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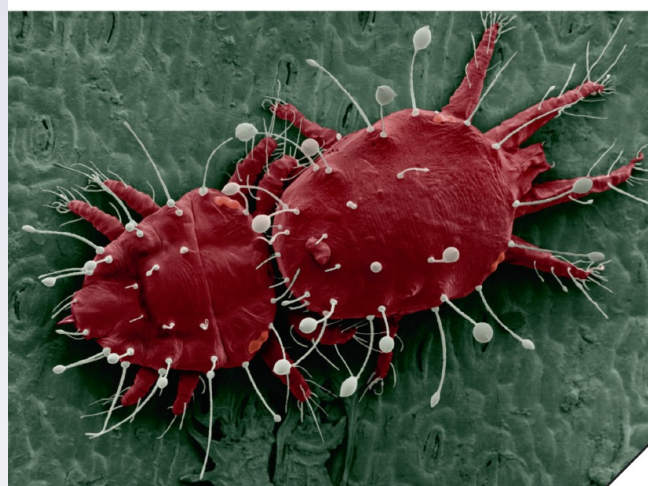
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IgE reactivity to *Acarus siro* extract in Korean dust mite allergic patients

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Abstract Although specific IgE to the storage mite *Acarus siro* is often detected, there are no detailed studies on IgE reactivity to *A. siro* in Korea. This study was undertaken to investigate the cross-reactivity to the mite species *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Tyrophagus putrescentiae*, and *A. siro* in Korean mite allergic patients. Specific IgE values were determined for the four mite species and a competitive inhibition test was performed for mite extracts using the ImmunoCAP system. The IgE value to *D. farinae* was the highest among the four mite species tested. There was a strong correlation in the IgE value between house dust mites (*D. pteronyssinus* and *D. farinae*) and between storage mites (*A. siro* and *T. putrescentiae*). IgE reactivity to *A. siro* was inhibited by *D. farinae* and *T. putrescentiae* extract. *Dermatophagoides farinae* extract was the strongest inhibitor of IgE binding to *A. siro* extract, indicating that IgE reactivity to *A. siro* extract is a cross-reaction caused by sensitization to *D. farinae*. Strong IgE reactive components were observed in *D. farinae* and *T. putrescentiae* extract by SDS-PAGE and IgE immunoblotting. However, no strong IgE-binding component was observed for *A. siro*. *Dermatophagoides farinae* is the main source of mite allergens that cause sensitization in Korea. Serum IgE from some of the house dust mite-sensitized patients showed positive responses to storage mite allergens by cross-reaction. Therefore, it is necessary to pay special attention to the diagnosis of mite allergies.

Keywords Allergen · Cross-reactivity · Dust mites

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Introduction

The storage mite *Acarus siro* is an important pest of stored products and is found in a wide range of habitats. Therefore, exposure to *A. siro* can occur not only in domestic settings, but also in agricultural and occupational environments. The mite has various names (i.e., flour mite, grain mite, cereal mite, forage mite, meal mite, and cheese mite) according to its habitat. Sensitization to *A. siro* was reported in cheese-makers (Molina et al. 1975), farmers (Ingram et al. 1979), grain workers (Blainey et al. 1989), and bakers (Revsbech and Dueholm 1990). In particular, it is known as an important cause of ‘barn allergy’, a type III hypersensitivity in farm workers (Cuthbert et al. 1979), and ‘baker’s itch’, a dermatitis-like reaction in bakers (Parsons 1924).

Acarus siro was first described in Korea in 1984 by Lee and Choi (1980). Specific IgE to *A. siro* extract was detected in 60 patients (55.1 %) suffering from allergic rhinitis in the Pusan area (Han et al. 1999). Furthermore, cross-reactivity between *A. siro* and *Tyrophagus putrescentiae* was noted, even though only a limited degree of cross-reactivity with *Dermatophagoides pteronyssinus* was observed. The allergenic importance of this mite has not been investigated in Korea although IgE reactivity to *A. siro* is frequently detected because *A. siro* extract is included in the panel of automated allergy test kits, such as the Korean panel of RIDA and the AdvanSure multiple allergen simultaneous test system (Ownby and Bailey 1986).

To examine the significance of allergy to *A. siro* in Korea, we investigated cross-reactivity among dust mites *D. farinae*, *T. putrescentiae*, and *A. siro* using serum samples that showed positive IgE reactivity to *A. siro* allergen by the AdvanSure test.

