
Respiratory allergy to the indoor ant (*Monomorium pharaonis*) not related to sting allergy

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Background: Many studies are available on systemic reactions to ant sting, but few have described the direct role of ants in respiratory allergy. The nonstinging house ant, *Monomorium pharaonis* (pharaoh ant), is a highly infesting species in indoor environments.

Objective: To determine whether the pharaoh ant is an indoor source of aeroallergens.

Methods: Two patients with asthma who lived in homes with ant infestation were enrolled. Pharaoh ants were collected at the patients' homes, and crude extracts were prepared. Skin prick tests with ant extracts were performed. Specific IgE to pharaoh ant was measured by enzyme-linked immunosorbent assay (ELISA), and the allergenic components were determined by using immunoblot analysis. Cross-reactivity among pharaoh ant, imported fire ant, *Pachycondyla chinensis* ant, and other indoor allergens was evaluated by ELISA inhibition tests. Specific bronchial challenge testing was performed using pharaoh ant extracts.

Results: Both patients had positive skin test reactions to pharaoh ant extract and high levels of specific IgE antibodies to pharaoh ant. The ELISA inhibition test results demonstrated significant inhibition by pharaoh ant; however, *P. chinensis*, cockroach, and house dust mite showed no inhibition of the IgE binding to pharaoh ant. Two important IgE-binding components, 9.4 and 34 kDa, were identified by using immunoblot analysis. Pharaoh ant bronchial challenge test results showed typical early asthmatic reactions in 1 patient and dual asthmatic reactions in the other patient.

Conclusions: Ants can induce IgE-mediated bronchoconstriction regardless of sting in sensitized patients. Ants should be taken into consideration as a cause of respiratory allergy in patients living in homes with visual evidence of infestation.

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INTRODUCTION

Several studies have indicated that insects are important sources of aeroallergens. Insects involved in respiratory sensitization include cockroaches,^{1–3} locusts,⁴ crickets,⁵ moths,⁶ houseflies,⁷ and ants.³ Except for cockroaches, however, the clinical importance of sensitization to these insects remains uncertain owing to the lack of clinically relevant data.

The sting from an ant can cause a wide range of allergic responses in sensitized individuals. Imported fire ants (IFAs) have caused systemic reactions at high incidence in endemic areas.⁸ In Korea, venom from the *Pachycondyla chinensis* ant has occasionally induced anaphylactic reactions.^{9–11} However, the direct role of the ant in respiratory allergic diseases remains to be clarified.

The nonstinging house ant, *Monomorium pharaonis* (pharaoh ant), belonging to the Myrmicinae subfamily and the Formicidae family, is a highly infesting species in the urban indoor environment. We recently identified that the pharaoh ant can act as a source of aeroallergens. To our knowledge,

this is the first article to provide direct evidence that ants can play an important role in respiratory allergic diseases.

CASE REPORTS

Two patients were referred to the Allergy Clinic of Yonsei University Severance Hospital (Seoul, Korea) because of suspected respiratory allergy.

Patient 1 was a 46-year-old woman with cough, dyspnea, wheezing, rhinorrhea, and sneezing of a year's duration. She was a housewife, and her medical and family history was unremarkable. She denied smoking, having any pets, or having mold problems in her home. She identified visible ants in the living room and kitchen of her home, and she suspected that they were the cause of her complaints. She had no known history of ant sting or bite injury. Chest radiography findings were normal, and the serum total IgE level was 83 IU/mL. The percentage of eosinophils in induced sputum was 10% of the total inflammatory cells. Her forced expiratory volume in 1 second (FEV₁) was 2.12 L (89.5%), with a forced vital capacity (FVC) of 2.50 L (89.9%). The provocative concentration of methacholine that caused a decrease in FEV₁ of 20% (PC₂₀) was 2.2 mg/mL.

Patient 2 was a 58-year-old woman with cough, wheezing, and chest discomfort. She was diagnosed as having bronchial asthma 5 years earlier. Despite regular administration of antiasthma medications, her symptoms intermittently wors-

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ened. She was a housewife and a lifetime nonsmoker, and her medical history was not significant except for the asthma. She denied smoking, having any pets, or having mold problems in her home. She also identified ants in her home, and she suspected that the ants were the cause of her symptoms. She had no known history of ant sting or biting injury. Her serum total IgE level was 242 IU/mL, and her sputum eosinophil percentage was 52%. Her FEV₁ was 96.0%, with a FVC of 101.1% and a PC₂₀ of 10.0 mg/mL.

