

Citrus red mite (*Panonychus citri*) is the most common sensitizing allergen of asthma and rhinitis in citrus farmers

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Summary

Objective To evaluate type I hypersensitivity to citrus red mite (*Panonychus citri*), its prevalence, and relationship to respiratory dysfunction, a cross-sectional survey was performed among citrus farmers on Cheju Island, Korea.

Materials and methods Questionnaires, and skin prick test responses to 11 common inhalant allergens and citrus red mite were performed in 181 citrus farmers, and serum-specific IgE antibodies to citrus red mite were measured by ELISA in sera of 123 subjects. To determine airway hyperresponsiveness, methacholine bronchial provocation tests were performed in 55 subjects who complained of recurrent lower respiratory symptoms.

Results The prevalence of asthma based on presence of asthmatic symptoms on the questionnaire and airway hyperresponsiveness to methacholine, and allergic rhinitis based on presence of nasal symptoms on the questionnaire and positive skin-test response were 12.1% and 19.3%, respectively. The positive rate of skin responses to one or more of 11 common inhalant allergens excluding citrus red mite was 17.1%, and if citrus red mite was included, 25.9% of farmers had positive responses. On skin prick tests, citrus red mite (16.5%) was the most common sensitizing allergen, followed by cockroach (11.0%), *Dermatophagoides pteronyssinus* (9.9%), and *D. farinae* (9.3%). Among farmers with asthma and allergic rhinitis, the positive skin responses to citrus red mite were noted in 54.5% and 68.5%, respectively. Serum-specific IgE antibodies to citrus red mite were detected in 45 farmers (36.5%) of the 123 tested, and there was significant correlation between specific IgE level and weal (A/H ratio) to citrus red mite ($r = 0.57$, $P < 0.001$). The prevalence of asthma was higher in subjects with positive skin responses or high serum-specific IgE antibodies to citrus red mite than in those without skin response or serum specific IgE ($P < 0.05$, respectively).

Conclusion Citrus red mite is the most important allergen in citrus farmers with asthma and rhinitis in which causative allergen has not been identified. It should be included in the skin test battery for screening the causative allergen in farmers exposed to citrus red mite.

Keywords: asthma, citrus farmer, citrus red mite, rhinitis

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Introduction

Spider mites (Family Tetranychidae) can be pests of citrus throughout the world, especially in subtropical areas [1]; the

two most common species are the citrus red mite (CRM, *Panonychus citri*) and the Texas citrus mite (*Eutetranychus annecki*). CRM, one of the mites which causes most damage to citrus, rapidly develops injurious populations in early spring, with two peaks in late spring and mid fall [1]. Cheju Island is located in the southern part of the Korean mainland, where about half a million people live. Citrus trees

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were introduced before 1945, and citrus fruit has been one of the main products for about 35 years. More than 50 thousand adults have been commercially involved in citrus cultivation, and few have moved to other areas.

It has been established that in the occupational environment, the non-pyroglyphid storage mites are often found to cause allergic problems [2,3], though the pyroglyphid mites, *Dermatophagoides*, are chiefly responsible for house dust allergy [4]. There have been several reports of occupational allergy to Tetranychidae [5–7], and we recently reported 16 cases of CRM-induced asthma in citrus farmers who visited our clinic [8]. In this study, a cross-sectional survey was performed to evaluate type I hypersensitivity to CRM, its prevalence, and relationship to respiratory dysfunction in citrus farmers on Cheju Island.

Materials and methods

Study subjects

A hundred and eighty-one farmers involved in citrus production on Cheju Island were enrolled in this study. They had worked on citrus farms for a mean period of 23.7 years (range 5–40); 78 were males and 103 were females, and their mean age was 50.1 years (range 18–87); 67 subjects (37.2%) had a smoking history (Table 1). They responded to a questionnaire, administered by physicians and allergists, and underwent skin prick tests (SPTs) including CRM extract and 11 common inhalant allergens. Subjects who had had asthmatic symptoms during the previous 12 months were evaluated by methacholine bronchial provocation test. Sera from 123 subjects were collected and stored at -20°C . The subjects gave their written informed consent as regulated by Seoul National University Hospital, Seoul, Korea.