Materials and methods

Serum samples

Serum samples were collected from patients at the Allergy–Asthma Clinic at Severance Hospital, Seoul, Korea for diagnosis. Patient consent was obtained before blood collection. Specific IgE to dust mites *D. pteronyssinus* (d1), *D. farinae* (d2), and storage mites *A. siro* (d70) and *T. putrescentiae* (d72) was determined using the ImmunoCAP system (Phadia, Uppsala, Sweden). For comparison, some of the serum samples were analyzed by the AdvanSure test (LG Life Sciences, Daejeon, Korea) for detection of specific IgE to dust mite. This study was approved by the institutional review board (4-2009-0717).

Allergen preparation

For the competitive analysis, mite extracts were prepared as described previously (Jeong et al. 2012a). Mites used in the study were reared in the Arthropods of Medical Importance Resource Bank, Yonsei University College of Medicine, Seoul, Korea except for *A. siro*, which was from Allergon AB (Angelholm, Sweden). In brief, the mite bodies were isolated using saturated salt water and allergens were extracted in a bicarbonate buffer. The extract was dialyzed extensively against distilled water and lyophilized. The extracts were reconstituted in a phosphate buffer, aliquoted, and kept at -70°C until use. Protein concentration was determined by the Bradford assay (Bio-Rad, Hercules, CA, USA).

Competitive ImmunoCAP inhibition

IgE reactivity of the mite extracts was examined by competitive IgE binding inhibition. The serum samples were pre-incubated with various concentrations of inhibitors and anti-human IgE reactivity was measured using the ImmunoCAP system. The percentage inhibition was calculated as $(1 - A_i/A_0) \times 100$, where A_i is IgE value (kU/l) with an inhibitor and A_0 is IgE value without an inhibitor.

SDS-PAGE and IgE immunoblotting analysis

Thirty micrograms of mite protein was separated on 15 % polyacrylamide gels under reducing conditions. Separated protein bands were detected using Coomassie Brilliant Blue or transferred electrophoretically onto polyvinylidenedifluoride (PVDF) membranes (0.45 μ m, GE Water & Process Technologies, Trevose, PA, USA) for the detection of IgE-binding components. After blocking with 3 % skim milk, the membrane was incubated with a 1:4 dilution of sera (pooled from five subjects). Subsequently, IgE reactive proteins were probed with alkaline phosphatase-conjugated goat anti-human IgE (1:1,000-diluted) (Sigma-Aldrich, St. Louis, MO, USA) and the color was developed with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (Promega, Madison, WI, USA).

Results

Specific IgE to dust mites

Specific IgE values for dust mites were determined using the ImmunoCAP system for 20 serum samples that were positive for *A. siro* allergen by the AdvanSure test (Table 1). The level of specific IgE to *D. farinae* was the highest in all 20 serum samples tested. IgE reactivity to *A. siro* showed a strong correlation with reactivity to *T. putrescentiae* (Pearson's correlation coefficient = 0.93), whereas IgE reactivity to *D. farinae* correlated with reactivity to *D. pteronyssinus* (Pearson's correlation coefficient = 0.92; Fig. 1). The strong correlation of IgE reactivity between house dust mites (*D. farinae* and *D. pteronyssinus*), and between storage mites (*A. siro* and *T. putrescentiae*) was shown. Pearson's correlation coefficient of the IgE value between *D. farinae* and *A. siro*, and between *D. farinae* and *T. putrescentiae* was 0.5 and 0.46, respectively.

Cross-reactivity between dust mites

Dermatophagoides farinae extract inhibited specific IgE binding to *A. siro* extract (Fig. 2). *Dermatophagoides farinae* extract was shown to be superior to *A. siro* at inhibitor concentrations from 0.4 to 50 μ g/ml. *Tyrophagus putrescentiae* extract also inhibited IgE binding to *A. siro* to a maximum of 72 %. The competitive inhibition study therefore indicated that IgE reactivity to *A. siro* is mainly due to cross-reaction.

