

Detection and Quantification of Pharaoh Ant Antigens in Household Dust Samples as Newly Identified Aeroallergens

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Key Words

Asthma • Pharaoh ant • Allergen • Environment

Abstract

Background: The widespread house ant, *Monomorium pharaonis* (pharaoh ant, PA), was recently identified as a potential cause of respiratory allergies. However, there are no reports of the distribution of PA allergens in various environments. We developed specific ELISA inhibition assays and measured the distribution and amount of PA antigens in household dust samples. **Methods:** Floor dust was collected at 3-month intervals from 56 homes in Seoul over a 1-year period. PA antigens in fine dusts were quantified by ELISA inhibition assays using rabbit anti-PA sera, and specific IgE to PA antigens in residents' serum was measured by ELISA. **Results:** In 18 of the 56 homes (32.1%), PA antigen was detected in at least 1 floor dust sample either from the living room or the kitchen. Levels of PA antigens showed seasonal variations with peaks in autumn and winter. The detection rate of PA antigens was significantly higher in homes with visual evidence of PA infestations (70%) than in homes without such infestations (23.9%; $p < 0.05$). However, a significant amount of PA antigens was still detected in uninfested

homes. Thirteen of 113 (11.5%) residents were positive for PA-specific IgE. PA-specific IgE was detected more frequently in residents living in PA antigen-positive homes (19.6%) than in antigen-negative homes (4.8%; $p < 0.05$). **Conclusion:** A considerable level of PA antigens is distributed in the indoor environment. Therefore, inhalant exposure to PA antigens can occur during domestic activities. These results suggest that PAs might be a significant source of aeroallergens in households.

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Introduction

It has long been recognized that insects are significant sources of aeroallergens [1–12]. Although cockroaches have attracted considerable attention, there have been very few studies on other insects as a source of indoor inhalant allergens [1, 2]. The changes in lifestyle and living environment might have encouraged the growth of indoor arthropods and insects, and increased the level of human exposure to new allergens. The recently reported respiratory allergy to the Asian ladybug, *Harmonia axyridis*, in the US is a good example of a new type of inhalant

allergy to insects [7–9]. The recognition of avoidable new allergens is an important part of clinical allergy practice.

The nonstinging house ant, *Monomorium pharaonis* (pharaoh ant, PA), is a species that infests the urban indoor environment. We recently reported that the PA can act as a source of indoor aeroallergens. Briefly, 18.2% of 318 patients with asthma showed positive skin responses to PA and 7.9% had an isolated response. Immunoblot analysis identified 2 important IgE-binding components of PA, and the ELISA inhibition test demonstrated significant inhibition by PA. However, other ant species, cockroaches and house dust mites (HDMs) did not inhibit the IgE binding to PA. Eight of 9 patients in whom PA bronchial challenge tests had been performed showed typical asthmatic responses [13, 14].

Despite its potential role in respiratory allergy, there is no information about the distribution of PA allergens in the indoor environment. Evaluation of the exposure to PA antigens is essential for determining the risk factors for sensitization and elicitation of symptoms in susceptible subjects. Furthermore, quantifiable measurement of the PA antigens in the environment is needed to determine whether inhalant exposure to ant antigens can occur as a result of domestic activities.

The aim of this study was to demonstrate the existence of PA antigens in households, and to evaluate the distribution of PA antigens. For this purpose, specific ELISA inhibition assays were developed and the amount of PA antigens was measured in samples of household dust.

Materials and Methods

Subjects

A total of 56 homes in Seoul were selected based on the following: (1) the residents' willingness to actively participate in this study; (2) no plans to move house during the study period; (3) no use of insecticides or pesticides with the exception of a mosquito repellent. An adult resident of each home provided written informed consent to be included in this study. Eighteen participants were patients with allergic rhinitis and/or bronchial asthma, who were treated at the Allergy Clinic of the Yonsei University Severance Hospital, and the remaining 38 were friends of these patients.

A modified questionnaire developed by the International Study of Asthma and Allergic diseases in Children [15] was translated into Korean and given to all family members. The resident was regarded as having a respiratory allergy if he/she had experienced wheezing during the previous 12 months or had been diagnosed with allergic rhinitis during the previous 12 months. Skin prick tests were performed to determine atopy by using a panel of 12 common aeroallergens, and blood sampling was carried out after permission.