Ten atopic control subjects with allergic asthma who were sensitive to house dust mites (HDMs) (5 controls were exclusively sensitive to HDMs and 5 were cosensitive to HDMs and other allergens, such as animal dander or pollens) and 10 nonatopic control subjects were enrolled.

MATERIALS AND METHODS

Preparation of Pharaoh Ant Extract

Pharaoh ants were collected from the patients' houses, and their species were confirmed by Professor B.-J. Kim (Wonkwang University, Iksan, Korea). The ants were ground up and extracted into phosphate-buffered saline solution (PBS) (pH 7.4) 1:20 wt/vol at 4°C for 48 hours, followed by centrifugation at 12,000 rpm for 1 hour. The supernatant was dialyzed against distilled water at 4°C for 48 hours and then used as crude extract. The protein concentration of the extract was 500.3 µg/mL, as determined using the BioRad method. For skin prick testing, the extract was diluted with PBS and then mixed with an equal amount of sterile glycerin.

Allergy Skin Prick Tests

Skin prick tests were performed using pharaoh ant extract (1:100 wt/vol), IFA extract (Hollister-Stier Laboratories LLC, Spokane, WA), and 50 common aeroallergens, including pollens, molds, animal danders, cockroaches, and HDMs

(AT Ltd, Worthing, West Sussex, England). After 15 minutes, the mean diameter of the wheal formed by the allergen was compared with that formed by histamine.

Specific Antibody Determination and Cross-reactivity Study

The presence of specific IgE, IgG, and IgG4 to pharaoh ant was determined by enzyme-linked immunosorbent assay (ELISA). Microtiter plates were coated with 50 µL of 0.05M carbonate buffer (pH 9.6) containing 10 µg/mL of pharaoh ant extract at 4°C. After blocking with 200 µL of 1% bovine serum albumin in PBS-Tween, the plates were incubated with 50 µL of either patient or control sera (undiluted for IgE, 1:100 for IgG, and 1:2 for IgG4). After washing, plates were incubated with biotinylated anti-human IgE (Vector Laboratories, Burlingame, CA), anti-IgG, and anti-IgG4 (Sigma-Aldrich Corp, St Louis, MO) at a dilution of 1:1,000 vol/vol, followed by incubation with streptavidin-peroxidase (Sigma-Aldrich Corp) at a dilution of 1:1,000 wt/vol. After washing, ABTS solution (25 mg of 2,2'-azino-bis-3-ethylbenzthiazoline-sulfonic acid in 50 mL of 50mM citrate phosphate buffer [pH 4.2] containing 50 µL of 30% hydrogen peroxide) was added as a substrate, and 50 µL of 2mM sodium azide was added to stop the reaction. The colorimetric reaction was measured by the absorbency at 405 nm on an ELISA reader (Dynatec, Alexandria, CA). The positive cutoff value was decided as mean + 2 SDs of the absorbency value of the healthy control serum samples.

Levels of specific IgE to *Dermatophagoides farinae* were also measured by using the ELISA method. Patient serum (undiluted) was added to a *D farinae*-coated immunoplate, and the remaining steps were performed as described previously herein.

To determine the cross-reactivity of IgE response to pharaoh ant, ELISA inhibition tests were performed. The pa-

Table 1. Clinical Features of the 2 Patients and 10 Atopic Asthma Control Subjects