IgE reactive components of mite extract

To compare the IgE-reactive components of dust mites we performed SDS-PAGE and IgE immunoblot analysis using serum samples which displayed the strongest IgE reactivity to

Table 1 Specific IgE (kU/L) levels to four dust mites

Patient no.	d1 ^a	d2	d70	d72	Gender/age	Diagnosis	Sensitization profile ^c
1	61.1 (5) ^b	>100 (6)	3.36 (2)	3.41 (2)	F/9	Atopic dermatitis	
2	0.65 (1)	6.65 (3)	4.14 (3)	5.19 (3)	M/70	Allergic rhinitis, Asthma	
3	14.5 (3)	31.7 (4)	2.00 (2)	2.04 (2)	F/25	Atopic dermatitis	Pork
4	0.07 (0)	4.48 (3)	1.49 (2)	2.01 (2)	M/52	Allergic rhinitis	
5	88.5 (5)	>100 (6)	2.91 (2)	4.42 (3)	F/29	Atopic dermatitis	Cod, chicken
6	1.21 (2)	4.22 (3)	0.32 (0)	0.62 (1)	M/50	Allergic rhinitis, Asthma	
7	0.13 (0)	4.48 (3)	1.08 (2)	1.75 (2)	M/80	Hypereosinophilia	
8	23.5 (4)	77.8 (5)	4.67 (3)	6.42 (3)	F/12	Atopic dermatitis	Cat dander
9	26.0 (4)	61.1 (5)	9.48 (3)	8.98 (3)	F/29	Allergic rhinitis, Atopic dermatitis, Anaphylaxis	Sallow willow, egg white, crab, shrimp
10	0.08 (0)	0.54 (1)	0.20 (0)	0.24 (0)	F/45	Hypereosinophilia	
11	6.50 (3)	11.9 (3)	1.26 (2)	1.65 (2)	M/32	Allergic rhinitis, Chronic urticaria	<i>Alternaria</i>
12	0.97 (2)	3.46 (2)	0.54 (1)	0.74 (2)	M/44	Allergic rhinitis, Asthma	Sweet vernal grass, timothy grass, orchard grass
13	0.30 (0)	7.78 (3)	1.92 (2)	2.90 (2)	F/54	Chronic urticaria	
14	78.8 (5)	>100 (6)	2.70 (2)	3.28 (2)	F/17	Allergic rhinitis	Dog dander
15	72.5 (5)	>100 (6)	3.77 (3)	4.10 (3)	F/8	Atopic dermatitis	Dog dander
16	31.6 (4)	73.4 (5)	2.64 (2)	3.00 (2)	M/8	Allergic rhinitis	
17	39.9 (4)	>100 (6)	5.83 (3)	3.24 (2)	M/5	Asthma	Dog dander, ragweed
18	0.04 (0)	0.02 (0)	0.00 (0)	0.01 (0)	M/7	Polycythemia vera	
19	44.9 (4)	>100 (6)	0.65 (1)	1.04 (2)	M/21	Allergic rhinitis, Asthma, Atopic dermatitis	
20	0.28 (0)	5.06 (3)	0.96 (2)	1.60 (2)	M/55	Hypereosinophilia	

^a d1 *Dermatophagoides pteronyssinus*, d2 *Dermatophagoides farinae*, d70 *Acarus siro*, d72 *Tyrophagus putrescentiae*

^b Classes of specific IgE level (0–6) are given in parenthesis

^c Allergens which showed positive reaction by multiple IgE test were summarized (except dust mites)