At the first home visit, a trained field technician recorded the home characteristics by asking the adult resident questions regarding the building structure, living duration, type of heating, frequency of cleaning and the presence of cockroaches, ants, fungi and pets. The indoor temperature and relative humidity were recorded at each home visit.

Dust Collection and Extraction

From April 2001 to January 2002, home visits were carried out every 3 months in 56 homes. Dust was collected as previously described [16]. Briefly, a trained technician collected floor dust in a standardized manner from a 1-m² area of the floor in the living room and the kitchen for 2 min using a vacuum cleaner (LG Electronics, Changwon, Korea). Five sticky traps (16 × 9 × 3 cm; Earth Chemical Co., Japan) were placed on the floor of the living room and the kitchen and left in each home for 1 day to capture any inhabitant ants.

The dust samples were sieved through a mesh screen to remove the large particles and fibers. Forty milligrams of fine dust was extracted overnight with 2 ml of 1% bovine serum albumin (BSA) diluted in phosphate-buffered saline (PBS)-Tween 20 at room temperature before being centrifuged at 15,000 rpm for 30 min. The supernatants were then stored at –20°C.

Preparation of PA Extract

The PA antigens were extracted as previously described [13]. The PAs were ground up and extracted with a PBS (pH 7.4) solution 1:20 w/v at 4°C for 48 h, followed by centrifugation at 12,000 rpm for 1 h. The supernatant was dialyzed against distilled water at 4°C for 48 h, lyophilized at –70°C and used as a crude ant extract. The protein concentration in the crude extract was determined by using the Bradford method.

Rabbit Immunization

Two white female rabbits were injected subcutaneously with 1 ml of a 50:50 mix of Freund's complete adjuvant (Sigma-Aldrich Corp., St. Louis, Mo., USA) and 10 mg of the crude PA extract. At 3-week intervals, injections of 10 mg of PA extract with Freund's incomplete adjuvant were repeated 3 times. Seven days after the final injection, the rabbits were sacrificed, and the anti-PA serum was pooled and stored at –20°C in aliquots to be used for the ELISA inhibition and immunoblot inhibition analyses.

ELISA Inhibition Assay

Microtiter plates were incubated overnight at 4°C with 0.1 µg of the PA extract in 100 µl of 0.1 M carbonate buffer (pH 9.6). On the next day, 100 µl of each dust extract was added to 100 µl of rabbit serum (diluted 2,000-fold in 1% BSA/PBS-Tween 20) and incubated for 2 h at room temperature. To produce the standard inhibition curve, 100 µl of the PA extract, *Dermatophagoides farinae* or *Blattella germanica* extracts (Korea National Research Resource Collection of Allergens, Seoul, Korea) with the antigen concentrations of 100, 10, 5 µg PA protein/ml, and then 6 doubling dilutions were also incubated with rabbit serum. After washing and blocking, the plate was incubated for 1 h with 100 µl of the preincubated mixture of rabbit serum prepared as described above. After washing, the plate was incubated with 100 µl of biotinylated goat anti-rabbit IgG (Pierce, Rockford, Ill., USA) followed by incubation with streptavidin-peroxidase (Sigma-Aldrich Corp.). After washing, 100 µl of 3,3',5,5'-tetramethylbenzi-

dine-0.01% H₂O₂ (KPL, Gaithersburg, Md., USA) was added as a substrate, and the reactions were stopped by adding 100 µl of a stop solution (KPL). Optical densities were measured at 450 nm using an automated UV microplate spectrophotometer (Dynatec, Alexandria, Calif., USA). The standard curve was constructed using a 4-parameter logistic curve fit and the antigen content in each sample was calculated. The inhibitory ELISA was considered significant if the 50% inhibitory concentration of the inhibitor was less than 50 µg/ml.

IgG immunoblot Inhibition Experiments

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis were performed under reduced conditions. The PA extract was electrophoresed in 13.5% separation gel with 4% stacking gel. The proteins were transferred onto a nitrocellulose membrane (Amersham, Buckinghamshire, UK). The rabbit serum, diluted 2,000-fold, was preincubated with PA, *D. farinae*, *B. germanica* or dust extracts for 2 h at room temperature, and incubated with the nitrocellulose-blotted PA antigens. Bound specific IgG was detected with goat anti-rabbit IgG conjugated with alkaline phosphatase (Sigma-Aldrich Corp.), followed by 0.66% 4-nitroblue tetrazolium and 0.33% 5-bromo-4-chloro-3-indoly-phosphate system (Promega, Madison, Wisc., USA) as a substrate solution.