Participant No.	SPT wheal diameter, mm					slgE, OD*	slgG/slG4, OD*
	Histamine	Pharaoh ant	IFA	<i>D farinae</i>	Other		
Patients							
1	3 × 3	14 × 7	3 × 3	2 × 2	Neg	0.900	0.506/0.519
2	4 × 4	8 × 4	4 × 4	Neg	Neg	0.840	0.390/0.675
Controls							
1	4 × 3	Neg	Neg	20 × 18	Neg	0.009	0.441/0.027
2	4 × 4	Neg	Neg	10 × 18	Cat 16 × 14	0.010	0.702/0.516
3	4 × 4	Neg	Neg	18 × 16	Neg	0.001	0.305/0.021
4	4 × 4	Neg	Neg	18 × 14	Mugwort 10 × 7	0.009	0.536/0.063
5	5 × 3	Neg	Neg	20 × 17	Dog 9 × 7	0.009	0.256/0.013
6	3 × 3	Neg	Neg	12 × 8	Neg	0.007	0.379/0.021
7	4 × 4	Neg	Neg	13 × 8	Oak 13 × 11	0.005	0.576/0.114
8	4 × 3	Neg	Neg	7 × 6	Dog 4 × 3	0.002	0.392/0.457
9	5 × 5	Neg	Neg	6 × 4	Neg	0.001	0.531/0.045
10	4 × 4	Neg	Neg	5 × 4	Neg	0.007	0.270/0.018

Abbreviations: SPT, skin prick test; Neg, negative; slgE, pharaoh ant specific IgE; IFA, imported fire ant; *D farinae*, *Dermatophagoides farinae*; Mugwort, mugwort pollen; Oak, oak pollen.

* Levels are described as absorbency values in enzyme-linked immunosorbent assay.

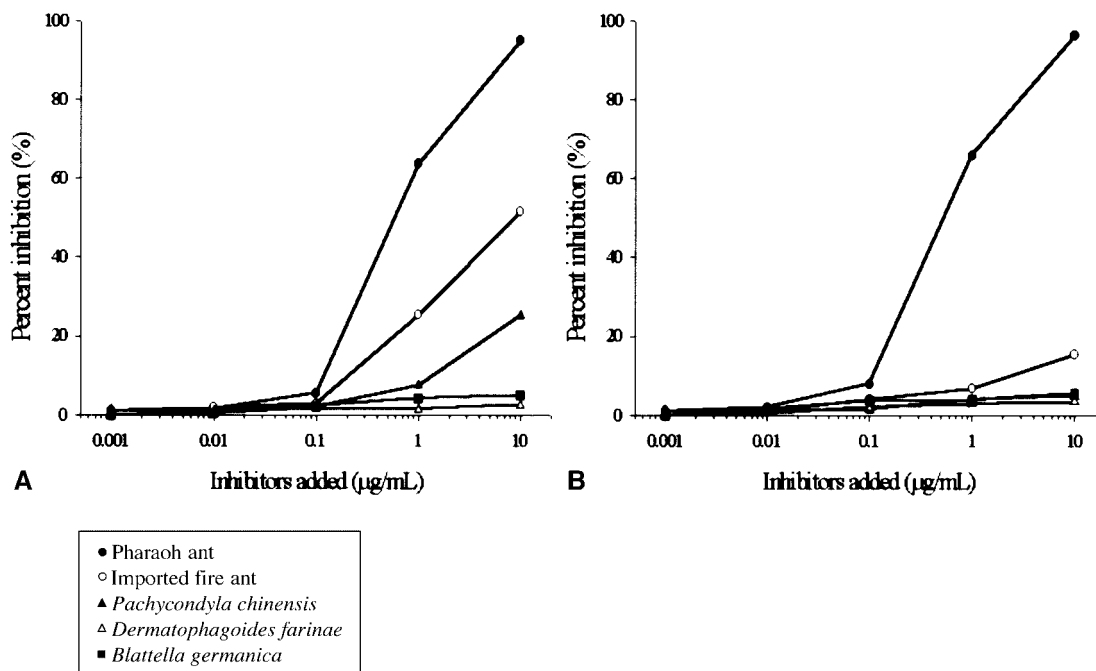


Figure 1. Enzyme-linked immunosorbent assay inhibition of pharaoh ant specific IgE. Serum samples from patient 1 (A) and patient 2 (B) were preincubated with inhibitors and then added to a pharaoh ant-coated immunoplate.

tient's undiluted serum was preincubated with inhibitors such as IFA (Bayer, Pittsburgh, PA), *P chinensis*, *Blattella germanica*, and *D farinae* extracts (kindly donated by Dr T.-S. Yong at Yonsei University) and then added to a pharaoh ant antigen-coated plate. The same steps were followed as in the ELISA.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and IgE Immunoblot Analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis were performed under reduced conditions. Pharaoh ant extract was electrophoresed in 13.5% separation gel with 4% stacking gel. Proteins were transferred onto a nitrocellulose membrane (Amersham, Buckinghamshire, England) which was incubated with the patient and pooled control sera diluted at a ratio of 1:4 in PBS-Tween. Bound specific IgE was detected by goat anti-human IgE 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, WI) as a substrate solution.