Questionnaire survey

Physicians together with allergists administered a questionnaire, which was developed from the Respiratory Disease Questionnaires of the American Thoracic Societies [9]. It was translated into Korean and included questions regarding demographic data, smoking status, occupational, and medical history. The medical history concerned asthmatic symptoms (paroxysmal cough, wheezing, and nocturnal dyspnea or dyspnea at rest), the onset of asthmatic symptoms, nasal symptoms (paroxysmal sneezing, watery rhinorrhea, and nasal obstruction), and the temporal relationship between exposures at workplace and aggravation of symptoms. If, according to the questionnaire, subjects had experienced two or more asthmatic and/or nasal symptoms, they were assumed to be positive in this respect.

Preparation of CRM extract

CRMs were provided from the Citrus Research Center on Cheju Island. They were extracted with phosphate-buffered saline (PBS, pH 7.5, 1:5 w/v) at 4°C for 1 h followed by centrifugation at 2000 *g*. The supernatants were passed through syringe filter (MSI, Westobar, MA, USA), and for SPT, the 1:5 w/v extract was mixed with an equal amount of sterile glycerin. The supernatant was dialysed (the cutoff molecular weight was 6 kDa) against 4 L of distilled water at 4°C for 48 h, and lyophilized at -70°C for the preparation of antigens used in ELISA and ELISA inhibition test.

Allergy skin prick tests

SPTs with 11 common inhalant allergens (*D. pteronyssinus*, *D. farinae*, *Aspergillus* spp., *Alternaria* spp., tree pollen mixture 1 and 2, grass pollen mixture, mugwort and ragweed pollen, animal epithelium, and cockroach, Allergopharma Co., Leinbek, Germany), CRM (1:10 w/v), and histamine (1 mg/mL, Allergopharma Co.) were performed on the volar side of both forearms. After 15 min, mean diameter of a weal formed by the allergen was compared with that formed by histamine. If the former was the same or larger than the latter (A/H ratio ≥ 1.0), the reaction was deemed to be positive. If responses to the questionnaire indicated chronic nasal symptoms and positive skin response to one or more of inhalant allergens including CRM was noted, allergic rhinitis was diagnosed.

ELISA for specific IgE antibodies to CRM extract

The presence of specific IgE antibodies to CRM was determined by ELISA. Microtitre plates (Dynatech, VA, USA) were first coated with 50 μL /well of CRM (5 μg /well) extracts, 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% BSA-PBST for 1 h at room temperature. The wells were then incubated for 2 h at room temperature with 50 μL of either the patient's or control sera (undiluted) from 30 asthmatic patients showing a negative SPT response to common inhaled allergens as well as to CRM. After washing three times with PBS-T, 50 μL of the 1:1000 v/v biotin-labelled goat antihuman IgE antibody (Vector Co., Berlingham, CA, USA) were added to the wells and incubated for 2 h at room temperature. The wells were then washed three times with PBS-T and incubated with 1:1000 v/v streptavidin-peroxidase (Sigma Co., St Louis, MO, USA) for 30 min before another washing step which was followed by incubation with 100 μL ABTS (2,2'-azinobis-3-ethyl-benzthiazoline sulphuric acid in a citrate phosphate buffer) for 10 min at room temperature. The

Table 1. Characteristics of study subjects

	Asthmatics	Allergic rhinitics	Total subjects
Number	22	35	181
Age, mean years (range)	52.2 (29–70)	45.6 (18–77)	50.1 (18–87)
Sex (male/female)	12/10	19/16	78/103
Smoking history (%)	45.4	28.6	37.0
Occupational history, mean years (range)	23.8 (9–40)	22.2 (9–40)	23.7 (5–40)
Skin prick test* (%)	59.0	100	25.9
<i>D. pteronyssinus</i> (%)	27.2	40	9.9
<i>D. farinae</i> (%)	22.7	34.2	9.3
Tree I (%)	0	0	0
Tree II (%)	0	0	0
Grass (%)	0	2.8	1.1
Ragweed (%)	0	2.8	1.1
Mugwort (%)	9	8.5	3.3
<i>Alternaria</i> spp. (%)	4.5	2.8	1.1
<i>Aspergillus</i> spp. (%)	0	0	1.1
Animal epithelium (%)	0	0	0
Cockroach (%)	18.1	34.2	11.0
Citrus red mite (%)	54.5	68.5	16.5
SPT (–CRM†) (%)	36.3	62.8	17.1
SPT (+CRM) ‡ (%)	59.1	100	25.9
CRM only§ (%)	22.7	37.2	6.6
Asthma¶ (%)	–	34.2	12.1
CRM-sensitive asthma** (%)	50	31.4	6.0

