

Sensitivity to citrus red mite and the development of asthma

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Background and objective: The citrus red mite (*Panonychus citri*, CRM) is an important allergen in the development of asthma and/or rhinitis in citrus farmers and a common sensitizing allergen among exposed children. The aim of this study was to evaluate CRM-induced skin responses and serum-specific IgE, IgG4, and IgG1 responses, and their role in the development of asthmatic symptoms among exposed adolescents.

Methods: A total of 100 adolescents between the ages of 15 and 16 years (59 males and 41 females) were randomly recruited. All of the subjects responded to the ISAAC questionnaire themselves. Skin prick tests to CRM were carried out and serum-specific IgE, IgG4, and IgG1 levels were measured using ELISA.

Results: Fourteen subjects (14%) had experienced wheezing during last 12 months. The wheal size formed by CRM was larger than 3 mm in 23 subjects (23%). Serum-specific IgE levels were elevated in 21 subjects (21%), IgG4 levels were elevated in 32 subjects (32%), and IgG1 levels were elevated in five subjects (5%). The prevalence of wheezing during last 12 months was significantly higher in subjects with positive skin responses and serum-specific IgE responses induced by CRM than in those with negative skin responses and low serum IgE responses (30.4% versus 9.0%, $P < .05$; 33.3% versus 8.7%, $P < .05$). The prevalence of wheezing, however, did not differ according to serum-specific IgG4 levels (9.4% versus 16.2%, $P > .05$). Skin responses to CRM showed a significant correlation with serum-specific IgE levels, but not with IgG1 and IgG4 levels. Serum CRM-specific IgE levels correlated with specific IgG1 and IgG4 levels, but no significant correlation was found between serum IgG1 and IgG4 levels.

Conclusion: The specific IgE responses induced by CRM were prevalent among adolescents living near citrus farms, and may be important in the development of asthma. The specific IgG4 and IgG1 responses induced by CRM play a minimal role in the development of asthma symptoms.

Ann Allergy Asthma Immunol 2000;85:483–488.

INTRODUCTION

The citrus red mite (CRM) (*Panonychus citri*), which belongs to the family spider mite (*Tetranychidae*) infests citrus leaves, damaging them and in some

cases causing defoliation.¹ This mite is broadly distributed around the world, especially in Asia, California and Florida in North America, South Africa, and the Mediterranean where citrus fruits are widely cultivated.² A recent investigation has demonstrated that CRM is an important causative allergen among asthmatics, among citrus-cultivating farmers.³ It is also a common sensitizing allergen in children living around citrus orchards.⁴ Because citrus fruits have been cultivated worldwide and many people have been exposed to this mite, it is worthwhile to pay attention to CRM as a common environmental allergen.

Atopy is usually defined as genetically enhanced IgE production to common allergens; however, immunologic responses to allergens are not confined to the IgE antibody. Specific IgG responses to aeroallergens, foods, venoms, and occupational sensitizers are also detectable in sera from exposed individuals, regardless of atopic status.⁵ There has been much controversy as to the role of specific IgG subclasses induced by aeroallergens in the development of allergic diseases.

The aim of this study was to investigate the specific IgE, IgG4, and IgG1 antibody responses induced by CRM and their relationships with asthma and rhinitis symptoms among adolescents exposed to this mite.

SUBJECTS AND METHODS

Subjects

A total of 100 adolescents between the ages of 15 and 16 years (59 males and 41 females) were randomly recruited. All of them had lived in rural areas with citrus farms since infancy. The subjects were divided into two groups according to environmental exposure: (1) the high-exposure group (61 subjects) who lived near citrus farms (≤ 1 Km) and (2) the low-exposure group (39 subjects) who lived far from citrus farms (> 1 Km). All of the subjects responded to a questionnaire themselves and underwent skin prick tests to CRM. Sera were collected to measure CRM-specific IgE, IgG4, and IgG1 levels. All of the subjects gave their informed consent, which is required by the Seoul National University Hospital. The study protocol was approved by the Ethics Committee of the Seoul National University Hospital.

