

House dust mite-specific IgE antibodies in induced sputum are associated with sputum eosinophilia in mite-sensitive asthmatics

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Background: Although allergen-specific IgE antibodies have been considered to play an important role in the pathogenesis of atopic asthma, the role of IgE antibodies in the development of airway inflammation is not well defined.

Objective: To evaluate the association between allergen-specific IgE antibodies and inflammation of the asthmatic airway.

Methods: We measured house dust mite (HDM; *Dermatophagoides farinae*)-specific IgE antibodies in both serum and induced sputum from 16 HDM-sensitive asthmatic patients, and evaluated their association with sputum eosinophilia and eosinophil cationic protein (ECP) levels in induced sputum.

Results: Levels of HDM-specific IgE antibodies in induced sputum were significantly higher in asthmatic patients than in controls ($P < .01$). In asthmatic patients, levels of HDM-specific IgE antibodies were significantly higher in induced sputum samples with eosinophilia (sputum eosinophil count $\geq 5\%$ of 200 counted non-squamous cells) than in those without eosinophilia ($P < .05$). There were no significant differences in serum levels of HDM-specific IgE antibodies between asthmatic patients with sputum eosinophilia and asthmatic patients without sputum eosinophilia. In asthmatic patients, sputum ECP levels were significantly correlated with levels of HDM-specific IgE antibodies ($r = 0.60$, $P = .01$) in induced sputum but not with those in serum.

Conclusion: We conclude that allergen-specific IgE antibodies in induced sputum from atopic asthmatics are associated with sputum eosinophilia. This result suggests that IgE-dependent mechanisms are involved in eosinophilic inflammation of the airway in atopic asthmatics.

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INTRODUCTION

Allergen-specific IgE antibodies have been considered to play an important role in the pathogenesis of atopic asthma.¹ Epidemiological studies support the view that sensitization to allergens, especially indoor allergens, is important in the development of asthma.^{2,3} Airway inflammation is a critical feature in the pathogenesis of asthma.⁴ However, the role of IgE antibodies in the development of airway inflammation is not well defined.⁵

Allergen-specific IgE antibodies in nasal secretions and tears from patients with allergic rhinitis and conjunctivitis more accurately predict current allergic symptoms than those found in serum.^{6,7} A study on airway secretion from asthmatic patients could provide more information on the role of allergen-specific IgE antibodies in the pathogenesis of atopic asthma. However, measurement of allergen-specific IgE antibodies in airway secretion is considered more difficult than measurement in serum due to difficulty in obtaining airway secretion from asthmatic patients and due to the relatively low concentration of IgE antibodies in airway secretion.⁸

In a previous study, we reported that analysis of induced sputum could be a

useful, non-invasive method for studying allergen-specific IgE antibodies in airway secretion from asthmatic patients.⁹ To evaluate an association between allergen-specific IgE antibodies and inflammation of the asthmatic airway, we measured house dust mite (HDM)-specific IgE antibodies in serum and induced sputum from 16 HDM-sensitive asthmatics and evaluated their association with sputum eosinophilia and eosinophil cationic protein (ECP) in induced sputum.

MATERIALS AND METHODS

Subjects

We collected induced sputum samples from 16 HDM-sensitive asthmatic patients showing positive reaction to HDM (*Dermatophagoides farinae*; Bencard Co., Brentford, UK) on skin prick test (Table 1). All patients had a typical clinical history of asthma and documented reversibility of forced expiratory volume in one second (FEV₁) greater than 15%. All patients were nonsmokers and had mild asthma, and none had received inhaled or oral corticosteroids in 3 months prior to the study. Twelve healthy non-atopic, non-asthmatic controls were recruited from the medical students and staff of Ajou University Hospital. All subjects underwent skin prick test with common aeroallergens (house dust mite, grass pollens, tree pollens, weed pollens, cat, dog, and molds; Bencard Co, Brentford, UK). A positive skin test was defined as the mean wheal diameter being 3 mm greater than that of negative control (normal saline). All patients showed positive skin reactions to HDM allergen alone except two patients who showed positive skin reaction to both cat and HDM allergens.

