

Seasonal variation of IgG subclass antibodies to house dust mite in sera from mite-sensitive asthmatic patients

Dong-Ho Nahm, MD*; Hae-Sim Park, MD*; Cheol-Woo Kim, MD†; Jung-Won Park, MD†; and Chein-Soo Hong, MD†

Background: House dust mite is an important cause of bronchial asthma. Seasonal variation of environmental house dust mite allergen levels and the specific IgE antibody to house dust mite have been reported.

Objective: We studied the changes in IgG subclass antibodies to house dust mite associated with seasonal variation of house dust mite allergen levels in houses of mite-sensitive asthmatic patients.

Methods: In 14 mite-sensitive asthmatic patients, house dust mite allergen (Der f 1) contents in bedding were measured monthly, and IgG subclass antibodies to house dust mite, *Dermatophagoides farinae* (*D. farinae*), were determined by enzyme-linked immunosorbent assay (ELISA) every 3 months from July to December.

Results: The concentration of Der f 1 in dust from bedding reached maximum levels in August and September, and significantly decreased in November and December compared with August and September ($P < .05$). Levels of *D. farinae*-specific IgG4 antibodies significantly decreased in December compared with September ($P < .05$) with no statistically significant change between September and June ($P > .05$). Levels of *D. farinae*-specific IgG2 antibodies decreased significantly in December compared with June ($P < .05$). The levels of IgG1 and IgG3 antibodies to *D. farinae* showed no significant differences during the study period.

Conclusion: These findings suggest that seasonal changes in natural exposure to house dust mite allergen might lead to concurrent changes in specific IgG4 antibodies to house dust mite in mite-sensitive asthmatic patients and each IgG subclass antibodies to house dust mite might have a different kinetics.

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INTRODUCTION

IgE antibody responses to house dust mite allergen have been considered to play an important role in the development of bronchial asthma.^{1,2} Although traditionally house dust mite has been considered a perennial allergen, seasonal variation in mite numbers and allergen levels in house dust has been reported.³ Recently, significant varia-

tion of nonspecific airway hyperreactivity and specific IgE antibody to house dust mite in mite-sensitive asthmatic patients were reported in association with seasonal changes in house dust mite allergen.^{4,5}

Asthmatic patients can produce antibodies of all four IgG subclasses to house dust mite.^{6,7} Among the four IgG subclasses, IgG4 antibody to allergens has been the one most extensively studied.⁸ It has been shown to increase after immunotherapy and has been suggested to play a role as a blocking antibody that can interfere with the binding of IgE to allergen.⁹ The protective role or any pathogenetic significance of IgG4 antibodies to allergen has not been established yet.¹⁰ IgG an-

tibody response to house dust mite in asthmatic patients has been interpreted as a natural immune response secondary to chronic environmental exposure to mite allergen.¹¹ Measurement of allergen-specific IgG antibodies has been suggested as a marker for exposure to a certain agent in a particular setting such as occupational asthma.¹²

The regulation mechanism of IgG subclass antibodies to allergen has not yet been fully determined.⁸ It has been shown that IgG1 and IgG4 antibodies to grass pollen in allergic patients are synchronized with seasonal variation of IgE antibody in response to seasonal pollen exposure.¹³ Variation of IgG subclass antibodies to house dust mite and their kinetics in association with seasonal changes in environmental house dust mite allergen levels have been rarely studied.

To further evaluate the influence of natural exposure to house dust mite allergen on IgG subclass responses to house dust mite and their kinetics, we observed the changes in IgG subclass antibodies to house dust mite in 14 mite-sensitive asthmatic patients according to the changes in house dust mite allergen levels in their houses.

MATERIALS AND METHODS

Patients

Fourteen mite-sensitive asthmatic patients who showed positive skin prick test to *Dermatophagoides farinae* (*D. farinae*), participated in this study. All patients, nine males and five females, had a clinical history of asthma and nonspecific bronchial hyperreactivity. Mean age was 25 (range: 16 to 42). These patients were the same subjects included in our previous study on sea-

* Department of Allergy and Clinical Immunology, Ajou University School of Medicine, Suwon, Korea.

† Division of Allergy and Immunology, Department of Internal Medicine, Yonsei University Medical College, Seoul, Korea.