Specific IgE Determination

The presence of specific IgE (sIgE) to the PA antigens was determined by using ELISA [8]. Microtiter plates were coated with a 0.05 M carbonate buffer containing 10 µg/ml of the PA extract. After blocking, the plate was incubated with either resident or control serum from 10 healthy volunteers with negative skin test responses to the common aeroallergens and PA. The plate was then incubated with biotinylated anti-human IgE (Vector Laboratories, Burlingame, Calif., USA) followed by incubation with streptavidin-peroxidase. After washing, ABTS solution was added as a substrate, and 2 mM NaN₃ was added to quench the reaction. The colorimetric reaction was measured by absorbance at 405 nm on an ELISA reader. The positive cutoff value was decided as the mean + 2 SD of the absorbance value of the nonatopic control serum samples.

Statistical Analyses

To determine the reproducibility of the ELISA inhibition assay, the slopes and intercepts of the standard curves produced by 4-parameter logistic fit were compared after logit transformation using analysis of covariance. The home visiting periods were categorized into 4 seasons: spring (April), summer (July), autumn (October) and winter (January). The seasonal variation in the PA antigens was assessed with a repeated measures analysis. The median values of the PA antigens in the dust samples were compared using the Wilcoxon signed-ranks test or the Mann-Whitney test. The amounts of antigen below the detection limits were assigned a value of half the detection limit in order to include them in the analysis. Detection rates of the PA antigens in dusts were compared using the χ^2 test. All statistical analyses were performed using SAS version 8.1 software.

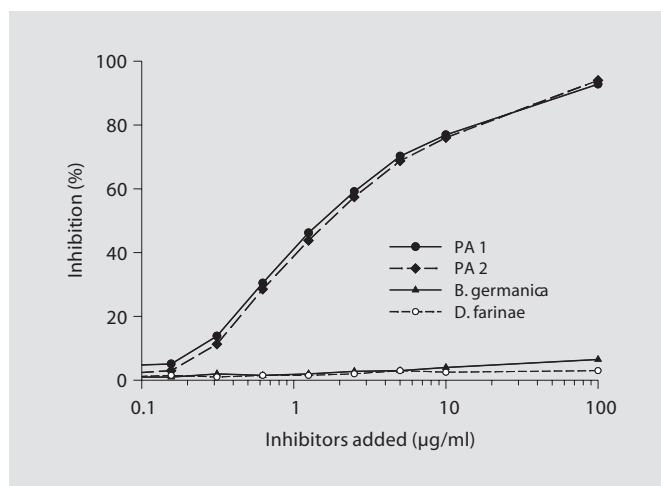


Fig. 1. ELISA inhibition of IgG binding of rabbit anti-ant serum to the PA antigens. Rabbit serum was preincubated with an inhibitor [2 experiments with PA extracts (PA 1 and 2), *D. farinae* and *B. germanica*] and then added to the PA antigen-coated immunoplate. The inhibition curves by PA 1 and 2 represent 2 standard curves, with no difference in the slopes and intercepts after logit transformation.

Results

Specificity of Rabbit Serum and Validation of the ELISA Inhibition Assay

The ELISA inhibition test indicated that the binding of rabbit IgG to the PA antigens was inhibited by preincubation with the PA antigens in a dose-dependent manner. However, the other indoor aeroallergens, *D. farinae* and *B. germanica*, did not inhibit the binding of IgG to the PA antigens (fig. 1). In this method, the lower detection limit of the PA antigens was 0.1 µg/ml. Analysis of covariance showed that there was no significant difference between the slopes and intercepts of the logit-transformed curves from 4 consecutive assays ($p > 0.05$). These results show that the ELISA inhibition assay is suitable for measuring the PA antigens.

