

IgE binding components in *Tetranychus urticae* and *Panonychus ulmi*-derived crude extracts and their cross-reactivity with domestic mites

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Summary

Background Recent investigation has revealed that spider mites such as *Tetranychus urticae* and *Panonychus ulmi* are important allergens in the development of occupational asthma among apple farmers.

Objective To evaluate IgE binding components in *T. urticae* and *P. ulmi*-derived crude extracts and their cross-reactivity with *Panonychus citri*, *Tyrophagus putrescentiae* and *Dermatophagoides pteronyssinus* in apple cultivating farmers.

Methods Thirty-one apple farmers with positive skin responses to *T. urticae* or *P. ulmi* were randomly recruited, and specific IgE levels in their sera were measured using ELISA. Cross-reactivity was evaluated by ELISA inhibition. IgE binding components were evaluated by IgE immunoblotting.

Results A total of 11 IgE binding components in *T. urticae* and 10 in *P. ulmi* were found. Among them, the 17 kDa, 27 kDa, 33 kDa, 37 kDa, 41 kDa, 56 kDa, and 75 kDa allergens in *T. urticae*, and the 33 kDa, 41 kDa, and 51 kDa allergens in *P. ulmi* were identified as dominant allergens. *T. urticae*-specific IgE binding was completely inhibited by 100 µg/mL of *T. urticae* (99.7%), but only partially inhibited by *P. citri* (83.0%), *P. ulmi* (71.6%), *T. putrescentiae* (69.7%), and *D. pteronyssinus* (60.1%). *P. ulmi*-specific IgE binding was completely inhibited by additions of *P. citri* (92.3%) and *P. ulmi* (91.2%), but only partially inhibited by the addition of *T. urticae* (61.5%), *T. putrescentiae* (57.7%), and *D. pteronyssinus* (54.4%).

Conclusion There were seven dominant allergens found in *T. urticae* and three in *P. ulmi*. *T. urticae*- and *P. ulmi*-specific IgE bindings were partially inhibited by crude extracts derived from *D. pteronyssinus* and *T. putrescentiae*.

Keywords: spider mite, *Tetranychus urticae*, *Panonychus ulmi*, *Panonychus citri*, *Tyrophagus putrescentiae*, *Dermatophagoides pteronyssinus*, allergen, cross-reactivity

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Introduction

Allergic diseases due to domestic mites are a major health problem worldwide. House dust mites, particularly *Dermatophagoides pteronyssinus* (DP) and *D. farinae* (DF), are

important sources of allergens inside homes in humid regions [1,2]. *Tyrophagus putrescentiae* (TP) is recognized as one of the important storage mites in rural regions [3,4]. According to an epidemiologic study in Korea, DF, DP, and TP cohabit in more than 25% of the houses examined, and TP has been reported to be the third most common domestic mite in Korea [5].

Spider mites (Tetranychidae) were negligible pests in

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fruit cultivation before World War II. However, these mites have increased in the fruit cultivation industry since pesticides were introduced to eliminate fruit moths 40 years ago [6,7]. Our investigations have demonstrated that the citrus red mite (*Panonychus citri*) is the most important causative allergen among citrus-cultivating farmers with asthma and/or rhinitis [8,9]. The European red mite (*Panonychus ulmi*) and the two-spotted spider mite (*Tetranychus urticae*) are important allergens in the development of occupational asthma among apple-cultivating farmers [10]. Taxonomically, spider mites belong to the order Acari, suborder Prostigmata, and the family of Tetranychidae, but the domestic mites such as TP and DP belong to the suborder Astigmata. However, previous reports have not demonstrated any clear allergenic relationship between spider mites and domestic mites [11,12]. The aim of this study was to evaluate IgE-binding components derived from crude extracts of *T. urticae* and *P. ulmi*, and determine their cross-reactivity with *P. citri*, TP and DP among apple farmers.

Methods

Subjects

In January 1999, 725 farmers involved in apple production in Korea were cross-sectionally surveyed and skin prick tests performed with *T. urticae* and *P. ulmi* extracts [10].

