms or lung function, expressed as FEV₁ monitoring (Figures 3A–3C) or maximal FEV₁ change at 15 minutes after exposure (Figure 4), even though three patients with intrinsic asthma displayed mild reductions in FEV₁ ranging from 120 to 500 ml.

Physiological and Immunological Responses to Der p Exposure in Intrinsic Asthma

We then evaluated whether antigen exposure of patients with intrinsic asthma resulted in subclinical airway inflammation, as reflected by changes in nonspecific AHR or sputum eosinophils. In patients with allergic asthma (Figure 5), a significant increase in histamine AHR was observed 24 hours after antigen exposure (42% reduction in median PC₂₀: median [range] 0.92 mg/ml [0.08 – 7.34 mg/ml] vs. 1.59 mg/ml [0.39 – 13.72 mg/ml] at baseline, $P = 0.003$). Four patients with allergic asthma had moderate-to-severe AHR (i.e., $< 1 \text{ mg/ml}$); however, removing them from the analysis did not affect statistical inference (After versus Before, $P = 0.03$). In contrast to patients with allergic asthma, no significant change in AHR was observed in patients with intrinsic asthma ($P = 0.193$) (Figure 5). Two patients with

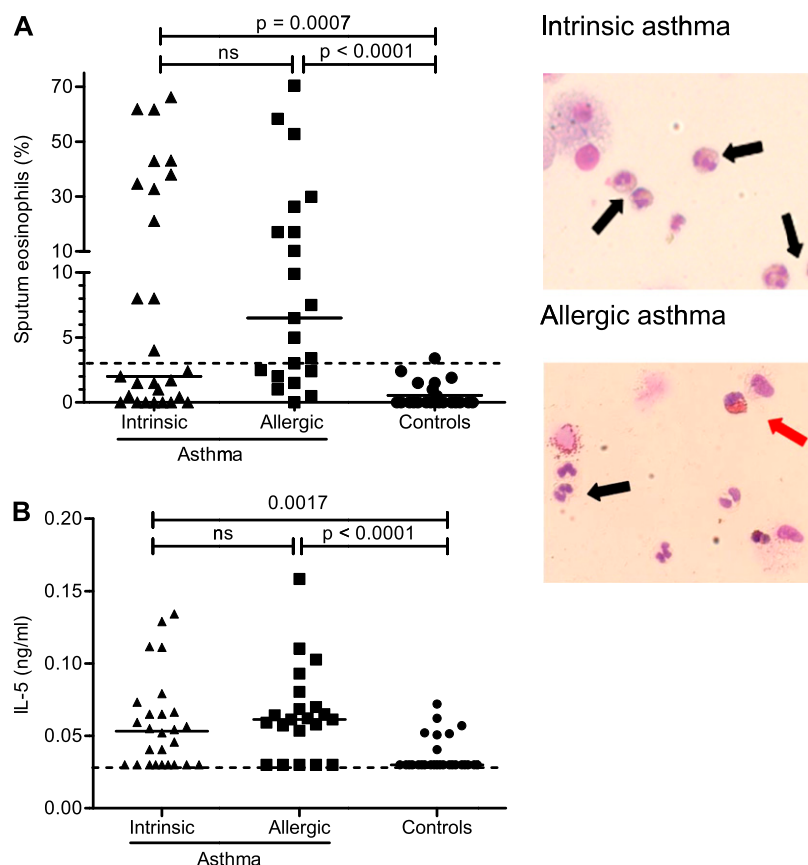


Figure 2. Eosinophils and IL-5 in sputum from patients with intrinsic asthma or allergic asthma and from nonatopic control subjects. (A) Eosinophils expressed as % total leukocytes on sputum cytopins. Dashed line reflects the cut-off values of normal eosinophilia (3% leukocytes). Insets represent photomicrographs (magnification 400 $\times$, red arrow indicates eosinophils, black arrows indicate neutrophils) in one patient with intrinsic (upper inset) and one patient with allergic (lower inset) asthma. (B) Sputum IL-5 in patients with intrinsic asthma, patients with allergic asthma, and nonatopic control subjects. IL-5 was assessed by ELISA with a detection limit ~ 0.03 ng/ml; samples below this limit were considered as not different from it. Horizontal lines depict medians. Data were obtained for $n = 26$ patients with intrinsic asthma, $n = 21$ patients with atopic asthma, and $n = 20$ control subjects. ns = not significant.

intrinsic asthma had no significant AHR ($PC_{20} > 16$ mg/ml) at the time of the study (not shown), one of these presenting AHR 24 hours after Der p exposure (from > 16 mg/ml to 7.68 mg/ml, not included in the figure).