Immunoblot inhibition experiments were performed to determine if the important indoor allergens *D. farinae* and *B. germanica* contained cross-reactive epitopes against rabbit IgG to PA. While preincubation of the rabbit serum with PA dose-dependently inhibited IgG binding to the PA antigens, the IgG reactivity was not blocked after preincubation with mite or cockroach extracts (fig. 2). The specificity of the ELISA inhibition test using the rabbit serum was also confirmed by the immunoblot inhibition tests. Partial inhibition of IgG binding was

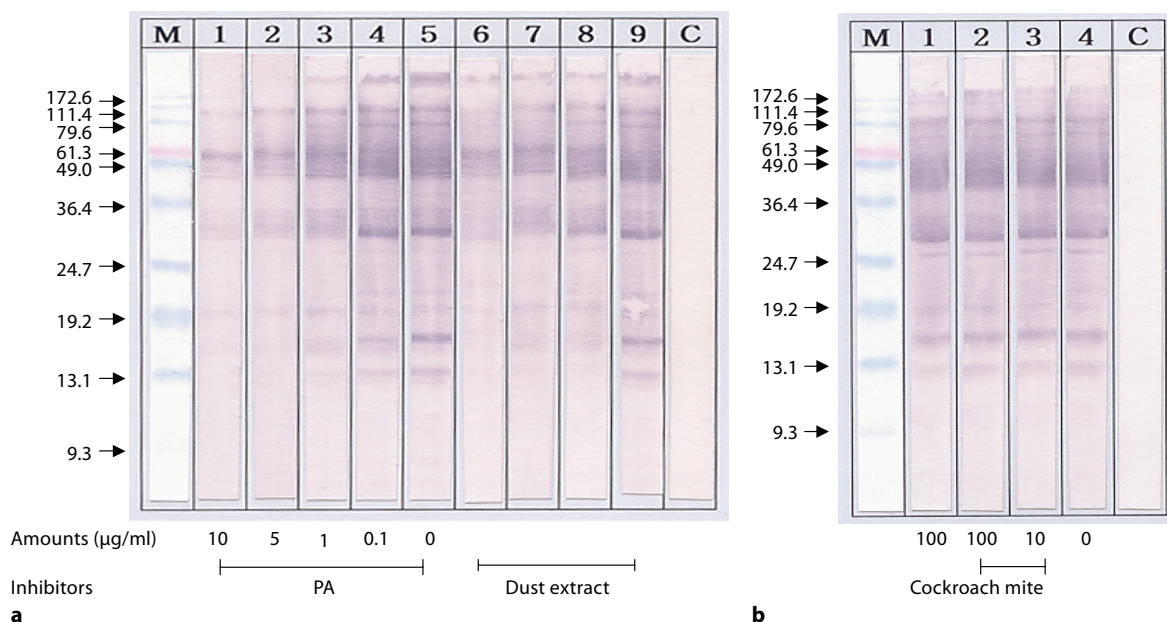


Fig. 2. IgG immunoblot inhibition experiments. Preincubation of rabbit serum with the PA extracts dose-dependently inhibited IgG binding to the PA antigens (**a**, lane 1–5), but not with mite (*D. farinae*; **b**, lane 2, 3) or cockroach (*B. germanica*; **b**, lane 1) extract. Preincubation with the PA antigen-positive dust extracts also dose-dependently inhibited the binding of IgG (**a**, lane 6–8). No inhibition was observed after preincubation with dust extracts testing negative for the PA antigen (**a**, lane 9). M = Marker protein; C = buffer control.

noted after preincubation of rabbit serum with the PA antigen-positive dust samples in the ELISA inhibition tests. No inhibition was observed after preincubation with the PA antigen-negative dust sample (fig. 2).

Measurement of PA Antigens in Household Dust Samples

A total of 101 floor dust samples (56 from living room, 45 from kitchen) were collected during each round of home visits. Overall, 404 dust samples were used to measure the level of the PA antigen. Fifty-five dust samples (13.6%) had detectable amounts of the PA antigens. The detection rate of PA antigens was higher in autumn (18.8%, 19/101) and winter (19.8%, 20/101), and lower in spring (5.9%, 6/101) and summer (9.9%, 10/101).