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Received for publication February 14, 2000.
Accepted for publication June 2, 2000.

Questionnaire Survey

A questionnaire developed by International Study of Asthma and Allergic diseases in Children (ISAAC)⁶ was used with some modifications, and included demographic data, asthma and rhinitis symptoms, and environmental exposure to CRM. The questions on respiratory symptoms concentrated on wheezing episodes and nasal symptoms without upper respiratory tract infections. The questions on environmental exposure to CRM concerned the distance from the home to the citrus farms (>1 km or ≤1 km).

Preparation for CRM Extracts and Skin Prick Tests

Citrus red mites were extracted as previously described.⁷ In brief, the mites were mixed with modified Coca's solution with a 1:5 wt/vol ratio. After incubation at room temperature for 24 hours, the mixture was centrifuged at 3,000 *g* for 30 minutes. The supernatant was dialyzed (the cut-off molecular weight was 6,000 D) against 4 L of distilled water for 48 hours at 4 °C, and then lyophilized at -70 °C to prepare the antigens for ELISA. The same amount of glycerin was added with a final concentration of 1:20 wt/vol, and this was used for the skin prick tests.

None of the subjects had received antihistamines orally during the five days preceding the skin prick tests. A positive control of histamine (1 mg/mL, Allergopharma Co., Germany), along with a negative control of diluent (Allergopharma Co., Germany), was included in all tests. Fifteen minutes after the prick test, the mean diameter of the wheal formed by CRM was measured. If the diameter of the wheal measured 3 mm or larger compared to diluent, the skin response to CRM was defined as positive.

ELISA for Specific IgE, IgG4, and IgG1 Levels

The serum-specific IgE levels induced by CRM were determined using ELISA as previously described.³ In brief, microtiter plates (Dynatech, USA) were first coated with 50 µL/well of allergen extracts (5 µg/well), and left at 4 °C overnight. Each well was washed three times with 0.05% Tween phosphate buffered saline (PBS-T), and the remaining binding sites, were blocked by incubation with 350 µL of 3% bovine serum albumin for one hour at room temperature. The wells were then incubated for 2 hours at room temperature with 50 µL of either the patients' sera or control sera

(undiluted) from 30 CRM-unexposed nonatopic healthy controls. After being washed three times with PBS-T, 50 µL of the 1:1000 vol/vol biotin-labeled goat anti-human IgE antibody (Vector Co., USA) was added to the wells and incubated for 2 hours at room temperature. The wells were then washed three times with PBS-T and incubated with 1:1000 vol/vol streptavidin-peroxidase (Sigma Co., USA) for 30 minutes before another washing step. This was followed by incubation with 100 ml ABTS (2,2-azinobis-3-ethyl-benzthiazoline sulfuric acid in a citrate phosphate buffer) for 10 minutes at room temperature. The reaction was stopped by the addition of 100 µL 2 N sodium azide, and the absorbance was read at 410 nm by an automated microplate reader. All assays were performed in duplicate. For detecting specific IgG4 and IgG1 antibody levels, 1:1000 vol/vol goat anti-human IgG4 and IgG1 (Sigma Chemical Co., USA) were used instead of anti-human IgE and the same steps were then followed as above. The cutoff values of serum CRM-specific IgE, IgG4, and IgG1 levels were determined from the mean plus the two-fold standard deviations of the absorbance value (OD) from the control subjects.

Statistical Analysis

Positive rates between the two groups were analyzed using the chi-squared test. The mean values between the two groups were analyzed using the Student's unpaired *t* test. The statistical significance of the correlation between the two groups was calculated using Pearson's correlation coefficients. A *P* value of .05 or less was regarded as significant.