In patients with allergic asthma, sputum eosinophilia tended to increase ($P = 0.08$) and blood eosinophils increased significantly ($P = 0.009$) after allergen challenge (Table 3, Figures E6A and E6B). In contrast, sputum eosinophilia did not change in patients with intrinsic asthma exposed to Der p when expressed as percent of leukocytes (Table 3) or absolute numbers (not shown).

Specificity of Sputum IgE to Der p Major Allergens in Intrinsic Asthma

To address the apparent discrepancy in intrinsic asthma between the presence of Der p-specific IgE and the absence of clinical or inflammatory responses to *in vivo* Der p exposure, we evaluated whether sputum IgE from these patients were able to bind to relevant allergen components from house dust mite and subsequently to trigger (*in vitro*) cellular responses. We observed that sputum IgE antibodies from patients with intrinsic asthma were able to bind to recombinant Der p1 and Der p2, at

a significantly higher level as compared with control subjects ($P = 0.045$ and $P = 0.024$, Figures 6A and 6B, respectively). A trend for increased levels of Der p2-IgE was also detected in patients with allergic asthma compared with control subjects but did not reach statistical significance.

Sputum-Specific IgE to Other Allergens in Intrinsic Asthma

As performed for Der p-IgE, specificity of the ELISA to detect cat-specific IgE was assessed by neutralizing the signal by preincubating serum (Figure E3B) or sputum (Figure E3D) with increasing concentrations of cat extract. Significant ELISA inhibition was also observed for grass-IgE in serum (Figure E3B) and to a lower extent in sputum (maximal inhibition ~ 25 –50%, not shown).

No significant increase in sputum cat- or grass-IgE was observed in patients with intrinsic asthma (Figures E7A and E7B) or in patients with allergic asthma sensitized to these allergens.

Functionality of Sputum Der p-IgE from Intrinsic Asthma To Activate Basophils

As sputum IgE antibodies from patients with intrinsic asthma were able to recognize relevant components of Der p allergens,

TABLE 2. SPUTUM CELL COUNTS AT BASELINE

	Intrinsic Asthma ($n = 29$)	Allergic Asthma ($n = 24$)	Control Subjects ($n = 25$)
Total cells, $\times 10^6$ /g	7.47 (1.77–32.00)*,†	3.7 (1.50–20.30)	4.78 (1.00–8.86)
Eosinophils, %	2.0 (0.0–66.2)†	6.5 (0.0–70.4)†	0.0 (0.0–3.4)
Neutrophils, %	40.9 (1.7–75.7)*	18.0 (1.0–56.9)	26.5 (4.7–76.8)
Macrophages, %	40.3 (0.0–85.7)†,*	67.5 (24.1–90.0)	73.6 (21.70–92.9)

Data are presented as median (range).

* $P < 0.05$ vs allergic asthma.

† $P < 0.05$ vs control subjects.

‡ $P < 0.05$ vs intrinsic asthma.