Among these, 31 subjects with weal size, formed by *T. urticae* or *P. ulmi*, of larger than 3 mm were randomly recruited in this study (Table 1). Ten of the enrolled subjects had work-related asthma symptoms, which had been aggravated during their work in apple orchards and relieved during their holiday or vacation. Mean age (range) of subjects with and without these symptoms was 48.9 (41–67) and 50.6 (36–69) years, respectively. Mean occupational duration (range) of the two groups was 16.2 (9–28) years and 15.1 (7–30) years. Subjects gave written informed consent as required by the Seoul National University Hospital, Seoul, Korea.

Preparation of *T. urticae*, *P. ulmi*, *P. citri*, TP, and DP extracts

T. urticae, *P. ulmi*, and *P. citri* were extracted as previously described [8–10]. In brief, the mites were mixed with modified Coca's solution in a 1 : 5 w/v ratio. After incubation at room temperature ($\approx 20^\circ\text{C}$) for 24 h, the mixture was centrifuged at 3000 g for 30 min. The supernatant was dialysed (the cut-off molecular weight was 6000 Da) against 4 L of distilled water for 48 h at 4 °C, and lyophilized with a final concentration of 1 mg/mL at -70°C for the preparation of the allergens used in skin prick tests, ELISA and ELISA inhibition.

TP and DP extracts for ELISA and ELISA inhibition tests were kindly provided by Dr J. W. Park (Institute of

Table 1. Characteristics of study subjects

Characteristics of subjects	WRS (+)	WRS (–) (n = 10)	P (n = 21)
Age*, years	48.9 (41–67)	50.6 (36–69)	> 0.05
Occupational duration, years	16.2 (9–28)	15.1 (7–30)	> 0.05
Smoking	40%	52.4%	> 0.05
Skin reactivity†			
<i>D pteronyssinus</i>	0.58 $\pm$ 0.62	0.68 $\pm$ 0.45	> 0.05
<i>T putrescentiae</i>	0.34 $\pm$ 0.41	0.58 $\pm$ 0.52	> 0.05
<i>P ulmi</i>	0.98 $\pm$ 0.36	1.04 $\pm$ 0.51	> 0.05
<i>T urticae</i>	1.07 $\pm$ 0.33	1.03 $\pm$ 0.49	> 0.05
Serum specific IgE‡			
<i>D pteronyssinus</i>	1.92 $\pm$ 0.34	1.75 $\pm$ 0.13	> 0.05
<i>T putrescentiae</i>	1.96 $\pm$ 0.34	1.93 $\pm$ 0.26	> 0.05
<i>P ulmi</i>	2.59 $\pm$ 0.15	2.27 $\pm$ 0.41	0.02
<i>T urticae</i>	2.48 $\pm$ 0.57	2.04 $\pm$ 0.41	0.02
<i>P citri</i>	2.51 $\pm$ 0.44	2.18 $\pm$ 0.45	> 0.05

WRS, work-related asthma symptoms.

*mean (range).

†weal formed by allergen/weal formed by histamine, mean $\pm$ SD

‡log (absorbance, $\times 1000$), mean $\pm$ SD

Allergy, Yonsei University College of Medicine, Korea), and the extracts were prepared as described previously [13]. Using 2-site ELISA to measure the group 1 major allergen of DP and DF, and by microscopic examination, it was confirmed that the TP extract used in this study was not contaminated by DP or DF.

Skin prick tests and ELISA

None of the subjects had received antihistamines orally for the five days preceding the skin tests. A positive control of histamine (1 mg/mL, Allergopharma Co., Hamburg, Germany), and a negative control of diluent (Allergopharma Co.) were included in all tests. The mean diameter of the weal formed by an allergen was measured 15 minutes after the prick test.

The presence of serum-specific IgE antibodies was determined using ELISA as previously described [8,9]. All assays were performed in duplicate. The cutoff values of high-specific IgE bindings were determined from the mean plus twice the standard deviation of the absorbance values, which showed normal distribution, of 30 unexposed non-atopic healthy controls.