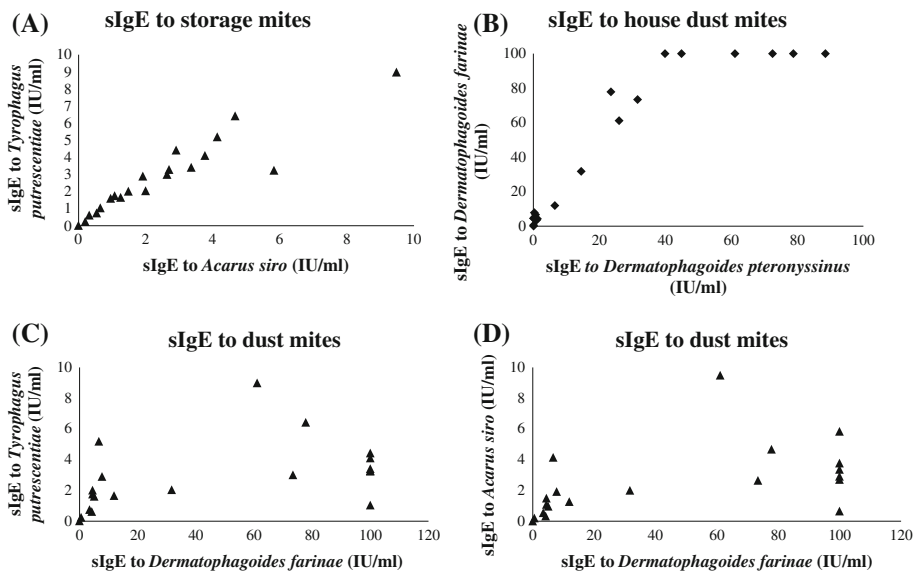
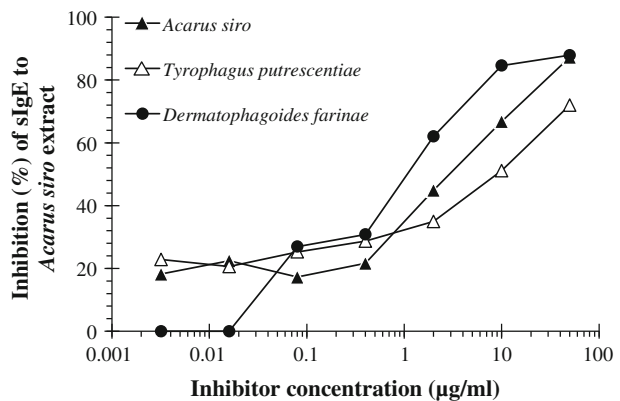


Fig. 1 Specific IgE reactivity to dust mites. Correlation of specific IgE between storage mites (a), house dust mites (b), and house dust mite and storage mite (c *Tyrophagus putrescentiae* and d *Acarus siro*)

Fig. 2 CAP inhibition analysis of *Acarus siro* allergens. IgE binding to *A. siro* extract was inhibited by various concentrations of dust mite extract



A. siro (Class 3 by CAP assay). Different protein banding and IgE binding patterns were observed for three different mite extracts (Fig. 3). For *D. farinae* extract, allergens of 10–15 kDa showed the strongest IgE reactivity, followed by a 23 kDa allergen. An allergen of 40 kDa was apparent in the *T. putrescentiae* extract. However, no strong IgE binding component was detected in the *A. siro* extract. In the case of *A. siro*, it is believed that allergenic components with conformational epitopes are important for IgE binding because SDS-PAGE was performed under denaturing conditions whereas the ImmunoCAP study was not.

We also performed an inhibition study of *A. siro*-specific IgE using recombinant Der f 1, Der f 2 and Der f 10 as the inhibitors (data not shown). However, we did not observe any significant inhibition by the recombinant allergens.

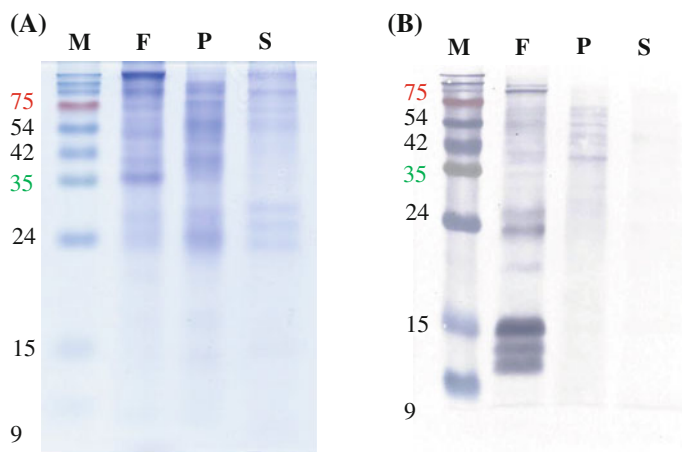


Fig. 3 SDS-PAGE (a) and IgE immunoblot (b) analysis of dust mites. Proteins (30 µg) were separated on 15 % polyacrylamide gels under reducing condition. M, molecular mass standard; F, *Dermatophagoides farinae*; P, *Tyrophagus putrescentiae*; S, *Acarus siro*