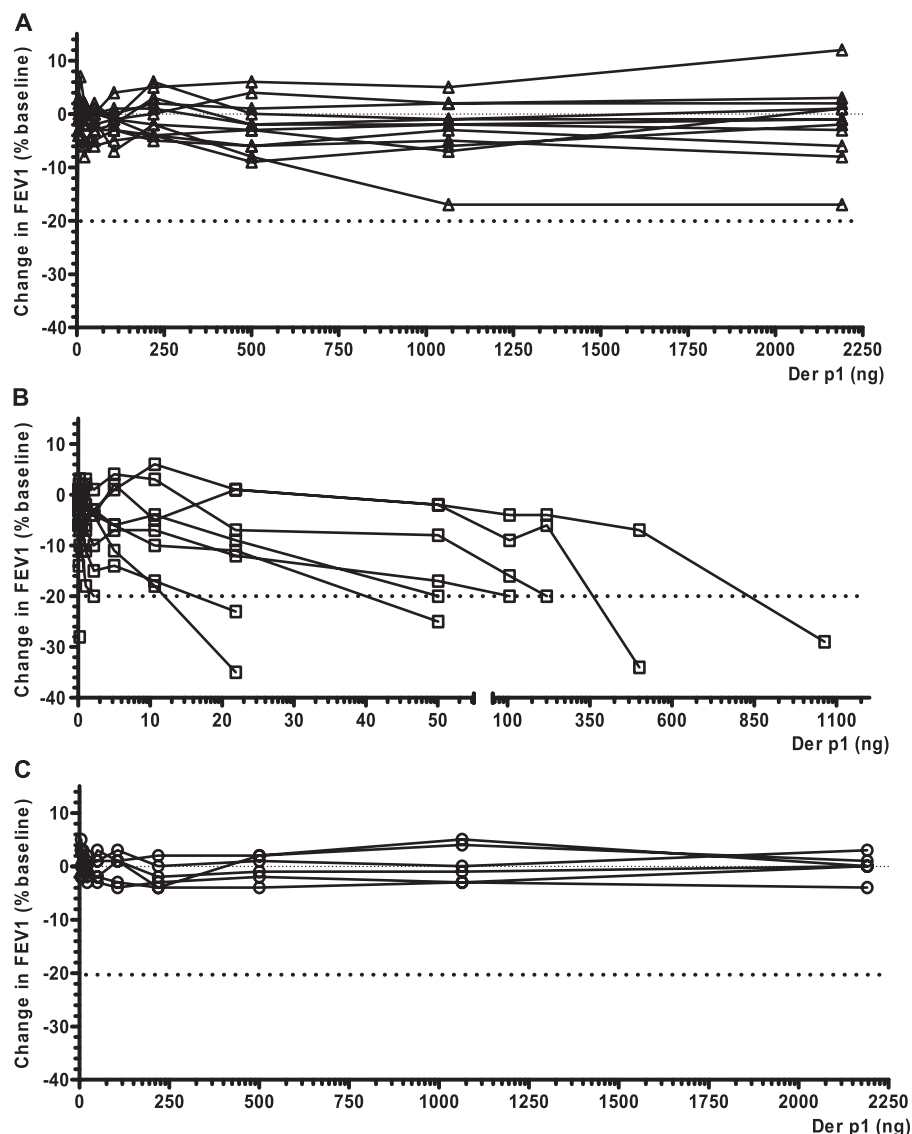


Figure 3. Whole-lung specific challenge to *Dermatophagoides pteronyssinus* (Der p) allergen in patients with (A) intrinsic asthma, (B) allergic asthma, and (C) healthy control subjects. Dashed line reflects positive cut-off value (20% fall in FEV₁). X axis represents cumulative dose of Der p1 (ng of Der p1, log scale). Der p stock solution (100 index of reactivity) contained 8 μ g/ml of Der p1.

we explored whether these sputum Der p-IgE could trigger antigen-dependent activation of effector cells, taking basophils as models of immediate allergic response. Absence of interference of DTT (at the used concentration of 0.05%) with basophil activation was checked, whereas increase in CD203c expression ($\sim 45\%$) was observed at higher concentrations (not shown). In the predefined suboptimal concentration of Der p (i.e., 4 ng/ml, see METHODS), incubation of basophils from one Der p-sensitive atopic donor with sputum (from patients with intrinsic or allergic asthma) preincubated with Der p (4 ng/ml) resulted in a sharp increase in CD203c expression as marker of basophil activation, as compared with Der p only (Figure 7, left panel). Similarly to results with sputum, incubation of basophils with serum containing Der p-specific IgE (> 100 kU/L) resulted in a similar increase in CD203c (serum + Der p: 95% CD203c⁺CRTH2⁺, vs. serum only: 6.4%; Der p: 7.6%).