RESULTS

Table 1 shows the characteristics of the study subjects. Fourteen subjects (14%) had experienced wheezing and 32 subjects (32%) had experienced chronic rhinitis symptoms over the last 12 months. Twenty-three subjects (23%) showed positive skin responses to CRM. Serum CRM-specific IgE levels were elevated in 21 subjects

Table 1. Characteristics of Study Subjects

Characteristics	Number of Subjects (%)
Male:female	59:41
Asthma symptoms	
Ever experiencing wheezing	17 (17%)
Wheezing during last 12 months	14 (14%)
Wheezing during exercise	16 (16%)
Nocturnal cough	21 (21%)
Chronic rhinitis symptoms*	
Ever experiencing rhinitis symptoms	39 (39%)
Rhinitis symptoms during last 12 months	32 (32%)
Skin responses to CRM	23 (23%)
Serum CRM-specific antibody	
IgE	21 (21%)
IgG4	32 (32%)
IgG1	5 (5%)
Environmental exposure†	
≤1 Km	61
>1 Km	39

CRM: citrus red mite (*Panonychus citri*).

* Rhinitis symptoms: sneezing or nasal obstruction without upper respiratory infection.

† Distance from nearest citrus farms to house of subject.

(21%), specific IgG4 in 32 (32%), and specific IgG1 in five (5%).

The prevalence of wheezing during the last 12 months was 18% in the high-exposure group, and 7.7% in the low-exposure group. This showed a higher tendency toward wheezing in the high-exposure group, although the difference was not statistically significant ($P > .05$). Positive skin responses to CRM were higher in the high-exposure group than in the low-exposure group (32% versus 8.3%, $P < .05$). Serum specific IgE, IgG4, and IgG1 levels according to environmental exposure are shown in Figure 1. Serum CRM-specific IgE levels [absorbance (OD, $\times 1000$), geometric mean $\pm$ SD] were higher in the high-exposure group than in the low-exposure group (237 ± 276 versus 174 ± 102 , $P < .05$). Specific IgG4 and IgG1 levels [absorbance (OD, $\times 1000$), geometric mean $\pm$ SD], however, were not different between the two groups (107 ± 87 versus 108 ± 101 , $P > .05$; 97 ± 37 versus 90 ± 32 , $P > .05$, respectively).

Figure 2 shows serum CRM-specific IgE, IgG4, and IgG1 levels according to skin responses to CRM. Serum CRM-specific IgE levels [absorbance (OD, $\times 1000$), geometric mean $\pm$ SD] were higher among those with positive skin responses to CRM than among those with negative skin responses (399 ± 349 versus 173 ± 135 , $P < .0001$). Specific IgG4 and IgG1 levels [absorbance (OD, $\times 1000$), geometric mean $\pm$ SD] were not significantly different between the two groups (125 ± 110 versus 103 ± 86 , $P > .05$; 100 ± 29 versus 93 ± 28 , $P > .05$, respectively).

Serum-specific IgE levels [log-IgE (absorbance, OD, $\times 1000$)] were significantly correlated with wheal size formed by CRM (allergen/histamine ratio) (correlation coefficient = 0.47, $P < .0001$). However, Serum-specific IgG4 and IgG1 levels [log-IgE (absorbance, OD, $\times 1000$)] were not correlated with wheal size formed by CRM (correlation coefficient = 0.08, $P > .05$; correlation coefficient = -0.006 , $P > .05$, respectively). When individ-