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Table 1. Seasonal Changes in House Dust Mite Allergen (Der f 1; $\mu\text{g/g}$ of dust) Levels in House Dust from 14 Mite-Sensitive Asthmatic Patients*

June	July	August	Sept	Oct	Nov	Dec
3.01 ± 1.44	2.76 ± 1.36	3.97 ± 1.59	3.81 ± 1.36	1.51 ± 1.50	$1.29 \pm 1.37^\dagger$	$1.71 \pm 0.69^\dagger$

* Data were expressed as a geometric mean $\pm$ geometric standard error of mean.

† Significant decrease in Nov and Dec compared with Aug and Sept ($P < .05$).

sonal variation of specific IgE antibody to house dust mite.⁵ Written informed consent was obtained from each patient and the study was approved by the Medical Ethics Committee of Severance Hospital. The study was performed from June to December of 1993.

Measurement of House Dust Mite Allergen in House Dust

The patients' houses were visited monthly from June to December and dust samples from the surface of beddings were collected and processed as described previously.⁵ The level of group I allergen of *D. farinae* (Der f 1) in house dust extracts was measured simultaneously by ELISA according to a previously described method, using monoclonal antibodies (kindly provided by MD Chapman).¹⁴

Serum Sampling

Every 3 months during the study period, venous blood was sampled from all patients and the sera was stored at -20°C .

Measurement of *D. farinae*-Specific IgG Subclass Antibodies

Specific IgG subclass antibodies to *D. farinae* were measured by ELISA as previously described, with modifications.^{6,7,15} Wells of microplate (Costar Corporation, Cambridge, MA) were coated overnight with *D. farinae* extract ($2\text{ }\mu\text{g/well}$) in $50\text{ }\mu\text{L}$ of 0.05 M carbonate buffer (pH 9.6) at 4°C . All incubations were $50\text{ }\mu\text{L}$ at room temperature except as otherwise noted and after each incubation the microtiter plate was washed three times with phosphate-buffered saline containing 0.05% Tween 20 (PBST). After washing, the wells were incubated with $200\text{ }\mu\text{L}$ of 1% bovine serum albumin in PBST for one hour. Duplicated dilutions of serum samples ($1:20$ for IgG1

and IgG2, $1:2$ for IgG3 and IgG4) were added to *D. farinae*-extract coated wells. After one hour, the wells were washed and biotinylated monoclonal anti-IgG1 (HP6091), anti-IgG2 (HP6014), anti-IgG3 (HP6050), and anti-IgG4 (HP6025) antibodies (Sigma Chemical Co., St. Louis, MO) were added at a dilution of $1:500$ (vol/vol) and incubated for one hour. After washing the wells, peroxidase-conjugated streptavidin (Sigma Chemical Co) at a dilution of $1:1000$ (wt/vol) was added and incubated for 30 minutes and washed. Then $100\text{ }\mu\text{L}$ of 0.01 M ABTS ($2,2'$ -azinobis-3 ethyl-benzthiazoline sulfonic acid) in 0.07 M citrate-phosphate buffer, pH 4.2 containing 0.03% H_2O_2 was added. The color reaction was stopped with $100\text{ }\mu\text{L}$ of 0.002 M sodium azide and the plates were read at 410 nm using a microtiter reader (Dynatech Laboratories, Inc., Alexandria, VA). All assays included the same positive control serum from a mite-sensitive asthmatic patient and negative control as a pooled serum from nonatopic healthy controls. The levels of IgG subclass antibodies to house dust mite were expressed as an ELISA binding index = $(S - B)/(N -$

B) as previously described.^{6,16} S is the mean absorbance of the sample, B is the mean background absorbance of dilution buffer only, and N is the mean absorbance of the negative control sample. All the measurements of each IgG subclass antibodies were done simultaneously to minimize the assay variation, and the interplate and intraplate variations were below 15% .

Statistics

Data were expressed as the mean $\pm$ standard error of the mean except for house dust mite allergen levels, which were expressed as a geometric mean $\pm$ geometric standard error of mean. The changes in house dust mite allergen and IgG subclass antibodies to house dust mite were compared by a Wilcoxon signed-rank test. A P value of less than $.05$ was considered significant.