This experiment was also performed with basophils from three patients with intrinsic asthma. Basophils from two of them were less responsive to anti-IgE triggering, whereas the third displayed significant activation (19%, 3.2%, and 84% of CD203c⁺ basophils, respectively). The (non)responder status did not relate to levels of serum IgE (13, 234, and 17 kU/L, respectively). In addition, stimulation of basophils from these three patients

with high concentration of Der p (200 ng/ml) and/or sputum did not result in cell activation (Figure 7, right panel). Of note, lower concentrations of Der p (i.e., 4 and 8 ng/ml), complexed

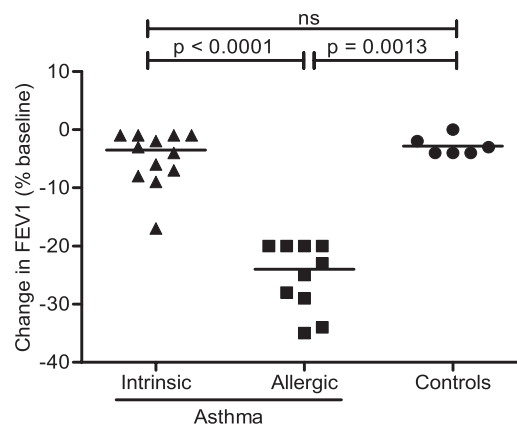


Figure 4. Change in FEV₁ (% baseline) at 15 minutes after the last antigen dose in patients with intrinsic and allergic asthma, as compared with healthy control subjects, during the whole-lung challenge. ns = not significant.

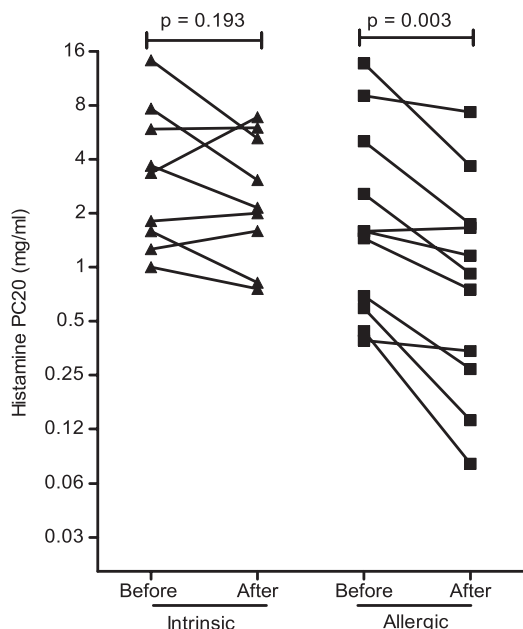


Figure 5. Nonspecific airway hyperresponsiveness to histamine (provocative concentration of histamine causing a 20% fall in FEV₁ [PC₂₀], mg/ml) before and 24 hours after lung specific challenge in patients with intrinsic (left) and allergic (right) asthma.

or not with sputum, did not lead to basophil activation, ruling out the possibility that antigen excess at this higher concentration (200 ng/ml) could have prevented basophil activation by IgE–allergen complexes.

To circumvent the effect of cytophilic (Der p–specific) IgE already bound to the surface of basophil (from Der p–sensitive donor), the assay was performed after acid stripping, which removed more than 95% of surface IgE in basophil-enriched PBMCs (CD3⁺ CRTH2⁺) as ascertained by anti-IgE staining (unstripped, 95.58 ± 0.72% [mean ± SEM] vs. stripped: 3.45 ± 0.82%). Resensitization of stripped basophils with human myeloma IgE (200 ng/ml/1 × 10⁶ PBMCs) restored IgE staining to 67.0 ± 24.0% CRTH2⁺ cells (Figure 8A). Passive resensitization could also significantly occur when incubating stripped basophils with pooled sputum samples from patients with asthma, although to a lower extent (9.86 ± 2.15% CRTH2⁺ cells, *P* = 0.015 compared with control), resulting in twofold increase in basophil activation after challenge with Der p allergen (~20%, as compared with < 8% with sputum or antigen alone) (Figure 8B).

DISCUSSION

In this study, we confirm that local IgE production occurs in the bronchial mucosa in intrinsic asthma and show for the first time that part of this IgE is directed toward house dust mite (Der p) allergens. In contrast with this local immune response, patients with intrinsic asthma did not develop clinically meaningful asthmatic responses on whole-lung challenge to Der p. However, we provide functional evidence that these sputum IgE antibodies from patients with intrinsic asthma are able, when complexed to Der p allergen, to activate basophils from an atopic donor.