Bronchial Provocation Tests

Specific bronchial provocation tests (BPTs) were performed with the pharaoh ant extract. Subjects inhaled the aerosolized extract dissolved in 0.4% phenol and 0.9% saline for 2 minutes by using the tidal breathing method. A control challenge with phenol-saline was performed before antigen provocation, and then serial increments of ant extracts (from

1:100,000 to 1:1,000 wt/vol) were administered at 10-minute intervals until a 20% or greater decrease in FEV₁ was obtained or until the highest concentration of the extract was inhaled. Both FEV₁ and FVC were measured every 10 minutes during the first hour and then every hour for the remaining 7 hours after the challenge. A decrease in FEV₁ of 20% or more from the baseline value was considered a positive reaction. Patient 2 was challenged with *D farinae* (from 1:10,000 to 1:1,000 wt/vol) as a control on a separate day. The BPTs with pharaoh ant extract were also performed in 3 HDM-sensitive asthmatic patients with similar airway hyperresponsiveness (PC₂₀ of 3.01, 5.31, and 9.82 mg/mL).

RESULTS

Skin Prick Tests

Skin prick test responses to pharaoh ant were positive in both patients. Patient 1 had a 4+ response to pharaoh ant and a 3+ response to IFA but negative responses to 50 common aeroallergens. Patient 2 had a 3+ response to pharaoh ant and IFA extract (Table 1). However, the 10 subjects with HDM-sensitive asthma and the 10 healthy controls exhibited negative responses to pharaoh ant extracts.

IgE Measurements and Cross-reactivity

Serum samples from the 2 patients showed high IgE ELISA values, with absorbencies of 0.90 and 0.84 OD for pharaoh ant compared with the cutoff level for a positive response

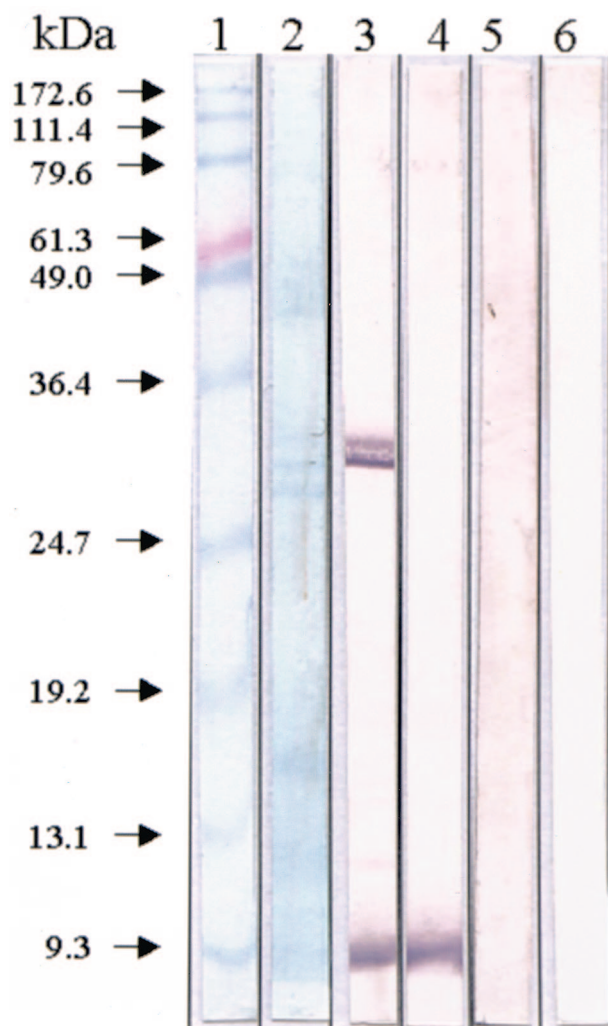


Figure 2. IgE immunoblot analysis using pharaoh ant extracts. Lane 1 shows molecular weight markers; lane 2, pharaoh ant extract profile onto membrane; lane 3, immunoblot with serum from patient 1; lane 4, immunoblot with serum from patient 2; lane 5, immunoblot with pooled sera from 10 healthy, nonatopic control subjects; and lane 6, buffer control.