*Weal formed by one or more of inhalant allergens including citrus red mite $\geq$ weal formed by histamine (1 mg/mL).

†Positive skin reaction to one or more of inhalant allergens excluding citrus red mite. ‡Positive skin reaction to one or more of inhalant allergens including citrus red mite. §Positive skin reaction only to citrus red mite. ¶Positive asthmatic symptoms on a questionnaire and bronchial hyperresponsiveness to methacholine. **Asthmatic subjects with positive skin responses to citrus red mite and work-related respiratory symptoms.

reaction was stopped by the addition of 100 μ L 2 mol/L sodium azide and the absorbance was read at 410 nm by an automated microplate reader. All assays were performed in duplicate. The cutoff value (0.24) of the positive IgE binding was determined from the mean plus twofold standard deviation of the absorbance value from 30 control subjects.

ELISA inhibition tests with CRM and *D. pteronyssinus*

Competitive ELISA inhibition was performed to determine the specificity of the IgE binding to the CRM antigen. The sera of the patients who had high specific IgE binding were pooled. Fifty microlitres of this pooled sera was preincubated with four concentrations (0.02–20 μ g/mL protein concentration) of *D. pteronyssinus* extract (Pharmacia, Uppsala, Sweden), as well as CRM extract, for 1 h at room temperature, and then incubated with the CRM-coated microtitre plate for 2 h. The same steps of the

experiments were followed as in the ELISA. After studying the control samples in which equal volumes of PBS, pH 7.5 were preincubated instead of inhibitors, and the inhibition of the specific IgE binding was expressed as:

$$100 - \frac{\text{abs. of samples preincubated with allergens}}{\text{abs. of samples preincubated with PBS}} \times 100$$

Methacholine bronchial provocation tests

Forced expiratory volume in 1 second (FEV₁) was measured with a portable spirometer (Micro Spirometer, Micro Medical, Rochester, UK), and the largest value of triplicate FEV₁ was adopted. Methacholine bronchial provocation testing was carried out, using the modified method of Chai *et al.* [10]. Concentrations of 1.25, 2.5, 6.25, 12.5, and 25 mg/mL of methacholine were prepared by dilution with buffered saline. A Rosenthal-French dosimeter (Laboratory for Applied Immunology, Baltimore, MD, USA) was used

to deliver the aerosol generated by a DeVilbiss 646 nebulizer (Somerset, PA, USA). Subjects with asthmatic symptoms inhaled five tidal breaths of increasing concentrations of methacholine until FEV₁ fell by more than 20% of its baseline value or the highest concentration was reached. The largest value of triplicate FEV₁ at 90 or 180 s after each inhalation was adopted for analysis. If the concentration of methacholine which caused a 20% fall in FEV₁ (PC₂₀) was less than 16 mg/mL, a subject was considered to be bronchial hyperresponsive to methacholine. If responses to the questionnaire indicated asthmatic symptoms and bronchial hyperresponsiveness to methacholine was noted, asthma was diagnosed.

Statistical analysis

Results were expressed as mean standard error (SE) unless otherwise indicated. The Chi-square, simple regression, and unpaired *t*-tests were applied using the Statview 512⁺ version on Macintosh (Abacus, Berkeley, CA, USA) to evaluate the statistical significance. A *P*-value of 0.05 or less was regarded as significant.