Discussion

Multiplex allergen diagnostic kits such as RIDA and AdvanSure are commonly used for the detection of allergen-specific IgE. Specific IgE to *A. siro* is frequently detected in such tests (personal communication, HS Kim Department of Dermatology, Yonsei University, College of Medicine, Seoul). However, it is well known that *A. siro* is not a dominant species of storage mite and that the dominant species in Korean house dust samples is *T. putrescentiae* (Ree et al. 1997; Jeong et al. 2006b). This fact motivated us to investigate possible cross-reactivity between house dust mites and storage mites. In this study, we investigated cross-reactivity among dust mites using serum samples from non-occupationally sensitized subjects in an urban area. Specific IgE values and inhibition studies indicate that *D. farinae* is the main source of sensitization in Korea, and specific IgE to storage mites are the consequence of cross-reactivity. This observation is in agreement with reports that *D. farinae* is the dominant species in Korea. Cross-reactivity between *T. putrescentiae* and *Dermatophagoides* spp. was clearly documented in a previous study (Park et al. 1999).

A high degree of cross-reactivity between *A. siro* and *T. putrescentiae* was previously reported (van der Heide et al. 1998) although the molecular identities of the allergens have not been described. To date, a single allergen, Aca s 13 (a fatty acid binding protein) from *A. siro* has been characterized (Eriksson et al. 1999) and officially listed in the WHO/IUIS allergen nomenclature database (www.allergen.org). Recently, Aca s 4 (α -amylase) was characterized, but it is not listed in WHO/IUIS allergen nomenclature database (Pytelkova et al. 2012). Allergens of 18, 19, 25, and 27 kDa have been reported to be the cross-reactive allergens (Han et al. 1999). The authors described 18–19 kDa components as group 2 allergens and 25–27 kDa allergens as group 1 allergens. However, group 1 allergens are not readily detected by IgE immunoblotting combined with SDS-PAGE under reducing conditions. Group 5 and group 13 allergens are known to be similar in molecular mass (Jeong et al. 2012b). Glutathione S-transferases are 25 kDa allergens with IgE cross-

reactivity (Huang et al. 2006). Group 3 (trypsin), group 6 (chymotrypsin), and group 7 (unknown) allergens are also similar in molecular weight.

The allergen responsible for the cross-reactivity of dust mites was not identified. Recombinant Der f 1, Der f 2, and Der f 10 did not inhibit the IgE binding to *A. siro* extract (data not shown) and IgE immunoblotting implies that conformational epitopes of an allergen may be important for cross-reactivity. Glutathione *S*-transferase may play a role in the cross-reactivity (Caraballo and Acevedo 2011) because Han et al. reported that 25 kDa allergens are the strongest IgE binding components of the storage mites *A. siro* and *T. putrescentiae* (Han et al. 1999).

Tropomyosin is a well-known panallergen responsible for the cross-reactivity among arthropods (Jeong et al. 2006a). However, tropomyosin (mite group 10 allergens) was shown to play a minimal role for the IgE cross-reactivity for its small amount in mite extracts (Jeong et al. 2007; Arlian et al. 2009). Mite group 2 allergens seem to be mainly responsible for the cross-reaction among mite species (Barber et al. 2012). Storage mite specific IgE reactivity, not only cross-reactive with house dust mite, has been reported (Barber et al. 2012; Zheng et al. 2012). However, most of IgE reactivity to storage mite is a cross-reaction with house dust mite in Korea. Mite group 2 allergens seem to be a main allergen responsible for the cross-reactivity in Korea (Park et al. 1999).

Acarus siro does not seem to be an important cause of respiratory allergy in Korea. However, the possibility that it causes baker's itch and ingestion-induced allergy investigate remains to be investigated. Ingestion of *A. siro*-contaminated food may cause anaphylaxis (Dutau 2002). *A. siro* was also detected in cereals (Dunn et al. 2008) and commercial dog food (Brazis et al. 2008). Interestingly, *A. siro* is a traditional inoculant used to ripen Mimolette cheese in France (Melnik et al. 2010).

In summary, we examined the cross-reactivity of dust mites using extracts of *D. farinae*, *D. pteronyssinus*, *A. siro*, and *T. putrescentiae*. Our data suggest that *D. farinae* is a source of primary sensitization and IgE reactivity to the other mites appears to be due to cross-reactivity. Moreover, conformational epitopes of some allergens are thought to be important for the cross-reactivity among mite species. Further studies are necessary to identify and characterize the allergens causing cross-reactivity. Use of recombinant allergens that are unique for the storage mite would be helpful for the diagnosis of occupational allergy.

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