ELISA inhibition tests

Competitive ELISA inhibition tests were performed to determine the allergenic cross-reactivity between the spider mites and the domestic mites. The tests were performed using the pooled sera of five subjects who showed high-specific IgE bindings to *T. urticae*, *P. ulmi*, TP, or DP. Fifty μ L of the pooled sera was preincubated with five concentrations (0.001, 0.01, 1, 10 and 100 μ g/mL) of *T. urticae*, *P. ulmi*, *P. citri*, TP, and DP extracts for one hour at room temperature. Sera were then incubated with *T. urticae*-, *P. ulmi*-, TP-, or DP-coated microtitre plates for two hours. The same steps as used in ELISA were then followed. After studying the control samples in which equal volumes of phosphate buffered saline (PBS), pH 7.5 were preincubated instead of inhibitors, the inhibition of specific IgE-binding was expressed as $[1 - (\text{absorbance of samples preincubated with allergens/absorbance of samples preincubated with PBS})] \times 100 (\%)$.

SDS-PAGE and IgE Immunoblotting

SDS-PAGE was carried out using the Laemmli's method under reducing conditions [14]. *T. urticae* or *P. ulmi* (1 mg/mL of crude antigen) was dissolved in sample buffer (12.5 mM Tris-HCl, pH 6.8, 5% glycerol, 0.4% sodium dodecyl sulfate (SDS), 1% 2-mercaptoethanol, 0.02% bromophenol blue) and boiled at 100 °C for 5 min. Five micrograms (per well) of the *T. urticae* or *P. ulmi* extracts

were separated in 15% polyacrylamide gels with a 4% polyacrylamide stacking gel (Mighty Small electrophoresis unit; Hoeffer, San Francisco, CA, USA) at 50 V for 30 min and at 180 V for two hours. The proteins were then transferred onto nitrocellulose membranes (pore size 0.45 μ m; Amersham, Little Chalfont, Buckinghamshire, UK) on a Transpor TE 42 Unit (Hoeffer) in transfer buffer (25 mM Tris, 192 mM glycine, and 20% methanol, pH 8.3) at 350 mA for one hour, and then blocked for one hour at room temperature with 5% non-fat milk in TBS-T (50 mM Tris with 0.2% Tween, pH 7.5). It was then incubated with patient and control sera (1 : 10 diluted in 1% BSA-TBS-T and 0.02% NaN_3) at room temperature for four hours and then incubated with horseradish peroxidase-conjugated anti-Human IgE antibody (Vector Laboratories, Inc, Burlingame, CA, USA). Control sera were obtained from non-atopic apple farmers with low serum-specific IgE levels to the spider mites. After washing, bound goat antibodies were detected with a BCIP/NBT kit (Promega, Madison, WI, USA).

Statistical analysis

Results were expressed as mean $\pm$ SD, unless otherwise indicated. Statistical significance of the two groups was compared using the Student's *t*-test or the chi-square test. Correlations were analysed and tested for statistical significance by calculating the correlation coefficient. Results were considered statistically significant when $P < 0.05$.

Results

Relationships between serum-specific IgE levels and work-related asthma symptoms

Table 1 shows the relationships of work-related asthma symptoms with serum-specific IgE and skin responses to *T. urticae*, *P. ulmi*, *P. citri*, TP, and DP extracts. No significant differences of skin response (allergen/histamine ratio) to *T. urticae*, *P. ulmi*, TP, and DP were observed between those with and those without work-related symptoms (1.07 ± 0.33 vs. 1.03 ± 0.49 ; 0.98 ± 0.36 vs. 1.04 ± 0.51 ; 0.34 ± 0.41 vs. 0.58 ± 0.52 ; 0.58 ± 0.62 vs. 0.68 ± 0.45 , $P > 0.05$, respectively). With respect to serum-specific IgE responses (log-absorbance, $\times 1000$), no significant differences to *P. citri*, TP, and DP were observed between those with and those without symptoms (2.59 ± 0.15 vs. 2.27 ± 0.41 ; 1.96 ± 0.34 vs. 1.93 ± 0.26 ; 1.92 ± 0.34 vs. 1.75 ± 0.13 , $P > 0.05$, respectively). However, the serum-specific IgE levels to *T. urticae* and *P. ulmi* were significantly higher in those with work-related symptoms than in those without such symptoms (2.48 ± 0.57 vs.

2.04 ± 0.41 , $P = 0.02$; 2.59 ± 0.15 vs. 2.27 ± 0.41 , $P = 0.02$).