RESULTS

Seasonal Changes in House Dust Mite Allergens in Bedding

The same as previously reported,⁵ Der f 1 levels in dust from bedding tend to increase gradually from June and reach a peak in August and September and

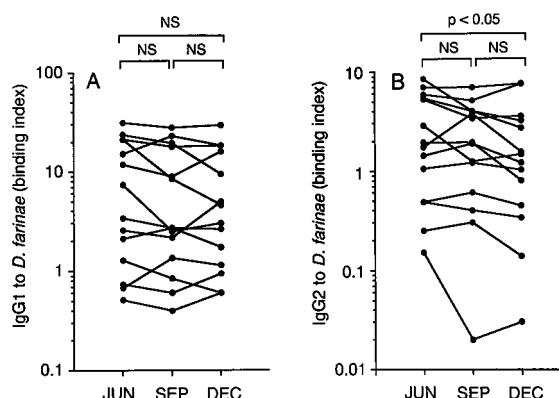


Figure 1. Seasonal changes in specific IgG1 (A) and IgG2 (B) antibodies to *D. farinae*.

Table 2. Seasonal Changes in Levels of *D. farinae*-Specific IgG Subclass Antibodies in 14 Mite-Sensitive Asthmatic Patients*

	June	September	December
Specific IgG1†	10.21 ± 2.79	8.51 ± 2.55	7.99 ± 2.42
Specific IgG2	3.03 ± 0.75	2.49 ± 0.56	2.27 ± 0.67‡
Specific IgG3	2.54 ± 0.59	4.59 ± 2.35	3.74 ± 1.31
Specific IgG4	26.15 ± 6.13	29.46 ± 5.51	19.74 ± 3.90§

* Data were expressed as a mean ± standard error of mean.

† Levels of IgG subclass antibodies to *D. farinae* were expressed as an ELISA binding index. ELISA binding index = (S - B)/(N - B). S is the mean absorbance of the sample, B is the mean background absorbance of dilution buffer only, and N is the mean absorbance of the negative control sample.

‡ Levels of specific IgG2 antibody to *D. farinae* significantly decreased in December compared with June ($P < .05$).

§ Levels of specific IgG4 antibody to *D. farinae* significantly decreased in December compared with September ($P < .05$).

gradually decrease until December. Der f 1 levels significantly decreased in November and December compared with August and September ($P < .05$; Table 1).

Seasonal Changes in *D. farinae*-Specific IgG Subclass Antibodies

Levels of *D. farinae*-specific IgG2 antibodies significantly decreased in December compared with June as shown in Figure 1 and Table 2 ($P < .05$). Levels of *D. farinae*-specific IgG4 antibodies significantly decreased in December compared with September ($P < .05$) with no statistically significant change between September and June ($P > .05$) (Fig 2, Table 2). Levels of specific IgG1 and IgG3 antibodies to *D. farinae* showed no significant differences during the study period (Fig 1, Table 2).

DISCUSSION

This study demonstrated that IgG subclass antibodies to house dust mite allergen varied with environmental changes of house dust mite allergen levels in mite-sensitive asthmatic patients. To our best knowledge, the present study is the first one observing seasonal variations in specific IgG subclass antibodies to house dust mite. Studies on IgG subclass antibodies to timothy pollen have reported that IgG1 subclass antibody to pollen as well as IgG4 subclass antibody to the same antigen varied with seasonal pollen exposure.¹³ In this study, however, there

was a significant seasonal variation in IgG2 and IgG4 antibodies to house dust mite without significant changes in IgG1 and IgG3 antibodies to the same antigen. The seasonal changes in IgG2 antibody to house dust mite without significant changes in IgG1 and IgG3 antibodies to the same antigen is interesting, because IgG1, IgG2, and IgG3 subclass antibodies have been known to have similar half-life (about 20 days).¹⁷ Although there has been a lack of knowledge on the control mechanism and kinetics of allergen-specific IgG subclass antibodies,⁸ this finding might be compatible with the previous report, which showed IgG2 to be the most predominant subclass among specific IgG subclass antibodies to house dust mite in sera from mite-sensitive asthmatic patients.¹⁸ Together with our previous report⁵ on

seasonal variation of specific IgE antibody to house dust mite in the same subjects, the parallel change in mite-specific IgG4 antibody with mite-specific IgE antibody may be explained by the suggestion that the synthesis of IgG4 antibody is under the same cytokine (interleukin-4) regulation as IgE antibody.¹⁹ These findings suggest that each IgG subclass antibodies to house dust mite might be under different control mechanisms.