(absorbency of 0.01 OD). However, no asthmatic control subject had a significant level of specific IgE to pharaoh ant. When the cutoff value for specific IgG4 antibody (0.047 OD) was derived from the mean + 2 SDs absorbency values of the 10 healthy control subjects, the 2 patients and 4 atopic controls were found to be positive for specific IgG4 antibody. Neither patient 1 nor patient 2 had a detectable level of specific IgE to HDM or *D farinae*.

The results of ELISA inhibition testing indicate that IgE binding to pharaoh ant was completely inhibited by preincubation with 10 μ g/mL of pharaoh ant in both patients (Figure 1). Partial inhibition was also noted with the addition of IFA antigen in patient 1. However, *P chinensis*, *B germanica*, and *D farinae* did not inhibit IgE binding to pharaoh ant.

SDS-PAGE and IgE Immunoblot Analysis

The SDS-PAGE analysis of pharaoh ant extract revealed various protein bands ranging from 9 to 100 kDa. Figure 2 shows the patterns of the IgE-binding components in individual patients, pooled serum samples from control subjects, and buffer control. Two important IgE-binding components of 9.4 and 34 kDa were identified by using immunoblot analysis. No atopic control subject showed positive binding of specific IgE to pharaoh ant antigens (data not shown).

Bronchial Provocation Tests

Patient 1 showed an isolated early asthmatic reaction after inhaling 1:100,000 wt/vol of pharaoh ant extract. After recovery from the initial drop in lung function within 1 hour, FEV₁ in patient 2 decreased from 1.72 to 0.91 L (47.1% fall) 6 hours after inhalation of pharaoh ant extract (Fig 3). No significant change in FEV₁ was noted in the BPT results of patient 2 with *D farinae* or in 3 control subjects with asthma after challenge with pharaoh ant extract.

Clinical Course

The airway hyperresponsiveness experienced by patient 1 disappeared 6 months after moving to a new home without ant infestation (PC₂₀ >25 mg/mL). At the 6-month follow-up visit, she had no symptoms despite taking no antiasthma medicines. Although complete eradication of ant infestation failed, patient 2 noted that the visual evidence of ants was reduced. Her symptoms gradually improved and were easily controlled by using low-dose inhaled budesonide. Her airway hyperresponsiveness was negatively converted at the 1-year follow-up visit (PC₂₀ >25 mg/mL).

DISCUSSION

Although previous studies³⁻⁷ have reported that many kinds of insects can cause respiratory sensitization, the clinical significance of IgE to these insects remains obscure owing to lack of clinical correlation. However, the impetus for the present study came from patients' complaining of a possible relationship between their symptoms and ant infestation in their homes. The present study reveals that the pharaoh ant is a causative agent of allergic asthma based on clinical course, environmental characteristics, exclusive skin prick test responses to pharaoh ant, and the demonstration of specific IgE and IgE-binding components.

We present herein direct evidence that ants were the cause of their asthma by data showing positive reactions to BPT. The specificity of the bronchial reaction in BPT was confirmed by the negative results of BPTs in control patients with asthma. In ELISA inhibition testing, minimal inhibitions of IgE binding to pharaoh ants were noted with the addition of other important indoor allergens, namely, HDMs or cockroaches. These results also suggest that the reaction to pharaoh ant is specific. To rule out potential contamination by other allergens, we measured the levels of major allergen of HDM (Der f 1 and Der p 1) and cockroach (Bla g 1) in pharaoh ant extracts using the 2-site ELISA method,¹² but no allergen was detected (data not shown).