Results

Prevalence of asthma and allergic rhinitis, and the sensitizing allergens

Fifty-five (30.3%) of the 181 farmers complained of asthmatic symptoms on the questionnaire survey, and when methacholine bronchial provocation test were performed on these subjects, 22 (12.1%) had bronchial hyperresponsiveness to methacholine (Table 1). Of those asthmatic subjects, 12 were males and 10 were females; their mean age was 52.2 years (range 29–70), and mean duration of asthmatic symptoms was 6.4 years (range 1–20). Of the 22 asthmatics, allergic rhinitis was diagnosed in 12 subjects (54.5%), who had recurrent nasal symptoms and positive skin response to inhalant allergens including CRM. Thirteen (59.1%) of the asthmatics had positive skin responses to one or more inhalant allergens including CRM, and five (22.7%) showed an isolated skin response only to CRM. For those asthmatics, the most common sensitizing allergen was CRM (54.5%), followed by *D. pteronyssinus* (27.2%), *D. farinae* (22.7%), and cockroach (18.1%), and serum-specific IgE antibody to CRM was detected in 12 (66.7%) of the asthmatics.

Eighty-three (45.8%) of the total subjects complained of recurrent nasal symptoms on the questionnaire survey, and 35 (19.3% of the total) of those subjects had positive skin test responses to one or more of inhalant allergens including CRM. Of those allergic rhinitics, 19 were males and 16 were females, and their mean age was 45.6 years (range 18–77);

the mean duration of nasal symptoms was 7.2 years (range 1–20). Twenty (57.1%) of the 35 subjects with allergic rhinitis had experienced asthmatic symptoms, and bronchial asthma was diagnosed in 12 (34.2%). On the SPTs, the most common sensitizing allergens in decreasing order were CRM (68.5%), *D. pteronyssinus* (40.0%), *D. farinae* (34.2%), and cockroach (34.2%). Of the allergic rhinitics, 18 (69.2%) had high serum IgE to CRM, and 13 (37.2%) showed an isolated skin response only to CRM.

Skin responses to CRM, and its relationship to respiratory dysfunction

On SPTs with 11 common inhalant allergens, 31 (17.1%) of the 181 farmers demonstrated positive skin responses. However, 47 (25.9%) had positive skin responses if CRM was included in the inhalant allergens. On the SPTs in the total 181 subjects, the most common sensitizing allergen was CRM (16.5%), followed by cockroach (11.0%), *D. pteronyssinus* (9.9%), and *D. farinae* (9.3%). No difference on the skin test response to CRM was found according to smoking status (16.4 vs 16.6%, *P* > 0.05). However, the positive skin test response to CRM was more prevalent in subjects with positive skin responses to the other allergens (58.1%) than in those without it (8%, *P* < 0.001).

The positive rate of asthmatic and nasal symptoms based on the questionnaire survey was higher in the subjects showing positive skin response to CRM than in those with negative response (80 vs 39.1%, *P* = 0.001; 63.3 vs 23.8%, *P* = 0.001, respectively). The prevalence of asthma based on those having both asthmatic symptoms and bronchial hyperresponsiveness to methacholine was also higher in the positive skin responders to CRM than in the negative skin responders (40 vs 6.6%, *P* = 0.001).

Specific IgE antibody to CRM, and its relationship to respiratory dysfunction

The serum-specific IgE bindings to CRM in 123 farmers and in 30 control subjects are illustrated in Fig. 1. When an ELISA was considered as positive (≥ 0.24 of absorbance value), 45 (36.5%) were positive. The relationship between the specific IgE bindings and the skin responses to CRM are illustrated in Fig. 2, which demonstrates a significant correlation between them (*r* = 0.5, *P* < 0.001). Eighteen (90%) of 20 positive skin responders to CRM had high serum IgE to CRM, although 27 (26.2%) of 103 negative skin responders to CRM had high serum IgE. No difference of specific IgE bindings was found according to smoking status (0.46 ± 0.07 vs 0.32 ± 0.04 , *P* = 0.07). Table 2 shows a significant association between the presence of specific IgE antibody and the SPT results to two kinds of house dust mites such as *D. pteronyssinus* and *D. farinae*.