In 18 of the 56 homes (32.1%), the PA antigen was detected in at least 1 floor dust sample from either the living room or the kitchen. For convenience, data from these 18 homes were included in further analyses. Figure 3 shows the distribution of the PA antigens in the dust samples

from the 18 antigen-positive homes. In the living rooms, the median level of the PA antigens was significantly higher in autumn (28.1 µg/g dust) and winter (32.7 µg/g dust) than in spring (0.1 µg/g dust) and summer (0.1 µg/g dust; $p < 0.05$; fig. 3a). The median level of PA antigens in the kitchen floor samples was also significantly higher in autumn (25.1 µg/g dust) and winter (41.1 µg/g dust) than in spring (0.1 µg/g dust) and summer (0.1 µg/g dust; $p < 0.05$; fig. 3b).

Ants were captured in more than 2 sticky traps in 10 of the 56 (17.9%) enrolled homes, and all captured ants were identified as PAs by an entomologist. We considered ant capture as visual evidence of PA infestation in the home. The detection rate of PA antigens was significantly higher in the homes with visual evidence of PA infestation (70%, 7/10) than in homes without infestation ($p < 0.05$). However, a significant amount of PA antigens was also measured in the homes without infestation (23.9%, 11/46). There were 3 homes with ant infestation but no detectable PA antigen. In summer, the level of PA anti-

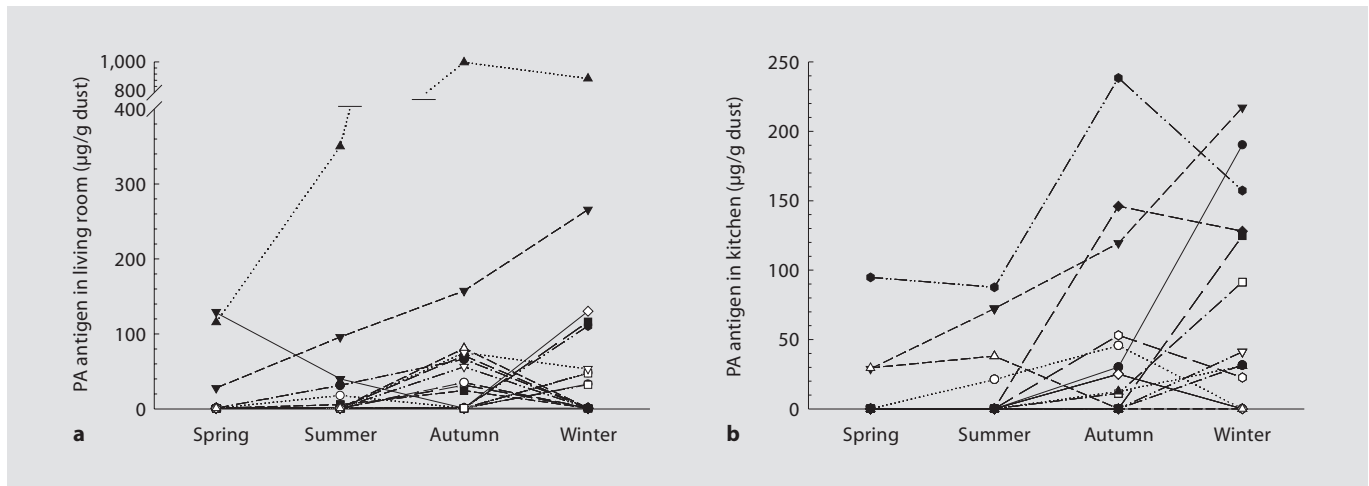


Fig. 3. Distribution of the PA antigens in the reservoir dusts of living room (a) and kitchen (b) floors in the 18 ant antigen-positive homes.

gens in the living room floor was significantly higher in those homes with PA infestation than in those without infestation (fig. 4).

PA-sIgE Determination

Of the 203 persons inhabiting the 56 homes, 52 did not answer the questionnaire or undergo the allergy skin test for personal reasons or because they were absent at the time of the home visit. Blood sampling was not performed in 38 residents due to refusal (30 residents) or young age (≤ 4 years, 8 residents). Therefore, the levels of sIgE to PAs were measured in the serum of the 113 residents who ranged in age from 5 to 64 years (mean 37.0 years; 50 males, 63 females). Of these residents, a questionnaire survey revealed 32 to have respiratory allergies and the remaining 81 to have no respiratory allergy. Thirteen of the 113 residents (11.5%) had significant levels of PA-sIgE. Of these 13 residents, 10 lived in PA-infested homes and 3 lived in noninfested homes. PA-sIgE was detected at higher rates in the residents from the homes with detectable PA antigens (19.6%, 10/51) than in those from homes with no measurable PA antigen (4.8%, 3/62; $p < 0.05$; fig. 5). PA-sIgE was more significantly detected in residents with a respiratory allergy (21.9%, 7/32) than in those with no respiratory allergy (7.4%, 6/81; $p < 0.05$).