IgE has been linked to asthma in epidemiological studies, and this appeared to be independent of the atopic status as referred to specific IgE responses to environmental antigens (15). Although specific sensitization to house dust mite is a strong risk factor for asthma, Pearce and colleagues (16) found a weak and inconsistent association between the prevalence of asthma and the prevalence of atopy in a broad sense. In our intrinsic asthma series, elevated IgE production was observed both in sputum and (without reaching statistical significance) in serum. Increased sputum eosinophils and IL-5 were also observed, further indicating Th2-polarized immune responses as reported previously both in intrinsic (5, 17, 18) and atopic asthma (19). Our intrinsic asthma population was also characterized by a limited but significant increase in sputum neutrophils. Increased neutrophilia in bronchial biopsies of patients with intrinsic asthma has been previously reported by others (20); however, this feature is not classically associated with intrinsic asthma but rather with severe asthma (21). Asthma severity was, however, not significantly different between our intrinsic and allergic asthma groups (i.e., *n* = 4 and *n* = 5 patients with Global Initiative for Asthma step 4-to-5 treatment, respectively). Moreover, evidence of active transcription of IL-4, IL-5, and IL-13 mRNA was also provided in a group of patients with intrinsic asthma by Humbert and colleagues (4, 22). We could also confirm in this study that IL-5 production is increased in patients with intrinsic asthma, as well as IL-4 in some patients, although this was not significantly increased as compared with control subjects. These Th2 cytokines are well known for the recruitment, activation, and survival of eosinophils within tissues (IL-5) and for induction of IgE switching in B cells (IL-4/IL-13). In this study, we focused more specifically on local IgE responses in intrinsic asthma through detailed sputum analyses, before and after local antigen exposure. Local IgE production has been documented previously in the bronchial mucosa of patients with nonatopic asthma (7, 17), but antigen specificity of these IgE remains elusive. We demonstrate here that a part of sputum IgE from patients with asthma with intrinsic phenotype does bind to house dust mite (Der p) allergens, at levels that are significantly higher than those found in healthy nonatopic subjects and similar to those observed in patients with atopic

TABLE 3. SPUTUM CELL COUNTS AND BLOOD EOSINOPHILS BEFORE AND 24 HOURS AFTER SPECIFIC LUNG CHALLENGE

	Intrinsic Asthma (<i>n</i> = 12)		Allergic Asthma (<i>n</i> = 11)		Control Subjects (<i>n</i> = 6)	
	Before	After	Before	After	Before	After
Sputum leukocytes						
Total cells, × 10 ⁶ /g	4.88 (1.77–13.48)	5.00 (2.77–18.00)	3.70 (1.50–20.30)	4.90 (1.44–34.20)	3.38 (2.65–7.16)	4.13 (2.83–4.82)
Eosinophils	1.9 (0.0–61.7)	2 (0.0–66.3)	5.7 (1.0–17.0)	9.9 (1.0–58.3)*	0.0 (0.0–0.0)	0.2 (0.0–0.5)
Neutrophils	51.3 (1.7–69.5)	35.6 (5.0–83.2)	19.3 (10.0–56.9)	11.1 (5.9–48.5)	23.9 (18.0–39.5)	22.5 (14.8–63.3)
Macrophages	38.4 (0.0–85.7)	51.6 (15.5–83.2)	68.2 (36.0–87.6)	62.1 (18.0–91.2)	75.1 (59.5–82.0)	76.5 (35.7–85.7)
Blood eosinophils	2.5 (0.8–14.7)	3.4 (1.4–22.4)	2.7 (0.4–6.8)	5.6 (0.9–7.1)†	0.9 (0.2–3.2)	1.2 (0.1–3.3)

Values are expressed in percentage of total leukocytes and shown as median (range) (%).

**P* = 0.08.

†*P* < 0.05 versus baseline (before challenge).