The positive rate of asthmatic and nasal symptoms were

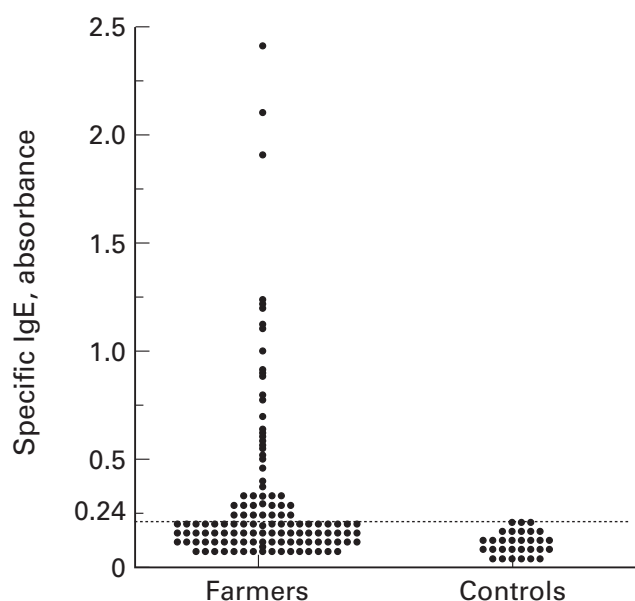


Fig. 1. Specific IgE bindings to citrus red mite by ELISA in sera from 123 farmers and 30 unexposed asthmatic controls.

significantly higher in the subjects with high serum IgE to CRM than in those without it (55.6 vs 20.5%, $P = 0.001$ and 62.2 vs 34.5%, $P = 0.01$, respectively). There was also a significant association between prevalence of asthma and presence of serum specific IgE antibody (26.7 vs 7.7%, $P = 0.004$).

ELISA inhibition tests

The dose–response pattern of the inhibitory effects with addition of the CRM antigens was illustrated in Fig. 3. Twelve (40%) of the 30 subjects with skin responses to CRM showed positive skin responses to *D. pteronyssinus* or *D. farinae*. In sera pool from five subjects with positive skin responses to both CRM and *D. pteronyssinus*, significant

Table 2. Relationships between skin response and specific IgE antibody to citrus red mite, and skin responses to house dust mites

Skin response	<i>D. pteronyssinus</i>		<i>D. farinae</i>	
	Positive	Negative	Positive	Negative
<i>Skin response to CRM</i>				
Positive ($n = 30$)	12	18	12	18
Negative ($n = 151$)	6	145	5	146
<i>Specific IgE to CRM</i>				
Positive ($n = 45$)	8	37	8	37
Negative ($n = 78$)	2	76	2	76

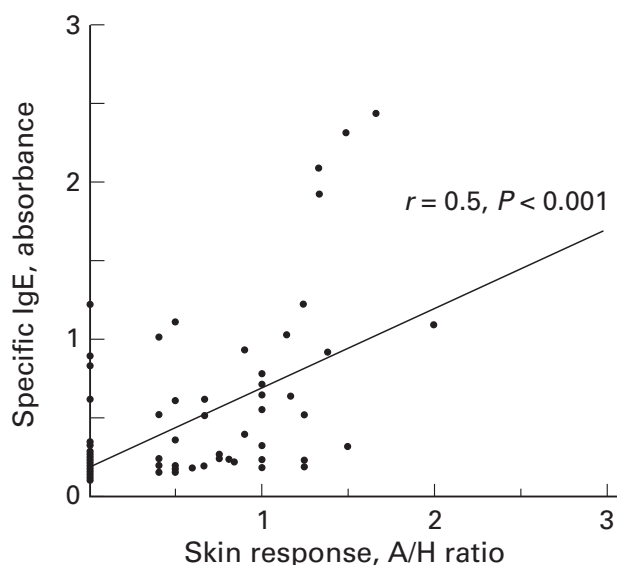


Fig. 2. Relationship between specific IgE antibodies by ELISA and skin responses to citrus red mite on skin prick tests.

inhibitions up to 52% were noted with additions of *D. pteronyssinus* in a dose-dependant manner. However, in sera pool from five subjects with an isolated skin response to CRM, significant inhibitions were noted with addition of CRM antigens, while minimal inhibitions were noted with additions of *D. pteronyssinus*.