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Table 1. Characteristics of the Study Subjects

	Healthy Controls	Asthmatic Subjects
Subjects (numbers)	12	16
Age (yr)	35 ± 11	36 ± 14
Sex (M/F)	5/7	10/6
FEV ₁ (% predicted)	101.4 ± 5.8	87.3 ± 18.0*
Sputum Eosinophil Count (%)†	0.5 ± 1.4	14.4 ± 17.7*
Sputum Albumin (mg/mL)	0.13 ± 0.07	0.48 ± 0.87*
Sputum ECP (ng/mL)	—‡	231.6 ± 177.3

Data are expressed as mean ± standard deviation.

* Significant difference between asthmatic patients and healthy controls ($P < .05$).

† % of 200 counted non-squamous cells.

‡ Not measured.

These two patients were not cat owners and their skin reactivities to cat allergen were smaller than to HDM allergen. This study was approved by the Ethics Committee of Ajou University Hospital and all subjects gave their written informed consent. The study was done from July to August in 1995 which is the season when environmental house dust mite allergen reaches peak levels in this country.¹⁰

Induction of Sputum and Serum Sampling

Sputum was induced using a previously described method.¹¹ Immediately before the sputum expectoration, each subject was pretreated with 200 µg salbutamol, administered by means of a metered dose inhaler. The subjects then inhaled nebulized sterile 3% saline solution for 20 minutes through an ultrasonic nebulizer (Omron Co., Tokyo, Japan). After being instructed to spit out saliva in the mouth and to gargle with fresh water first, the subjects were asked to cough and expectorate

sputum into a clean plastic container. Sera was collected and stored at -20°C .

Sputum Processing

Collected sputum was processed immediately as previously described.¹¹ Eosinophils were counted from at least 200 non-squamous cells on each slide. We defined the sputum eosinophilia as eosinophil count $\geq 5\%$ of 200 counted non-squamous cells, which is the value of mean +3 standard deviations of controls (Table 1). We chose this cut-off value on the basis of our previous study¹¹ and the validation of sensitivity and specificity by another group.¹²

Measurement of HDM-Specific IgE

Measurement of HDM-specific IgE antibodies in induced sputum was done according to a method previously described.⁹ Serum HDM-specific IgE (using *D. farinae* antigen) was measured by radioimmunoassay using AlaSTAT kit (Diagnostic Product Corporation, Los Angeles, CA).

Measurement of Total IgE Levels

Measurement of total IgE levels in induced sputum was done using a method previously reported.¹³ All samples were measured simultaneously. For the quantitative measurement, serially diluted IgE standards (Diagnostic Product Corporation, Los Angeles, CA) were included in each assay and the range of measurement was 0.1 to 4 IU/mL of induced sputum. Coefficient of intra-assay variation was below 10%. Serum total IgE was measured by radioimmunoassay using AlaSTAT kit (Diagnostic Product Corporation, Los Angeles, CA).

Albumin and ECP Measurements

Albumin levels in induced sputum were measured by nephelometry with a Beckman Array System (Beckman Instruments Inc., Brea, California). ECP levels in induced sputum from asthmatic patients were measured using the Pharmacia CAP system (Pharmacia Diagnostics Inc., Uppsala, Sweden). The lowest detection limit for ECP measurement was 2 ng/mL.

Statistics

Data were expressed as mean ± standard deviation. The Mann-Whitney *U* test was used to assess differences between two groups. Spearman's rank analysis was used to evaluate the correlation between data.

RESULTS

HDM-Specific IgE Antibodies in Serum and Induced Sputum

Levels of HDM-specific IgE antibodies were significantly higher in serum and induced sputum from mite-sensitive asthmatic patients than in those from controls ($P < .01$, Table 2). In all control subjects, total IgE levels in induced sputum were below the detection limit (<0.1 IU/mL). There was no significant correlation in levels of total IgE ($r = 0.05$, $P = .84$) and HDM-specific IgE antibodies ($r = 0.24$, $P = .36$) between serum and induced sputum from asthmatic patients.

Table 2. Levels of Total IgE and HDM-Specific IgE (sIgE) Antibodies in Serum and Induced Sputum

	Healthy Controls (n = 12)	Asthmatic Subjects (n = 16)
Serum total IgE (IU/mL)	70.7 ± 50.5	363.8 ± 214.2*
Serum sIgE (IU/mL)	0.24 ± 0.10	3.45 ± 2.80*
Sputum total IgE (IU/mL)	$<0.1^{\dagger}$	1.48 ± 1.30*
Sputum sIgE (absorbance)	0.015 ± 0.013	0.096 ± 0.115*

Data are expressed as mean ± standard deviation.

* Significant difference between asthmatic patients and healthy controls ($P < .01$).