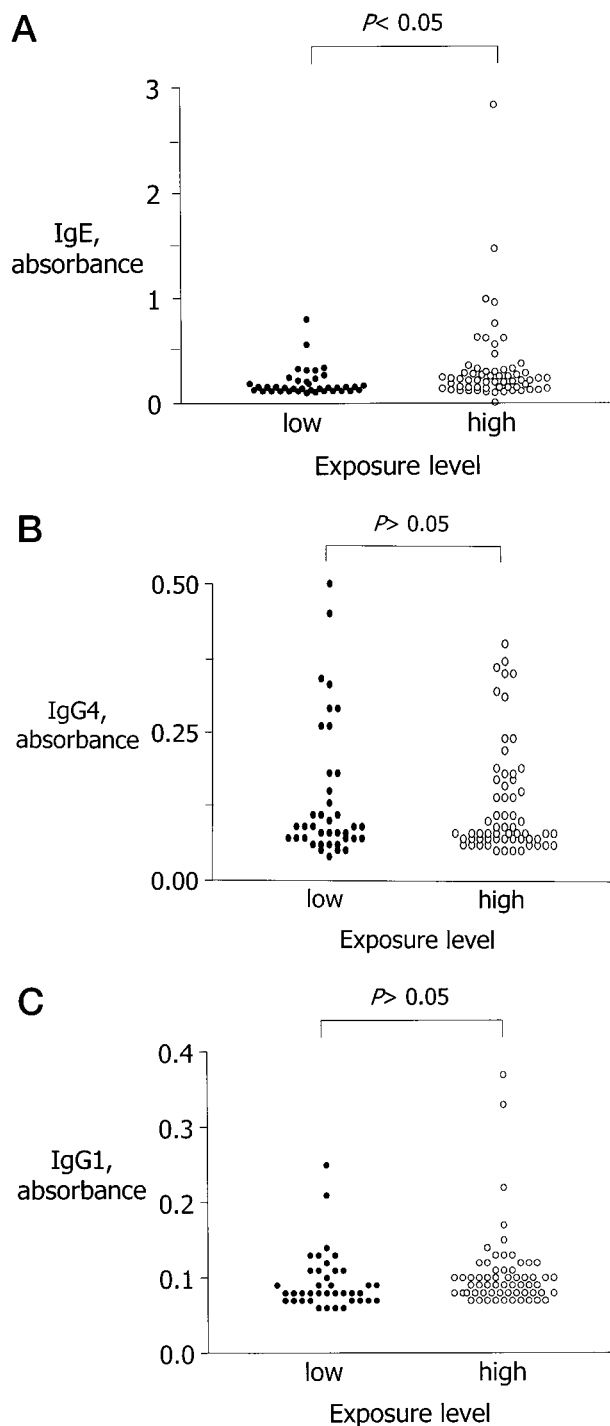


Figure 1. Individual values of serum specific IgE, IgG4, and IgG1 antibodies according to environmental exposure to the citrus red mite. This figure shows serum specific IgE (a), serum specific IgG4 (b), and serum specific IgG1 (c) levels (absorbance, OD) in the two groups with high and low-exposure to citrus red mite.

ual values of serum-specific IgE antibody were plotted against those of specific IgG4 or IgG1, a significant correlation was found between the specific IgE levels and the specific IgG4 and IgG1 levels (correlation coefficient = 0.24, $P < .05$; correlation coefficient = 0.49, $P < .0001$, respectively). No significant correlation, however, was found between specific IgG4 and IgG1 levels (correlation coefficient = 0.1, $P > .05$).

The prevalence of wheezing and chronic rhinitis symptoms during the last 12 months was higher among subjects with positive skin responses to CRM than among those with negative skin responses (30.4% versus 9%, $P < .05$; 52.2% versus 30%, $P < .05$). The prevalence of wheeze and chronic rhinitis during the last 12 months was significantly higher in subjects with high CRM-specific IgE levels than in those with low specific IgE levels (33.3% versus 8.9%, $P < .05$; 42.9% versus 29.1%, $P < .05$). The prevalence of asthma and rhinitis symptoms was the same in subjects with high and low specific IgG4 levels (9.4% versus 16.2%, $P > .05$; 28.1% versus 34.3%, $P > .05$, respectively).