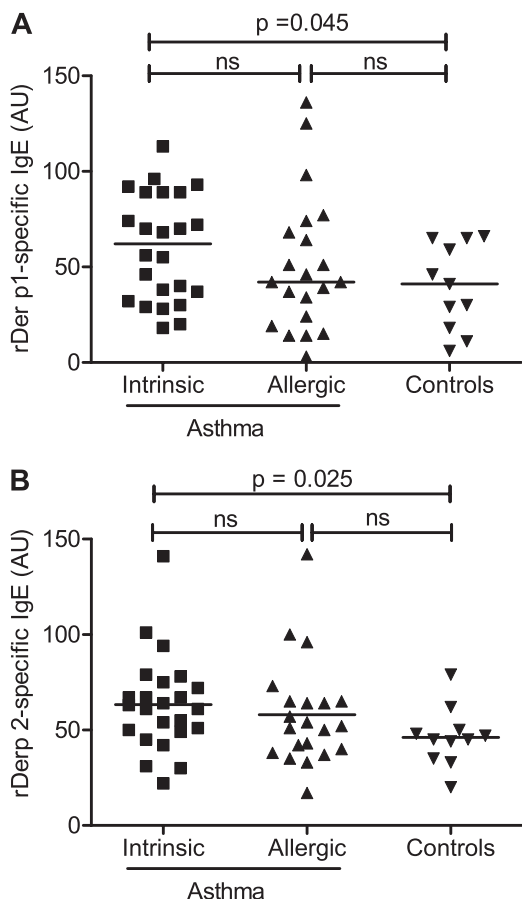


Figure 6. Specific IgE responses to *Dermatophagoides pteronyssinus* (Der p)1 and Der p2 major antigens in sputum from patients with intrinsic or allergic asthma and from nonatopic control subjects. Specific IgE to recombinant Der p1 (left) and rDer p2 (right) were assessed by ELISA. Horizontal lines depict medians. AU = arbitrary units; ns = not significant.

asthma with mite allergy. Local IgE responses to common environmental antigens without evidence of systemic sensitization have been previously reported in patients with intrinsic rhinitis (11, 18) and chronic rhinosinusitis with nasal polyps (23). In particular, Rondon and colleagues reported low but detectable levels of Der p-specific IgE in patients with persistent idiopathic

rhinitis (18, 24). Similarly, Sabirov and colleagues (23) observed that 6 out of 10 patients with nasal polyposis displayed IgE antibodies to *Alternaria* in tissue homogenates without evidence of serum-specific IgE. In line with a previous study (25), we observed that sputum IgE responses (both total IgE and Der p-specific IgE) did not correlate with serum responses, further suggesting that mucosal IgE production may be dissociated from systemic IgE. This observation is consistent with previous findings from Balzar and colleagues (26), who reported that IgE was preferentially expressed in the proximal airway mucosa (as compared with acinar/distal airways) from subjects with or without asthma and atopy, and that this local IgE bound to mast cells was only weakly correlated to serum/systemic IgE, but associated more closely with asthma outcomes such as lung function and severe exacerbations.

Our observation of specific IgE to Der p in sputum questions the possibility of a polyclonal IgE response, as suggested in nasal polyposis (27), a disease that is frequently associated with intrinsic asthma. However, results of specific IgE to cat dander and grass pollen in sputum from our patients with intrinsic asthma do not support this possibility. In addition, although one could not exclude that in the case of cat and grass allergens this relates to the sensitivity of the immunoassay, the absence of increased cat- or grass-IgE antibody levels in sputum from patients with atopic asthma who display serum-specific IgE to these allergens further supports a dissociation between local and systemic IgE responses. The fact that Der p allergens could be more potent than cat or grass allergens in inducing local IgE responses could rely on the proteolytic activity of Der p1 (cysteine protease), which may act directly on the airway epithelium to disrupt tight junctions and favor contact with sentinel antigen-presenting cells (28) and to promote Th2-polarizing function of dendritic cells leading to IgE synthesis (29). The observation that local production of IgE that includes IgE antibodies to dust mite could occur in healthy individuals is intriguing; it is known that local IgE (without known specificity) is detectable in such subjects and able to bind to tissue mast cells (26). Whether production of IgE, including specific IgE to Der p, in the airways covers a spectrum from normality toward allergen-induced asthma, with intrinsic asthma as intermediate phenotype, needs further investigation. This could reveal in contrast with the periphery/serum where specific IgE appears more dichotomous between normal and atopic subjects, even though some sensitized subjects could remain asymptomatic.