PA Antigen and Home Characteristics

The presence of the PA antigen in floor dusts was strongly associated with ant infestations in the home. The

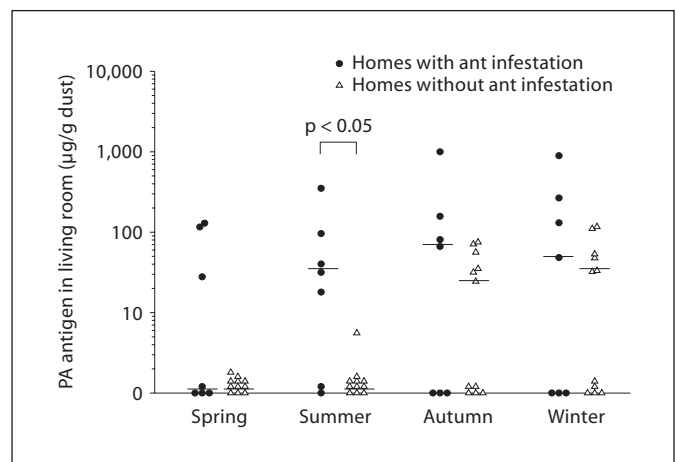


Fig. 4. Level of PA antigens in the living room floor dusts from the 18 PA antigen-positive homes according to the presence of an ant infestation in the home. The horizontal bars represent the median level of the PA antigens in the living room floor dusts.

location of the home was also an important factor for the presence of the PA antigen, with lower-level homes (first to third story) having a higher detection rate of the ant antigen than those homes at higher levels (over fourth story; 43.8 vs. 16.7%; $p < 0.05$). However, the amount of the PA antigens was not associated with the building type, living duration, temperature or relative humidity of the home.

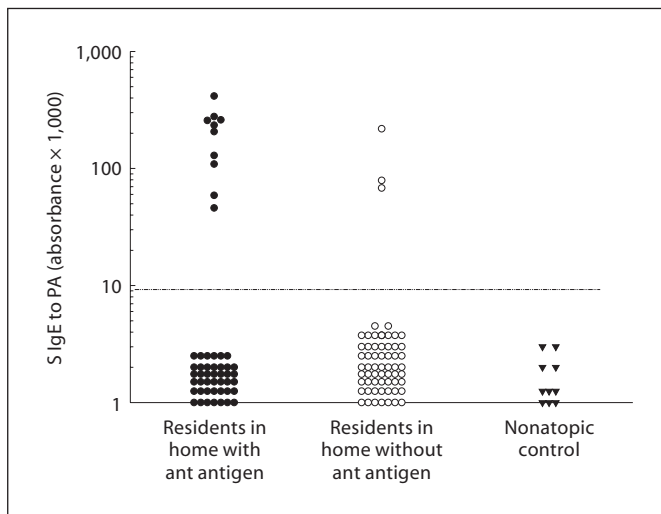


Fig. 5. sIgE binding to the PA antigens in serum samples from residents living in PA antigen-positive homes, PA antigen-negative homes, and nonatopic controls. The horizontal dotted line indicates the positive cutoff value of sIgE to PA, which was derived from the mean + 2 SD of the absorbance values of the healthy controls.

Discussion

PA has become a troublesome indoor insect [17]. PA was found to have infested approximately 18% of the homes in this study. However, this high prevalence of PA infestation does not represent the general status of PA infestations in Seoul because the subjects' homes were not randomly selected. Nevertheless, such an infestation rate was unexpectedly high. In addition, a significant level of PA antigens was distributed in the indoor environment, resulting in potential inhalant exposure.