† In all sputum samples from healthy controls, total IgE levels were below the lowest detection limit (<0.1 IU/mL).

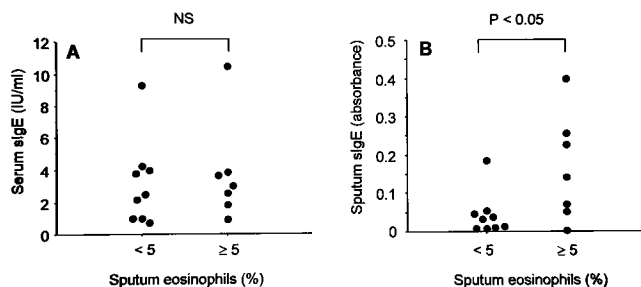


Figure 1. Levels of HDM-specific IgE (sIgE) antibodies in serum (A) and induced sputum (B) from HDM-sensitive asthmatic patients with and without sputum eosinophilia. NS = not significant.

Association Between HDM-Specific IgE and Sputum Eosinophilia

In asthmatic patients, levels of HDM-specific IgE antibodies were significantly higher in induced sputum samples with eosinophilia (sputum eosinophil count $\geq 5\%$ of 200 counted non-squamous cells) than in those without eosinophilia ($P < .05$, Fig 1). There was no significant difference in serum levels of HDM-specific IgE antibodies between asthmatic patients with sputum eosinophilia and asthmatic patients without sputum eosinophilia ($P > .05$, Fig 1). No significant differences were found in sputum total IgE and albumin levels between asthmatic patients with sputum eosinophilia and asthmatic patients without sputum eosinophilia ($P > .05$, Fig 2). In asthmatic patients, sputum eosinophil counts were significantly correlated with HDM-specific IgE levels in induced sputum ($r = 0.59$, $P = .02$), but not with those levels in serum ($r = 0.24$, $P = .36$).

Correlation Between HDM-Specific IgE and ECP Levels in Induced Sputum

In mite-sensitive asthmatic patients, ECP levels in induced sputum were significantly correlated with HDM-specific IgE levels ($r = 0.60$, $P = .01$) in induced sputum, but not with HDM-specific IgE levels in serum ($r = -0.12$, $P = .65$, Fig 3), total IgE levels ($r = 0.25$, $P = .36$), nor albumin levels ($r = 0.31$, $P = .25$) in induced sputum. Degree of airway obstruction, expressed as FEV_1 (%) values measured before sputum sampling, did not significantly correlate with HDM-specific

IgE levels in serum ($r = 0.08$, $P = .75$) nor with those levels in induced sputum ($r = -0.11$, $P = .67$).

DISCUSSION

This study demonstrates a significant association between sputum eosinophilia and allergen-specific IgE antibodies in induced sputum from mite-sensitive asthmatic patients but not between sputum eosinophilia and those antibodies in serum. These results are comparable with previous studies showing significant correlation between current symptoms and allergen-specific IgE in nasal secretions from subjects with allergic rhinitis and in tears from subjects with allergic conjunctivitis.^{6,7}

Eosinophil infiltration in the asthmatic airway correlates with clinical severity and it may be responsible for the development of an allergen-induced late asthmatic reaction.^{14,15} However, the role of IgE antibodies in

the development of late asthmatic response and eosinophilic airway inflammation is not well defined.⁵ Allergen-specific IgE antibodies were increased in bronchial lavage fluid from asthmatic patients who showed late asthmatic reaction after allergen bronchial challenge.¹⁶ Disodium cromoglycate treatment of asthmatic patients decreased eosinophils and allergen-specific IgE antibodies in bronchoalveolar lavage fluid.¹⁷ Treatment with anti-IgE monoclonal antibody in allergic asthmatic patients suppressed the early- and late-phase responses to inhaled allergen.¹⁸ In the mouse model, administration of the anti-IgE monoclonal antibody before allergen challenge inhibited the recruitment of eosinophils into the lungs and the production of IL-4 and IL-5.¹⁹ These reports are consistent with our *in vivo* study results demonstrating significant association between sputum eosinophilia and allergen-specific IgE antibodies in induced sputum from atopic asthmatic patients. In this study, sputum sampling was done during the peak season of natural house dust mite exposure,¹⁰ thus our patients might have been under chronic natural allergen challenge. These findings suggest that IgE antibodies are involved in eosinophilic inflammation of the airway in atopic asthmatics.