Correlations of serum specific IgE levels to *T. urticae* and *P. ulmi* with those to *P. citri*, TP and DP

High serum-specific IgE bindings to *T. urticae* and *P. ulmi* were observed in 13 (41.9%) and 16 (51.6%) of the 31 enrolled subjects, respectively. The correlations of specific IgE levels to *T. urticae* with those of the other mites in decreasing order were *P. ulmi* ($r = 0.552$, $P = 0.001$), *P. citri* ($r = 0.509$, $P = 0.004$), TP ($r = 0.345$, $P = 0.06$), and DP ($r = 0.258$, $P = 0.16$). Significant correlations were observed between the serum-specific IgE levels of *P. ulmi*, and *P. citri* ($r = 0.661$, $P = 0.0001$), but statistical significance was less marked between the specific IgE levels of *P. ulmi*, and TP and DP ($r = 0.419$, $P = 0.01$; $r = 0.342$, $P = 0.06$).

ELISA inhibition of *T. urticae*- or *P. ulmi*-specific IgE with *P. citri*, TP, and DP crude extracts

With ELISA inhibition, *T. urticae*-specific IgE binding was inhibited by 99.7% by the addition of 100 mg/mL of *T. urticae* extract, and by 83% with *P. citri* and 71.6% with *P. ulmi*. TP and DP extracts inhibited *T. urticae*-specific IgE by 69.7% and 60.1%, respectively. The 50% inhibitory concentrations (IC_{50}) of *T. urticae*-specific IgE in increasing order were 1.8 μ g/mL of *T. urticae*, 5.2 μ g/mL of *P. citri*, 9.1 μ g/mL of *P. ulmi*, 33 μ g/mL of TP, and 92.5 μ g/mL of DP extracts (Fig. 1a).

P. ulmi-specific IgE binding was completely inhibited by

the additions of 100 μ g/mL of *P. ulmi* extract (91.2%) and *P. citri* extract (92.3%). However, IgE binding was only partially inhibited by *T. urticae* (61.5%), TP (57.7%), and DP (54.4%). The 50% inhibitory concentrations (IC_{50}) of *P. ulmi*-specific IgE in increasing order were 2.4 μ g/mL of *P. ulmi*, 4.2 μ g/mL of *P. citri*, 55.5 μ g/mL of *T. urticae*, 68.1 μ g/mL of TP, and 95.1 μ g/mL of DP extracts (Fig. 1b).

TP-specific IgE binding was inhibited by 91.1% and 85.1% by the addition of 100 μ g/mL of TP and of DP extracts, respectively. However, TP-specific IgE binding was minimally inhibited by 100 μ g/mL of *T. urticae* (20.3%), *P. ulmi* (20.2%), and *P. citri* (22.6%) extracts (Fig. 2a). With respect to the cross-reactivity of DP, DP-specific IgE binding was inhibited by 96.6% by the addition of 100 μ g/mL of DP extracts. However, DP-specific IgE binding was only minimally inhibited by 100 μ g/mL of TP (25.9%), *T. urticae* (30.4%), *P. ulmi* (26.3%), and *P. citri* (23%) extracts (Fig. 2b).

IgE Immunoblotting of *T. urticae* and *P. ulmi* crude extracts

SDS-PAGE and IgE immunoblotting was performed upon 12 samples of sera from subjects with high-specific IgE binding to *T. urticae*. A total of 10 allergenic components in *T. urticae* crude extract were identified. More than 50% of the sera showed IgE reactivity to proteins of 17 kDa (66.7%), 27 kDa (75%), 33 kDa (66.7%), 37 kDa (66.7%), 41 kDa (83.3%), 56 kDa (66.7%), and 75 kDa (66.7%).