Clinical characteristics of subjects with CRM-sensitive asthma

Eleven of 181 (6.0% of the total, half of all asthmatics) were considered as having CRM-sensitive asthma as asthmatic symptoms had developed after work and had been aggravated in the workplace, and SPT was positive to CRM. Five were males and six were females, and their mean age was 46.1 years (range 29–63). The mean duration of latency between beginning of citrus cultivation and onset of asthmatic symptoms was 13.5 years (range 7–25). All had experienced recurrent nasal symptoms, which had been aggravated during working on citrus farms, and developed at an earlier time than asthmatic symptoms. Positive rate of skin response to one or more of the other inhalant allergens in these asthmatics was 54.5%, and the common sensitizing allergens were *D. pteronyssinus* (54.5%) and cockroach (54.5%). All except one case had high serum specific IgE antibody to CRM.

Discussion

In most epidemiological surveys of mite allergy, the only diagnostic criteria have been subjective symptoms and past

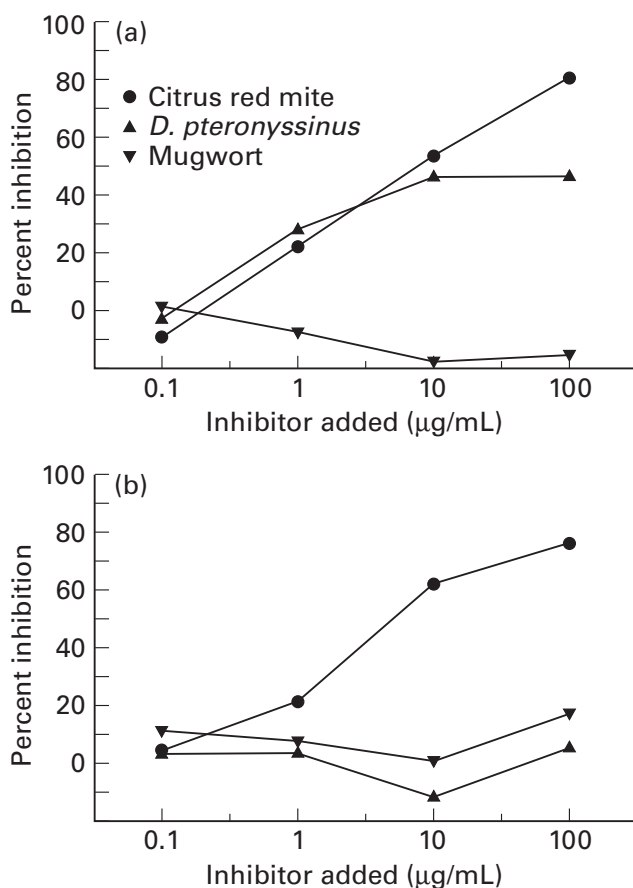


Fig. 3. Percent inhibition of citrus red mite IgE-ELISA with serial additions of citrus red mite, and *D. pteronyssinus* antigens. Sera pool showing concurrent positive response to citrus red mite and *D. pteronyssinus* was used in (a) and that showing an isolated positive response to citrus red mite was used in (b).

medical history of asthma [11–15]. Even though this low-cost approach is useful in a large population study, limitations such as a lack of objectivity, together with poor predictability of current asthma, and bronchial hyperresponsiveness cannot be avoided [11,16]. In our previous study [17], only 20.5% of children who reported asthmatic symptoms in a questionnaire were actually diagnosed as having bronchial hyperresponsiveness to methacholine, and, in this study, only 40% of adults with two or more asthmatic symptoms on the questionnaire showed bronchial hyperresponsiveness to methacholine. Toelle *et al.* [18] defined current asthma as the presence of asthmatic symptoms during the past 12 months plus bronchial hyperresponsiveness. In this study, we evaluated the prevalence of asthma according to Toelle and co-worker's definition.

Among citrus-cultivating farmers, the prevalence of asthma was 12.1%, which is higher than that of 4.6% found among children on the Korean mainland [17]. At the same time, the positive skin response to inhalant

allergens excluding CRM was lower in those islanders than in mainland children, but there was no difference in the proportion of extrinsic asthma, defined as current asthma plus positive skin reaction to allergens including CRM, between asthmatics on the island and those on the mainland [17]. In this study, the prevalence of asthma was higher in subjects with positive skin response or high serum IgE antibody to CRM than in those with negative skin response or low serum-specific IgE antibody. These findings suggest that CRM is an important causative allergen in the development of adult-onset asthma in citrus-cultivating farmers, which may be IgE-mediated.