In this study, the specificity and reproducibility of the ELISA inhibition assay was confirmed by immunoblotting inhibition and analysis of covariance of the standard curves. There are no reports on the distribution of indoor arthropods other than HDMs and cockroaches in Seoul. A microscopic evaluation of dust samples and bait traps was carried out in a field survey of 207 homes in Seoul in 1999. In that survey, we determined the distribution pattern of indoor arthropods and insects in the Seoul metropolitan area. HDMs were identified in 172 homes (83.1%), cockroaches in 80 (38.6%), PAs in 39 (19.8%), earwigs in 19 (9.2%), spiders in 14 (6.8%) and millipedes in 2 (0.9%) [unpubl. data]. It was concluded that HDMs, cockroaches and PAs were the main indoor arthropods in Seoul with the other local species being relatively in-

significant. Therefore, an ELISA inhibition test with other ants or local insect species was not performed because of the relatively low importance and lack of commercially available high-quality extracts.

Although the indoor environment provides favorable growth conditions for PAs all the year round, the ant population usually declines in winter because it requires considerable warmth and humidity [17]. However, this study found the level of PA antigens to be highest in winter. Several studies have also reported a lag period between the peak in population and the peak in allergen levels [18, 19]. Peak levels of PA antigens in winter might be representative of this lag and may be due to the persistence of ant allergens even after the number of ants has declined.

The most important factors for the detection of PA antigens were the level of the home and whether the home had a PA infestation. Although homes on the lower floors had higher detection rates of PA antigens, those on the higher levels also had detectable PA antigens. Indeed, a PA infestation and the PA antigens were detected in a home on the fifteenth floor in this study. Even if the detection of PA antigens was significantly higher in homes with visual evidence of a PA infestation, a significant amount was measured in homes with no PA infestation. It was considered possible that cross-reactivity with other ants or insect species occurred. However, this was ruled out indirectly by the previously mentioned specificity of this experiment. It is believed that these results might be due to the sensitivity of the assay; an immunological method was more sensitive in assessing the level of the PA antigens than the ant counts. It is well known that insect allergens can be present in an environment that has no physical signs of the insect. Chew et al. [20] reported that the cockroach allergen was detected in 48% of homes with no reported signs of cockroaches in the previous 12 months. Moreover, Pollart et al. [21] reported that 20% of homes not reporting signs of cockroaches had significant levels of the Bla g 2 allergen. This suggests that PAs should be considered a possible cause of disease in PA-sensitized symptomatic patients with a respiratory allergy, and the concentration of PA antigens should be measured even in the absence of an obvious ant infestation.

Exposure to allergens is essential to the process of specific sensitization. Exposure to an amount of the allergen greater than the threshold is required for sensitization. The risk levels for sensitization and symptom development are known for several inhalant allergens [1, 2, 20]. In this study, significantly higher detection of PA-sIgE was noted in residents living in PA antigen-positive homes

than in residents living in antigen-negative homes. We previously reported that PA allergens might have no cross-reactive allergenic determinants and could elicit sIgE response because there were many patients with monosensitization to PA, and the sIgE binding to PA was completely inhibited by PA itself but not by other allergens [14]. It was assumed that the PA-sIgE response in this study might be a unique reaction to PA. However, it is unclear if the detection pattern of PA-sIgE in this study reflects a dose-response relationship between allergen exposure and sensitization. Because PA-sIgE was not determined in all residents in the homes examined, the prevalence of PA-sIgE did not represent the general status and might have been overestimated. Further studies will be needed to clarify the sensitization and symptom provocation of exposure to PA allergens.

In conclusion, a considerable level of PA antigens might be distributed in an indoor environment, and inhalant exposure to PA antigens can occur as a result of domestic activities. PAs might be an important source of indoor aeroallergens. These species should be considered as a cause of respiratory allergy in patients living in urban areas even when there is no evidence of ant infestations in the home.

Acknowledgements

This study was supported by the Yonsei University College of Medicine, Internal Medicine Research grant and a grant (HMP-99-M-09-0006) of the Good Health R&D Project, Ministry of Health and Welfare, Korea.