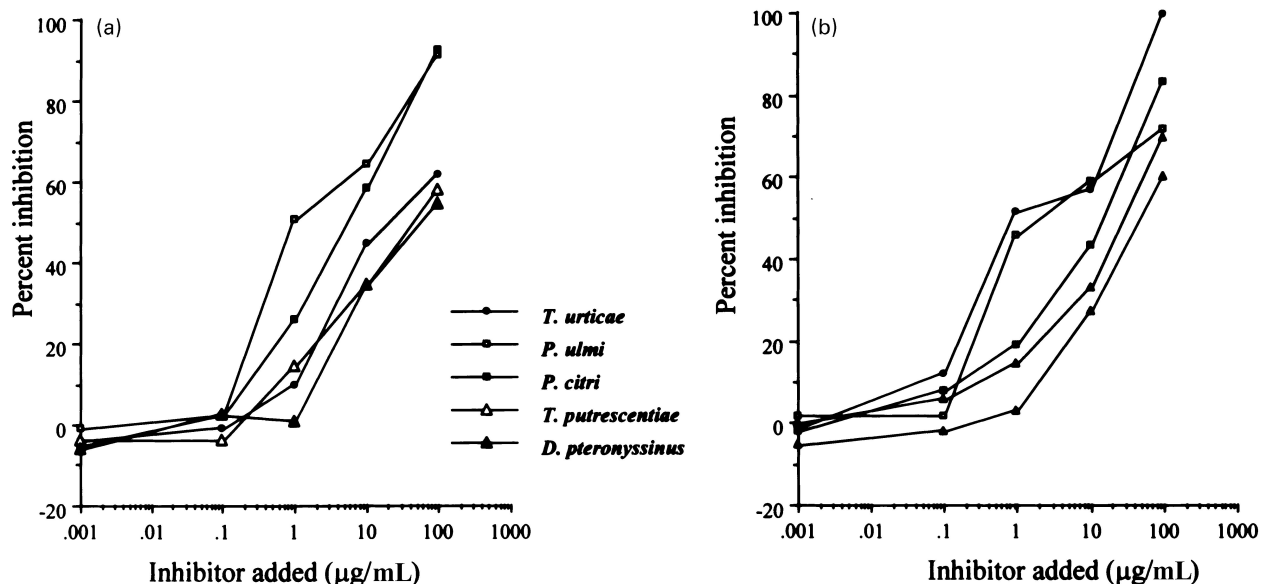


Fig. 1. Percent inhibition of *T. urticae*-specific (a) and *P. ulmi*-specific (b) IgE bindings with serial additions of *T. urticae*, *P. ulmi*, *P. citri*, *T. putrescentiae*, and *P. pteronyssinus* using sera from five subjects with high serum *T. urticae*-specific IgE levels