Permeability of airway mucosa is increased in asthmatic patients.²⁰ In patients with bronchial asthma, increased allergen-specific IgE antibodies in in-

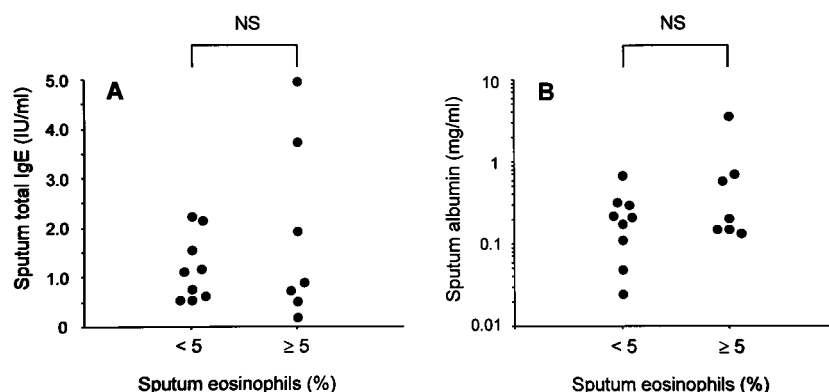


Figure 2. Levels of total IgE (A) and albumin (B) in induced sputum from HDM-sensitive asthmatic patients with and without sputum eosinophilia. NS = not significant.

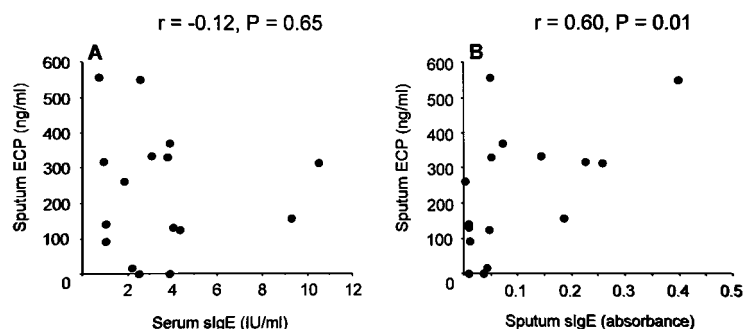


Figure 3. Correlation between ECP levels in induced sputum and HDM-specific IgE (sIgE) antibodies in serum (A) and induced sputum (B) from HDM-sensitive asthmatic patients.

duced sputum may be interpreted as a result of increased passive diffusion from systemic circulation. However, several observations are against such an interpretation. A previous study showed that IgE-forming plasma cells were mainly distributed in respiratory and gastrointestinal mucosa tissues and their regional lymph nodes but were scarce in spleen and subcutaneous lymph nodes.²¹ In addition, IgE antibodies in bronchoalveolar lavage and sputum were shown to be locally produced.^{8,22} Albumin has been widely used as a marker of airway permeability, because it is derived from systemic circulation without local production.⁸ In this study, allergen-specific IgE levels were significantly higher in induced sputum from asthmatic patients with sputum eosinophilia than in those from asthmatic patients without sputum eosinophilia, while no significant difference was noted in total IgE and albumin levels of induced sputum between the two groups. These results suggest that the association between allergen-specific IgE antibodies and eosinophilia in induced sputum is not due to increased permeability of airway mucosa.

Interestingly, previous studies of asthmatic subjects could not demonstrate a significant correlation between eosinophil counts in induced sputum and the number of eosinophils in bronchial biopsies.^{23,24} However, when the eosinophil count was expressed as normal and abnormal values on the basis of the results from healthy controls, significant agreement could be found

between sputum eosinophilia and presence of eosinophilic inflammation in bronchial mucosa.²³ Because, in this study, sputum eosinophil count was used to monitor the presence of eosinophilic inflammation in the bronchial mucosa of atopic asthmatics, we expressed the results of sputum eosinophil count in asthmatic patients as normal and abnormal values on the basis of data from healthy controls for statistical analysis. The validity of the cut-off value of sputum eosinophil count ($\geq 5\%$) used in this study also has been verified by another group using 41 asthmatic patients and 27 non-asthmatic subjects.¹²

We conclude that allergen-specific IgE antibodies in induced sputum from atopic asthmatics are associated with sputum eosinophilia. This result suggests that IgE-dependent mechanisms are involved in eosinophilic inflammation of the airway in atopic asthmatics.

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