DISCUSSION

In this study, among adolescents living in rural areas with citrus farms, the rate of positive skin responses to CRM was 23%, and the rate of positive serum-specific IgE responses was 21%. Cheju Island, where the study subjects had been living, is located off the southern coast of the Korean mainland and is famous for its citrus cultivation. Most adolescents living in the rural areas of Cheju Island have been there since infancy, rarely moving to other areas, and spend much of their time outdoors. Our previous study has revealed that CRM is the most common sensitizing allergen (16.5%) among citrus-cultivating farmers,³ and a common sensitizing allergen in children living around citrus farms.⁴ The environmental characteristics of Cheju Island could explain the higher rate of sensitization to CRM among adolescents in the rural areas.

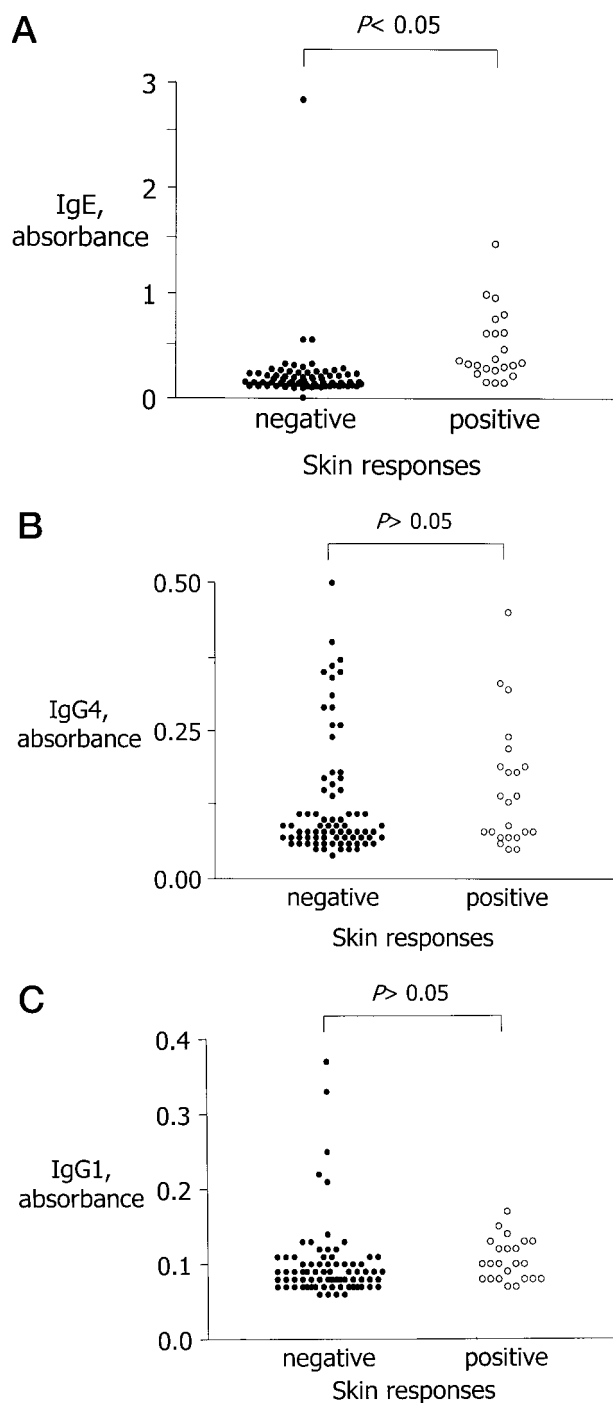


Figure 2. Individual values of serum specific IgE, IgG4, and IgG1 antibodies according to skin responses to the citrus red mite. This figure shows serum specific IgE (a), serum specific IgG4 (b), and serum specific IgG1 (c) levels (absorbance, OD) in the two groups with positive and negative skin responses to citrus red mite.