As we could show that allergen-specific IgE antibodies are present in the airways of patients with intrinsic asthma, along with the other main actors of allergic reactions, including activated

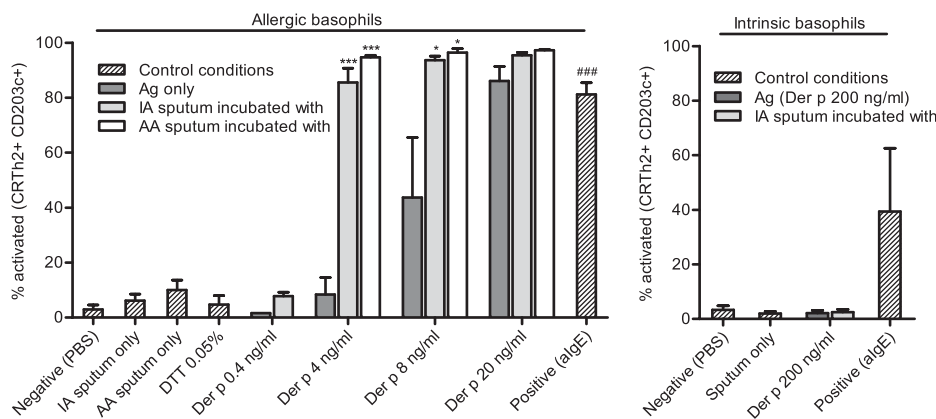


Figure 7. Activation of basophils from one *Dermatophagoides pteronyssinus* (Der p)-sensitive atopic donor (left panel) and from three patients with intrinsic asthma (IA) (right panel) by sputum, assessed by flow cytometry for CD203c expression. (Left panel) Basophil activation was assessed after incubation with Der p antigen (Ag) only or with sputum preincubated with various concentrations of Der p (4, 8, and 20 ng/ml). In this atopic donor, suboptimal Der p concentration was typically 4 ng/ml (see text). Data represent four independent experiments with sputum from patients with either IA or atopic asthma (AA). ***P < 0.0001 versus phosphate-buffered saline (PBS) control; **P < 0.01 versus control. (Right panel) In "intrinsic" basophils, stimulation was performed with higher concentration of Der p (up to 200 ng/ml). Data were obtained in three independent experiments with sputum from IA (n = 3 IA as basophil donors), not significant versus control. DTT = dithiothreitol.

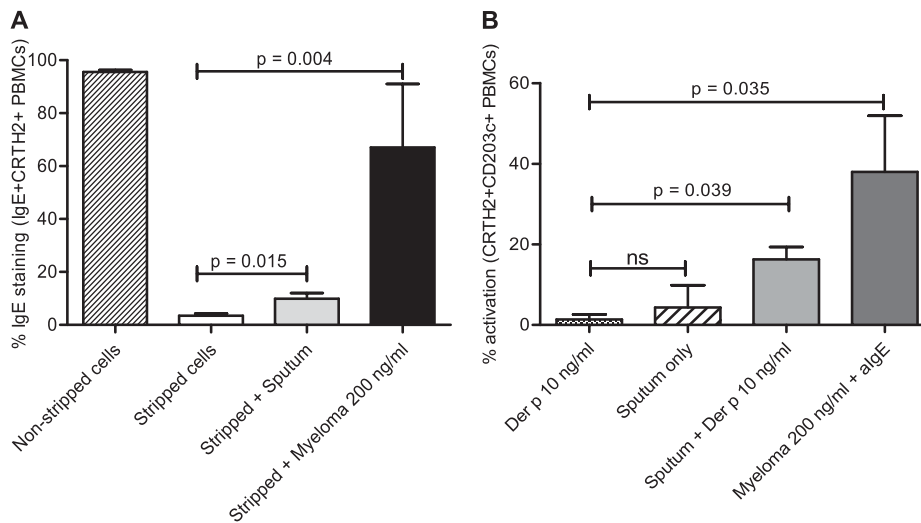


Figure 8. (A) IgE staining of basophils (IgE⁺ CRTH2⁺ peripheral blood mononuclear cells) from one atopic donor after acid stripping. Data represent mean $\pm$ SEM from three independent experiments and are expressed as % of IgE-bearing cells before (nonstripped) and after stripping (stripped), after resensitization with sputum or myeloma IgE. (B) Activation of IgE-stripped basophils by sputum. Basophils from one atopic donor were stripped and passively resensitized with 200 μ l of sputum from patients with asthma (three pooled samples, from patients with either intrinsic or allergic asthma) and then challenged with *Dermatophagoides pteronyssinus* (Der p) (10 ng/ml) or not (sputum only). Basophils resensitized with human myeloma IgE (200 ng/ml) and challenged with anti-IgE were used as positive control of the resensitization procedure. Data represented % mean $\pm$ SEM of activated cells (CD203c⁺ CRTH2⁺ PBMCs) from three independent experiments and were normalized for background.