References

- Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, Mitchell H, McNiff-Mortimer K, Lynn H, Ownby D, Malveaux F: The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N Engl J Med* 1997;336:1356–1363.
- Lewis SA, Weiss ST, Platts-Mills TA, Burge H, Gold DR: The role of indoor allergen sensitization and exposure in causing morbidity in women with asthma. *Am J Respir Crit Care Med* 2002;165:961–966.
- Lierl MB, Riordan MM, Fischer TJ: Prevalence of insect allergen-specific IgE in allergic asthmatic children in Cincinnati, Ohio. *Ann Allergy* 1994;72:45–50.
- Tee RD, Gordon DJ, Hawkins ER, Nunn AJ, Lacey J, Venables KM, Cooter RJ, McCaffery AR, Newman Taylor AJ: Occupational allergy to locusts: an investigation of the sources of the allergen. *J Allergy Clin Immunol* 1988;81:517–525.
- Bagenstose AH III, Mathews KP, Homburger HA, Saaveard-Delgado AP: Inhalant allergy due to crickets. *J Allergy Clin Immunol* 1980;65:71–74.
- Kimura JY, Matsuoka H, Ishii A: ELISA inhibition method in detection of mite and chironomid antigens in environmental samples of dust, soil and air. *Allergy* 1990;45:167–173.
- Ray JN, Pence HL: Ladybug hypersensitivity: report of a case and review of literature. *Allergy Asthma Proc* 2004;25:133–136.
- Albright DD, Jordan-Wagner D, Napoli DC, Parker AL, Quance-Fitch F, Whisman B, Collins JW, Hagan LL: Multicolored Asian lady beetle hypersensitivity: a case series and allergist survey. *Ann Allergy Asthma Immunol* 2006;97:521–527.
- Sharma K, Muldoon SB, Potter MF, Pence HL: Ladybug hypersensitivity among residents of homes infested with ladybugs in Kentucky. *Ann Allergy Asthma Immunol* 2006;97:528–531.
- Trudeau WL, Fernandez-Caldas E, Fox RW, Brenner R, Bucholtz GA, Lockey RF: Allergenicity of the cat flea (*Ctenocephalides felis felis*). *Clin Exp Allergy* 1993;23:377–383.
- Focke M, Hemmer W, Wohrl S, Gotz M, Jarisch R, Kofler H: Specific sensitization to the common housefly (*Musca domestica*) not related to insect panallergy. *Allergy* 2003;58:448–451.
- Barletta B, Puggioni EM, Afferni C, Butteroni C, Iacovacci P, Tinghino R, Ariano R, Panzani RC, Di Felice G, Pini C: Preparation and characterization of silverfish (*Lepisma saccharina*) extract and identification of allergenic components. *Int Arch Allergy Immunol* 2002;128:179–186.
- Kim CW, Choi SY, Park JW, Hong CS: Respiratory allergy to the indoor ant (*Monomorium pharaonis*) not related to sting allergy. *Ann Allergy Asthma Immunol* 2005;94:301–306.
- Kim CW, Kim DI, Choi SY, Park JW, Hong CS: Pharaoh ant (*Monomorium pharaonis*): newly identified important inhalant allergens in bronchial asthma. *J Korean Med Sci* 2005;20:390–396.
- The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee: Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). *Eur Respir J* 1998;12:315–335.
- Park JW, Kim CW, Kang DB, Lee IY, Choi SY, Yong TS, Shin DC, Kim KE, Hong CS: Low-flow, long-term air sampling under normal domestic activity to measure house dust mite and cockroach allergens. *J Investig Allergol Clin Immunol* 2002;12:293–298.
- Holldobler B, Wilson EO: The Ants: Classification and Origins. Cambridge, The Belknap Press of Harvard University Press, 1990, pp 4–141.
- Platts-Mills TA, Hayden ML, Chapman MD, Wilkins SR: Seasonal variation in dust mite and grass-pollen allergens in dust from the houses of patients with asthma. *J Allergy Clin Immunol* 1987;79:781–791.
- Mollet JA, Vailes LD, Avner DB, Perzanowski MS, Arruda LK, Chapman MD, Platts-Mills TA: Evaluation of German cockroach (Orthoptera: Blattellidae) allergen and seasonal variation in low-income housing. *J Med Entomol* 1997;34:307–311.
- Chew GL, Burge HA, Dockery DW, Muilenberg ML, Weiss ST, Gold DR: Limitations of a home characteristics questionnaire as a predictor of indoor allergen levels. *Am J Respir Crit Care Med* 1998;157:1536–1541.
- Pollart SM, Smith TF, Morris EC, Gelber LE, Platts-Mills TA, Chapman MD: Environmental exposure to cockroach allergens: analysis with monoclonal antibody-based enzyme immunoassays. *J Allergy Clin Immunol* 1991;87:505–510.