The relationship between environmental exposure and sensitization to indoor allergens has been previously studied. The results from a follow-up of a cohort of children by Sporik et al⁷ showed that exposure to high levels of house dust mite allergen increased the risk of sensitization. The present study demonstrated that subjects who had been living near citrus farms had enhanced skin and serum-specific IgE responses induced by CRM compared with those living far from citrus farms. No significant difference, however, was found in the serum CRM-specific IgG4 and IgG1 levels according to exposure levels. From these findings, we can assume that specific IgE responses induced by CRM-derived allergens may be determined by environmental exposure.

Several studies have suggested that the presence of specific IgG antibodies may represent a response to repeated allergen exposure. Park et al⁸ reported that the correlation between specific IgG antibodies to corn dust and exposure duration was significant among employees working in the animal feed industry. Aalberse et al⁹ found that specific IgG1 responses were predominant during the first few months of beekeeping, and IgE antibodies also appeared in more than 50% of the beekeepers during this period. IgG4 dominance became apparent particularly during the second year of beekeeping, and professional beekeepers had extremely high IgG4 antibody levels. In the present study, the serum-specific IgG4 levels induced by CRM were predominant compared to IgG1 levels (32% versus 5%). This finding may be explained by the fact that the adolescents tested in this study had been exposed to CRM for a long period of time.

Immune responses to inhalant allergens are not restricted to atopic subjects. While elevated serum IgE levels to common environment allergens are a distinctive feature of atopy, allergen-specific IgG responses can be detected in sera from both atopic and nonatopic subjects. IgE-inducing antigens have also proved to be excellent IgG4 in-

ducers, including not only many different atopic allergens, but also parasite allergens and castor bean allergens.¹¹ The reverse does not seem to always be the case, as evidenced by a food protein that induces strikingly high IgG4 responses without a marked contribution to food allergy.¹² Analysis by Western blotting of complicated allergen extracts reveals the IgG4 response to be more like the IgE response than the other Ig subtypes.¹³ On the level of the total allergen molecule, IgE and IgG4 responses are thus found to be associated.¹⁴ The explanation for this is that presumably both isotypes are IL-4 dependent and thus require the stimulation of Th2 cells.¹⁵⁻¹⁸ In this study, a significant correlation was observed between CRM-specific IgE levels and IgG4 and IgG1 levels, but not between specific IgG4 and IgG1 levels. This finding suggests that CRM-specific IgG4 and IgG1 responses are linked to the specific IgE responses induced by CRM.

Little has been known about the role of the specific IgG4 antibody in the development of clinical allergy. Human basophils express the low-affinity type II γ -receptor, CD32.¹⁹ Investigators have revealed, however, that IgG4 antibodies are functionally monovalent.¹⁰ The functional monovalency of IgG4 makes it unsuitable to act as a cytophilic antibody, like IgE, to become cross-linked by antigens.¹¹ These findings can explain our observation that skin responses to CRM were significantly correlated with serum-specific IgE responses, but not with IgG4 responses.

The fact that clinical improvement after immunotherapy induced specific IgG4 antibody suggests that allergen-specific IgG4 acts as a blocking antibody by competing with IgE for allergen binding and inhibiting IgE-dependent activation of mast cells and basophils.²⁰ In the study on occupational asthma and rhinitis in laboratory rats, Platts-Mills et al.²⁰ reported that asthmatic reactions significantly associated with serum-specific IgE levels, but not with IgG4 levels. In this study, the prevalence of asthmatic symptoms was significantly associated

with the skin responses and serum-specific IgE responses induced by CRM, but not with specific IgG4 responses. These findings suggested that the specific IgG4 responses induced by CRM play a minimal role in the development of asthma.

In conclusion, a positive rate of specific IgE responses to CRM was prevalent among adolescents living near citrus farms, and specific IgE responses could be determined by environmental exposure. Specific IgG4 and IgG1 responses induced by CRM play a minimal role in the development of asthma symptoms.

ACKNOWLEDGEMENT

This study was supported by a research grant from the Seoul National University Hospital, Seoul, Korea.

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