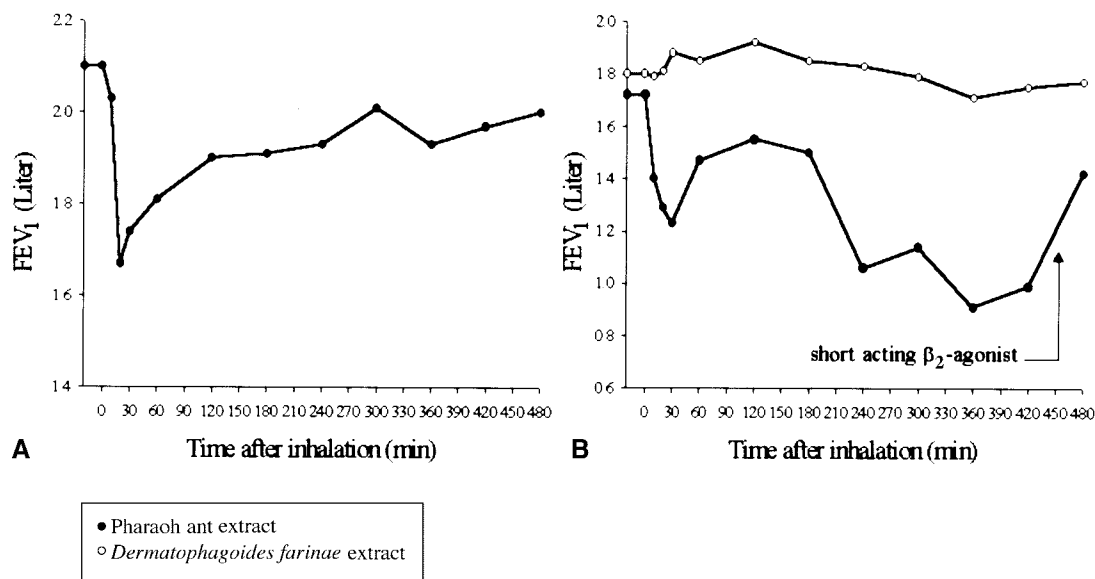


Figure 3. Bronchial provocation test results by exposure to pharaoh ant extract (1:100,000 wt/vol) or *Dermatophagoides farinae* extract (1:1,000 wt/vol) in patient 1 (A) and patient 2 (B). FEV₁ indicates forced expiratory volume in 1 second.

The role of specific IgG4 in patients with an inhalant insect allergy has not been clearly identified. A previous study¹³ demonstrated that levels of specific IgG increased after cockroach immunotherapy, which has been known to contribute to symptomatic improvements. However, a study of IgG antibody in chironomids allergy suggested that their prevalence represents a marker for the degree of exposure to insect allergens.¹⁴ In this study, specific IgG4 antibodies were detected in 2 patients and in 4 of 10 atopic controls, although they had never received immunologic treatment with pharaoh ant antigen. Furthermore, there was no direct evidence that the 4 atopic controls with positive specific IgG4 responses had been previously exposed to pharaoh ant antigens. Therefore, the specific IgG4 response to pharaoh ant seems to be complex, and further studies are needed to clarify the role of specific IgG4 in pharaoh ant allergy.

The partial inhibition of IgE binding to pharaoh ant with IFA, which is not present in Korea, suggests cross-allergenicity between pharaoh ant and IFA. On the other hand, pharaoh ant did not cross-react with *P chinensis*, which is the most important species that causes systemic reactions by ant stings in Korea.⁹⁻¹¹ We consider that these results may arise from taxonomic differences: pharaoh ants and IFAs belong to the Myrmicinae subfamily, whereas *P chinensis* belongs to the Ponerinae subfamily.^{11,15} Yun et al¹⁰ previously reported that there is no cross-reactivity between *P chinensis* and IFA antigen.

The pharaoh ant, the tiny yellow indoor ant, is of tropical origin and has been carried to all the inhabited regions of the world. It requires considerable warmth and does not nest out of doors except in southern latitudes, and its infestation has continuously increased.¹⁵ Airborne insect-derived particles,

such as shed scales, excreta, and disintegrated bodies, have been implicated as causes of respiratory allergy. Two IgE-binding components, 9.4 and 34 kDa, were found in pharaoh ant extract in this study. Further studies are needed to clarify the exact physical and biochemical properties of the allergenic components of pharaoh ant antigens. In conclusion, the results of this study suggest that the pharaoh ant can directly induce IgE-mediated bronchoconstriction in sensitized patients. This species should be taken into consideration as a cause of respiratory allergic diseases in patients living in houses with visual evidence of ant infestation.

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