Environmental control of house dust mite is the first-line management for mite-sensitive asthmatic patients.²⁰ For the evaluation of house dust mite allergen exposure in asthmatic patients, collection and processing of house dust has been widely used.²⁰ A potential theoretical problem in the current method for evaluation of house dust mite allergen exposure is that the measurement of the concentration of mite allergen in dust samples is not a direct measurement of the allergen entering the lungs.²¹ Measurement of airborne allergens is also not an accurate method for the evaluation of allergen exposure, because only a certain particle size of airborne allergen can enter the lungs.²¹ One enthusiastic report tried to measure house dust mite allergen in bronchoalveolar lavage fluid in a limited number of asthmatic patients and showed a significant decrease after allergen avoidance.²² It should be noted that any of the above methods cannot be performed easily in clinical practice; therefore, there has been a

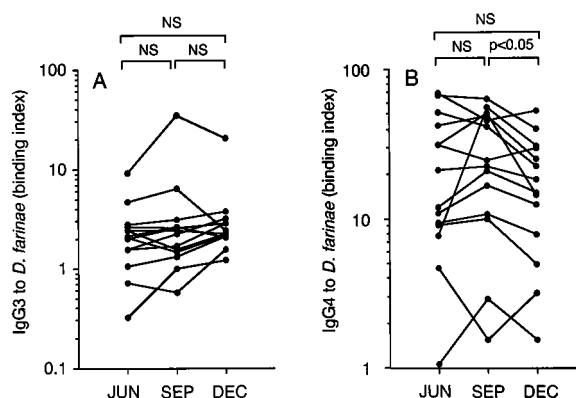


Figure 2. Seasonal changes in specific IgG3 (A) and IgG4 (B) antibodies to *D. farinae*.

need for the development of a simple indicator for current in vivo allergen exposure that can be used to evaluate the effectiveness of environmental house dust mite control.

Levels of allergen-specific IgE antibody have been suggested as an indicator of current exposure to house dust mite allergen.²³ A few supportive evidences exist for this. First, there is a significant correlation between specific IgE and environmental house dust mite allergen levels in mite-sensitive patients.²³ Second, avoidance of exposure to house dust mite allergen at high altitude, where no house dust mite was recovered, resulted in a decrease of serum house dust mite-specific IgE antibody levels.²⁴ Third, an increase in specific IgE antibodies to house dust mite was reported in institutionalized asthmatic children four days after they returned home.²⁵ Fourth, our previous report showed that a seasonal decrease in natural exposure to house dust mite allergen resulted in a concurrent decrease in the levels of specific IgE antibody to house dust mite.⁵

Another potential candidate as an the indicator of current exposure to allergen is the IgG4 antibody to allergen. Antigen-specific IgG4 antibody has been suggested as an indicator of exposure to a certain agent in a particular setting such as occupational asthma.²⁶ In this study, a parallel decrease in IgG4 antibodies to house dust mite associated with a seasonal decrease in house dust mite allergen levels in house dust was noted. Together with the previous report on the seasonal variation of IgG4 antibody to grass pollen in patients with grass pollen hypersensitivity,¹³ these findings suggest that serially measured allergen-specific IgG4 might be used as an indicator of exposure to allergen.

In conclusion, this study demonstrated that a sufficient long-term decrease in natural exposure to house dust mite allergen can result in a decrease in IgG4 antibody to house dust mite in mite-sensitive asthmatic patients. Further studies might be needed to evaluate the validity of specific antibodies to house dust mite as an indi-

cator of environmental exposure to house dust mite allergen in mite-sensitive asthmatic patients.

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- Request for reprints should be addressed to:*
Chein-Soo Hong, MD
Division of Allergy & Immunology
Department of Internal Medicine
Yonsei University College of Medicine
134 Shinchon-Dong, Seo Daemoon-ku
Seoul, 120-752
Korea
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