Although sensitization to inhalant allergens requires exposure to only a small dose, it is possible that exposure to high levels may be associated with the greatest risk of sensitization [19]. In this study, the most common sensitizing allergen on the skin prick tests was CRM (16.5%), followed by cockroach (11.0%), *D. pteronyssinus* (9.9%) and *D. farinae* (9.3%). The positive rate of skin response to cockroach was similar to that found among mainland children (11.0 vs 11.8%) [17]. However, positive rate to *D. pteronyssinus*, *D. farinae* and animal epithelium was significantly lower in the citrus farmers than among those children on the mainland (9.9 vs 27.5%; 9.3 vs 30.9%; 0 vs 20.4%) [17]. These differences may be due to the differences in environmental conditions, not to difference in genetic susceptibility. The positive skin response to each inhalant allergen has a wide range of regional differences, and among children in the mainland, the most common allergen was *D. farinae* and *D. pteronyssinus*, followed by cat epithelium and cockroach [17], and early exposure to these inhalant allergens may correlate with subsequent IgE sensitization [19]. Features of modern-day housing – well insulated but poorly ventilated, combined with increased soft furnishings – could have resulted in increased exposure to house dust mite and allergens shed by pets. Since childhood, however, citrus farmers on Cheju Island – which is located in the southern part of the Korean mainland, and very cold and windy in winter season – have lived in 'choga houses' which are not sealed against cold winters and are well-ventilated, and have usually spent their life outdoors. These environmental characteristics may explain that among the citrus farmers, sensitization rate to house dust mites and animal epithelium was low, but CRM was the most common sensitizing allergen.

In subjects with continuous exposure to the causative allergen, the latent period was shorter than in those who were intermittently exposed. Childhood asthma tends to begin before the age of 5 years; indoor allergens, especially house dust mites are responsible for its development [20–22]. As in the case of pollens, citrus farmers may be intermittently exposed to CRM-derived allergens, which have two peaks in late spring and mid fall, and are minimal

during other periods, especially winter. The latent periods may thus be longer in subjects with CRM-sensitive asthma than in those with childhood asthma due to indoor allergens, such as house dust mites.

Several types of occupational asthma, most, but not all, caused by high-molecular-weight allergens, occur at a higher rate among atopic workers [23,24]. In this study, sensitization rate to CRM, and the prevalence of CRM-sensitive asthma and rhinitis were significantly higher in farmers with positive skin responses to other inhalant allergens than in those without. This finding suggests two possibilities: first, atopy may be a risk factor for sensitization to CRM, and for the development of asthma and rhinitis caused by CRM. Second, a positive skin reaction to house dust mites may reflect cross-reactivity to CRM. There is an agreement in the suggestion that the closer the taxonomic relationship between the mites is, the greater the level of cross-allergenicity is [25–27]. CRM belongs to the order of mites (Acari), to the suborder of Prostigmata, to the family of spider mites (*Tetranychidae*), to the genus *Panonychus* and represents its own species. Burches *et al.* [6] reported that all subjects sensitized to spider mites were cross-reactive to house dust mites. In this study, the serum IgE level to CRM were higher in the subjects with concurrent positive skin responses to house dust mites than those without it. However, 18 (60%) of 30 farmers with positive skin response to CRM showed an isolated positive skin response to CRM, although 12 (40%) showed positive skin responses to house dust mites. ELISA inhibition test showed that minimal inhibitions were noted with additions of *D. pteronyssinus* in subjects with an isolated skin reaction to CRM, while in those with positive skin responses to both CRM and *D. pteronyssinus*, significant inhibitions were noted with additions of *D. pteronyssinus* antigens in a dose-dependent manner. These results suggest that CRM-derived extracts contain species-specific allergens in addition to common sharing allergens with house dust mites. Further investigations will be needed to identify species-specific, or common allergenic components within CRM extracts.

In conclusion, CRM is the most important allergen in citrus farmers with asthma and rhinitis in which causative allergen has not been identified. It should be included in skin test battery for screening causative allergen in citrus farmers.

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