T and inflammatory cells (16) and high-affinity IgE receptor (Fc ϵ RI)-bearing cells (30), we submitted these patients to a specific lung challenge. In contrast to patients with asthma with (systemic) Der p sensitization, patients with intrinsic asthma did not display any significant indices of asthmatic response to Der p exposure (i.e., change in symptoms, spirometry [FEV₁], sputum and blood eosinophils, or nonspecific AHR to histamine). In contrast with studies (18, 31) that reported significant clinical nasal responses to allergen exposure in patients with idiopathic rhinitis, we only observed marginal changes in lung function, suggesting low-grade IgE-mediated bronchial reactivity to Der p allergens in our patients with intrinsic asthma. We could not exclude that inhaled corticosteroid therapy (only withdrawn for 48 h before challenge, in $n = 11$ inhaled corticosteroid-treated patients among 12 challenged) might have reduced the reactivity of patients with intrinsic asthma, even if we could demonstrate that in the same conditions, clear-cut asthmatic responses occur in patients with allergic asthma (including in the majority of patients who were treated by maintenance inhaled corticosteroids, $n = 8$ among 11 challenged). Alternatively, this response could also reflect allergen-independent changes related to nonspecific airway hyperresponsiveness. Finally, another hypothesis is that signals that condition the development of clinically relevant asthmatic responses (e.g., in terms of symptomatic, meaningful changes in lung function) to antigen exposure, which infers cellular reactivity to IgE signaling, are linked with those underlying systemic sensitization. These signals may notably include factors that trigger dendritic cell migration to regional (mediastinal) lymph nodes.

Therefore, among hypotheses that could underlie the apparent discrepancy between evidence of specific IgE immune response to Der p and absence of clinical reactivity, putative impaired binding of sputum IgE from patients with intrinsic asthma to clinically relevant allergens or capacity to activate effector cells were assessed. First, we observed that sputum IgE from patients with intrinsic asthma significantly bind to recombinant Der p major allergens (Der p1 and Der p2), similarly to patients with allergic asthma. Second, the capacity of sputum IgE to activate basophils was evaluated to explore the functionality of locally produced IgE. Allergen-driven up-regulation of the activation marker CD203c (on CD3⁺

CRTH2⁺ PBMCs) was reported to occur through Fc ϵ RI cross-linking (32). It was also reported that CD203c-based basophil activation test could be slightly more sensitive than CD63, at least in venom allergy (33, 34). In this study, we conducted passive sensitization of basophils (from one Der p-sensitive atopic patient) with sputum IgE complexed with Der p, on both untouched and acid-stripped cells. This is to the best of our knowledge the first time that basophil activation test has been used in these settings (i.e., to explore the functionality of mucosal IgE). Our data demonstrate that sputum IgE are able (when complexed with Der p) to activate basophils. Although we cannot extrapolate our findings to tissue mast cells, these data provide functional evidence that locally produced IgE are able to trigger activation of effector cells implicated in allergic and asthmatic reactions. Our data also suggest that responses of basophils from patients with intrinsic asthma might be reduced, independently of serum IgE levels, but this possibility should be confirmed in a specific study addressing this issue on basophils.

The concomitant presence of mucosal specific IgE to mite allergens, and absence of clear clinical reactivity on lung exposure, which is intriguing, may further underline the complexity of asthma pathogenesis. The important role of allergy and Th2-skewed immunity was highlighted in murine experimental allergic asthma and, almost 20 years ago, in human atopic asthma (19). IgE antibodies might, however, not be sufficient alone to elicit asthmatic responses on antigen exposure. This hypothesis is close to that of Holt and Strickland (35), who suggest that during early stages of asthma development and during exacerbations, viral pathways are acting concomitantly to atopy-dependent pathways (e.g., through up-regulation of Fc ϵ RI expression on airway dendritic cells [36]). To explain the absence of reactivity *in vivo*, we propose that a “second signal” (e.g., viral-induced) that promotes IgE-mediated asthmatic responses through Fc ϵ RI is lacking in intrinsic asthma. An alternative hypothesis could involve signaling machinery of the basophil that is triggered by IgE, which might be deficient in intrinsic asthma as reported in nonresponders’ basophils (37). The role of local specific IgE could therefore be addressed by assessing factors that may drive cellular reactivity to the IgE pathway.

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