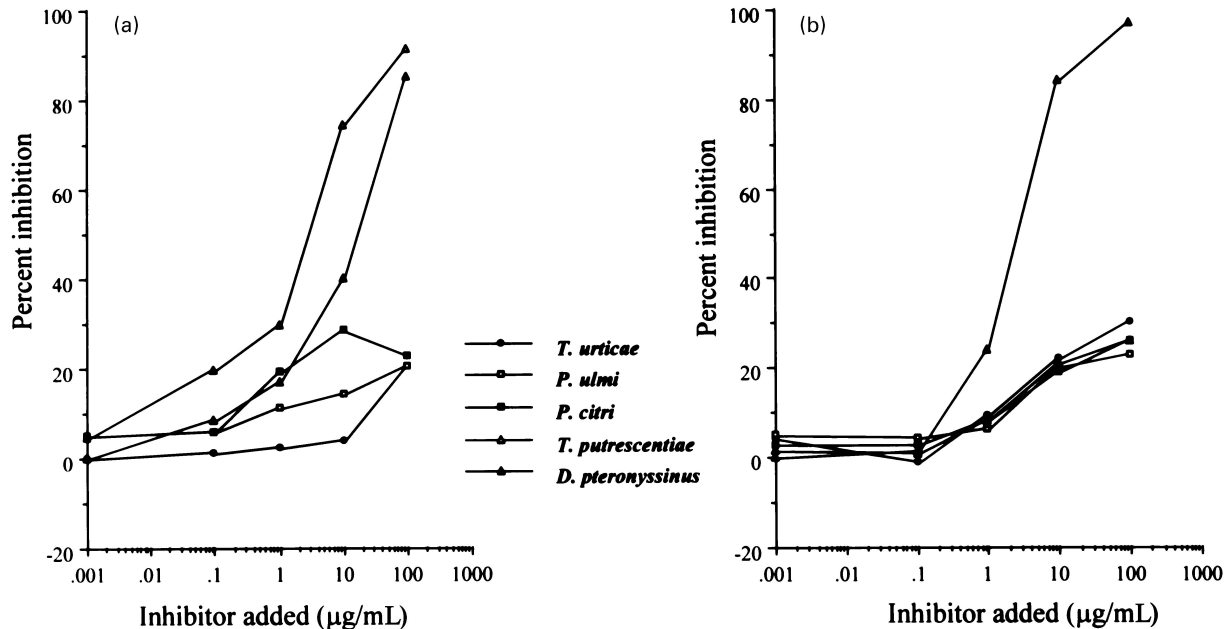


Fig. 2. Percent inhibition of *T. putrescentiae*-specific (a) and *D. pteronyssinus* (b) IgE binding with serial additions of *T. urticae*, *P. ulmi*, *P. citri*, *T. putrescentiae*, and *P. pteronyssinus* using sera from five subjects with high serum *P. ulmi*-specific IgE levels.

Among these, the density of the IgE binding band of the 17 kDa allergen was the most intense (Table 2 and Fig. 3).

SDS-PAGE and IgE immunoblotting results of 11 sera from subjects with high-specific IgE binding to *P. ulmi* are shown in Table 2 and Fig. 4. A total of 11 allergenic components in the *P. ulmi* crude extract were identified. More than 50% of the sera showed IgE reactivity to proteins of 33 kDa (72.7%), 41 kDa (54.5%), and 51 kDa (54.5%). Among these, the density of the IgE binding band of the 33 kDa allergen was the most intense.

Discussion

Immunologic mechanisms are involved in cases of occupational asthma due to high-molecular-weight allergens. The immunological mechanism can involve an IgE- or non-IgE-mediated reaction. To the best of our knowledge, there have been no reports about the pathogenic mechanism of occupational asthma induced by allergens derived from spider mites such as *T. urticae* and *P. ulmi*. In this study, serum-specific IgE levels to *T. urticae* and *P.*

Table 2. Frequency of *T. urticae*-specific and *P. ulmi*-specific IgE binding components in immunoblotting analysis

<i>T. urticae</i> (n = 12)			<i>P. ulmi</i> (n = 11)		
Molecular weight (kDa)	Frequency	%	Molecular weight (kDa)	Frequency	%
17	8	66.7	10	1	9.1
27	9	75	16	4	36.4
30	4	33.3	22	1	9.1
33	8	66.7	27	1	9.1
37	8	66.7	33	8	72.7
41	10	83.3	37	3	27.3
48	3	25	41	6	54.5
51	1	8.3	48	4	36.4
56	8	66.7	51	6	54.5
75	8	66.7	56	2	18.2
			75	5	45.5

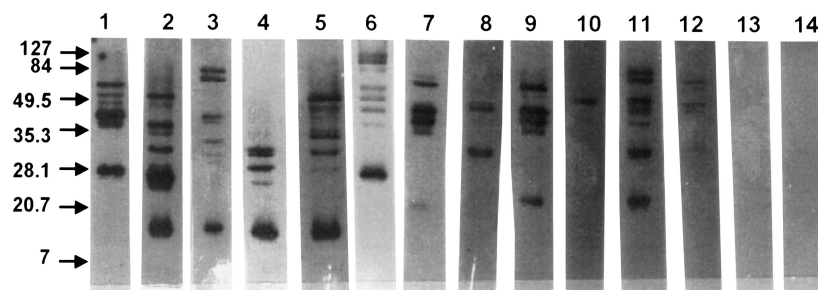


Fig. 3. Immunoblotting of *T. urticae*-specific IgE with 12 individual sera from farmers with high serum *T. urticae*-specific IgE levels. Lanes 1 through 12 are farmers' sera; lane 13, control serum; lane 14, buffer control.

ulmi were higher among subjects with work-related asthma symptoms than among those without such symptoms, although no significant differences in the specific IgE levels to TP and DP were observed between the two groups. This finding suggests that specific IgE responses to the allergens derived from spider mites may be responsible for the development of work-related asthma symptoms, and that serum-specific IgE levels to the spider mites may be one of the predicting factors to develop asthmatic symptoms in apple farmers sensitized to the spider mites.

Crude DP allergens are a source of many allergens. Extracts have been shown to contain as many as 32 different IgE-binding components, as assessed by SDS-PAGE and immunoblotting [15]. With respect to allergens derived from spider mites, the results of this study suggest that both *T. urticae* and *P. ulmi* extracts contain many different IgE-binding components and, among these, at least three IgE-binding components are dominant allergens by SDS-PAGE and IgE immunoblotting, although the sample size was small.

Spider mites belong to the order Acari, suborder Prostigmata, and to the spider mite family (Tetranychidae). *P. ulmi* and *P. citri* belong to the genus *Panonychus*, and *T. urticae* to the genus *Tetranychus*. Meanwhile, domestic mites such as TP and DP belong to the suborder Astigmata. TP has been recognized as one of the important storage mites [3,4] and was found to cohabit with *Dermatophagoides* spp. in more than 25% of Korean homes [5]. Our previous study revealed that the sensitization rate to TP was second most common sensitization among apple farmers

[10]. There have been some controversies concerning the cross-reactivity between TP and the *Dermatophagoides* spp. Some investigators have suggested minimal cross-reactivity in atopic subjects in rural areas [16–18], while others have reported moderate to higher cross-reactivity between TP and the *Dermatophagoides* spp. [13,19–21]. The results of the present study showed that TP-specific IgE binding was extensively inhibited by an extract of DP, but DP-specific IgE binding was only minimally inhibited by an extract of TP. This finding was in accord with the study result of Park *et al.* [13], performed in urban areas of Korea, which reported a marked inhibition of TP-specific IgE with DP extract, but found that DP-specific IgE was not inhibited by TP. These results suggest the possibility that crude extracts derived from the *Dermatophagoides* spp. contain species-specific allergens as well as allergens cross-reactive with TP, but TP-derived extracts does not have sufficient species-specific allergens.

The present study suggests that *P. ulmi*, *T. urticae* and *P. citri* are more closely related in terms of serum-specific IgE binding for the sera pool compared to DP and TP. Burches *et al.* [12] reported that specific IgE to *T. urticae* could not be detected in the absence of the specific IgE to DP. However, our previous study revealed that *P. citri*-specific IgE binding was partially inhibited by DP extract [9], and Astarita *et al.* [11] reported that *T. urticae*-specific IgE binding was not inhibited by DP in farm workers. In the present study, ELISA inhibition tests showed that *P. ulmi*-specific IgE binding was extensively inhibited by *P. ulmi* and *P. citri*, but only partially inhibited by *T. urticae*, TP,

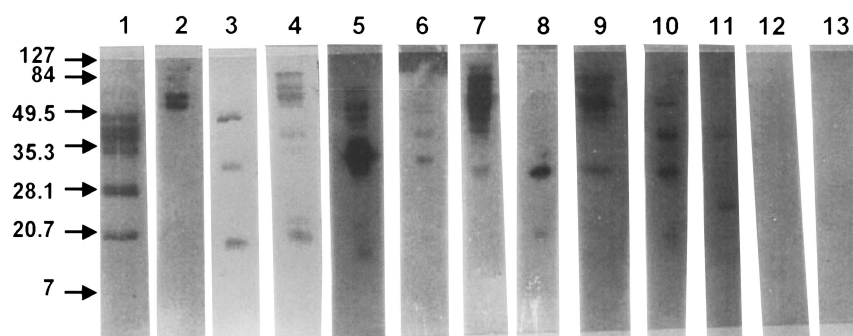


Fig. 4. Immunoblotting of *P. ulmi*-specific IgE with 11 individual sera from farmers with high serum *P. ulmi*-specific IgE levels. Lanes 1 through 11 are farmers' sera; lane 12, control serum; lane 13, buffer control.

and DP in decreasing order. Meanwhile, *T. urticae*-specific IgE binding was extensively inhibited by *T. urticae*, but only partially inhibited with *P. citri*, *P. ulmi*, TP, and DP in decreasing order. These results suggest that both *T. urticae*- and *P. ulmi*-derived extracts contain genus and/or species-specific allergens in addition to common shared allergens with the domestic mites. With regard to the allergenic cross-reactivity between spider mites and domestic mites, it seems that the closer the taxonomic relationship between mites is, the greater the level of cross-allergenicity.

In conclusion, there were seven dominant allergens found in *T. urticae* and three in *P. ulmi*. *T. urticae*- and *P. ulmi*-specific IgE bindings were partially inhibited by crude extracts derived from the domestic mites, such as *D. pteronyssinus* and *T. putrescentiae*.

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