

Anaphylaxis caused by the new ant, *Pachycondyla chinensis*: Demonstration of specific IgE and IgE-binding components

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Background: There have been no reports dealing with the pathogenic mechanism and IgE-binding components in patients with anaphylaxis caused by a sting from *Pachycondyla chinensis*.

Objectives: This study was conducted to observe the clinical features of patients with *P chinensis*-induced anaphylaxis. The roles of specific (s) IgE and sIgG4 antibodies were evaluated, and IgE-binding components were identified.

Methods: Seven patients with *P chinensis*-induced anaphylaxis and 15 unexposed control subjects were enrolled. *P chinensis* ants were collected at the patients' homes, and venom was prepared as *P chinensis* extract. Five patients complained of bee venom-induced anaphylaxis and had positive sIgE levels to yellow jacket venom, wasp venom, or both as well. Serum sIgE and sIgG4 were detected by means of ELISA. To identify IgE-binding components within *P chinensis* extracts, 12% SDS-PAGE with immunoblot analysis was applied.

Results: All patients had positive skin prick test responses to *P chinensis* antigen and positive sIgE levels. Five (71%) patients had positive sIgG4 levels. Eight IgE-binding components (58, 46, 31, 29, 27, 25, 22, and 12 kd) were noted, and the component at 12 kd was the most frequently found allergen (85%). IgE ELISA inhibition tests were performed on 2 groups of sera: one from patients with anaphylaxis induced by both *P chinensis* and bee venom (group A) and the other from patients with anaphylaxis induced by *P chinensis* venom alone without bee venom allergy (group B). ELISA inhibition tests with serum from group A showed significant inhibitions with addition of *P chinensis* extract, partial inhibitions with yellow jacket antigen, and minimal inhibitions with wasp or imported fire ant antigens. However, ELISA inhibition tests with serum from group B showed significant inhibitions with *P chinensis* antigen but no inhibition with wasp, yellow jacket, or imported fire ant antigens.

Conclusions: IgE-mediated reactions contributed to the development of *P chinensis*-induced anaphylaxis. Eight IgE-binding components and one major allergen (12 kd) were identified. Further studies will be needed to clarify the role of sIgG4 and to identify allergenic relationships with major bee and wasp allergens. (J Allergy Clin Immunol 2001;107:1095-9.)

Key words: *Pachycondyla chinensis*, major allergen, specific IgE and IgG4, cross-reactivity

The ant is a widespread insect that is comprised of 11 subfamilies, 297 genera, and approximately 8800 species.¹ In Korea 4 subfamilies, 33 genera, and 104 species of ants have formally been registered.² In the southeastern United States and Australia, imported fire ants (*Solenopsis invicta* and *Solenopsis richteri*) belonging to the Myrmicinae subfamily and Formicidae family have caused systemic reactions with a high incidence.^{2,3} In Korea the venom from the *Pachycondyla* species of the Ponerinae subfamily and Formicidae family has occasionally induced anaphylactic reactions,^{4,5} and it is the only ant to have caused anaphylaxis. It is known that *Pachycondyla chinensis* ants exist in Far East Asian countries, including Korea, Japan, and China. Yun et al⁶ reported one case of *P chinensis*-induced anaphylaxis, and its IgE-binding component in the subject's serum.

Regarding the role of specific (s) IgG4, especially in bee and wasp venom allergy, sIgG4 levels increased significantly after allergen-specific immunotherapy, which is associated with symptomatic improvement.^{6,7}

In this study we observed the clinical features of *P chinensis*-induced anaphylaxis, evaluated the role of sIgG4 and sIgE, and identified the major allergens. Also, allergenic relationships with bee and wasp allergens were evaluated.

METHODS

Study subjects

Seven patients who visited the emergency department of Ajou University Hospital because of anaphylaxis caused by *P chinensis* ant venom were enrolled in this study. Their initial symptoms included generalized urticaria, respiratory distress, wheezing, and hypotension with or without loss of consciousness. Serum was collected at initial diagnosis in the emergency department. The patients were classified into 2 groups depending on the presence of bee and wasp anaphylaxis. Group A included subjects having anaphylaxis caused by both *P chinensis* and bee venom, and group B included subjects having anaphylaxis caused by *P chinensis* venom alone without bee venom allergy. Unexposed control subjects were

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TABLE I. Clinical features of the study subjects

Patient No.	Sex	Age (y)	Atopy	Clinical manifestation	Total IgE (IU/mL)	Ant skin test (A/H ratio)	slgE/slG4 (OD)
1	F	43	Presence	Anaphylaxis	663	3+	1.18/0.97
2	F	20	Presence	Anaphylaxis, NSBR	655	3+	1.27/0.56
3	F	74	Presence	Anaphylaxis	353	ND	0.69/0.52
4	F	45	Absence	Anaphylaxis	76	ND	0.82/0.34
5	F	33	Presence	Anaphylaxis, urticaria	397	ND	0.71/0.48
6	F	48	Absence	Anaphylaxis, cold urticaria	595	3+	1.16/0.92
7	F	44	Presence	Anaphylaxis, NSBR, AR	135	2+	0.69/0.89

Anaphylaxis criteria include generalized urticaria, respiratory distress, wheezing, and hypotension with or without loss of consciousness.

A/H, Skin reactivity presented as allergen to histamine wheal ratio; NSBR, nonspecific bronchial hyperresponsiveness; ND, not done; AR, allergic rhinitis.

Abbreviations used

PBST: 0.05% PBS-Tween

RT: Room temperature

TBST: TRIS-buffered saline with Tween

enrolled from a pool of healthy subjects without histories of ant venom allergy.

Preparation of *P chinensis* ant extract

P chinensis ants were collected from patients' homes, and their species were confirmed by Professor B. J. Kim (Wonkwang University, Iksan, Korea; personal communication). They were ground up and extracted into PBS (pH 7.5) 1:5 wt/vol at 4°C overnight, followed by centrifugation at 5000 rpm. The supernatant was passed through a syringe filter (MSI). For skin prick testing, the 1:5 wt/vol extract was mixed with an equal amount of sterile glycerin. The supernatant was dialyzed (molecular weight cut-off point, 6 kd) against 4 L of PBS at 4°C for 48 hours, lyophilized at -70°C, and used for ELISA and immunoblot analysis. Bee venom allergens used in this study were supplied by Bayer Co (West Haven, Conn).

Allergy skin prick test

Skin prick tests with 50 common inhalant allergens, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Aspergillus* species, *Alternaria* species, mugwort and ragweed pollens, animal epithelium, cockroach (Bencard), *P chinensis* extract (1:10 wt/vol), and histamine (1 mg/mL) were performed on the backs of all the subjects. After 15 minutes, the mean diameter of the wheal formed by the allergen was compared with that formed by histamine. If the former was the same or larger than the latter (allergen/histamine ratio ≥ 1), it was considered positive and presented as 3+. If the allergen/histamine ratio was less than 1, it was presented as 2+.

Measurement of total serum IgE levels

The total serum IgE level was measured with a DPC kit according to the manufacturer's directions.

ELISA for slgE or slgG4 antibody to *P chinensis*

The presence of slgE and slgG4 antibodies to *P chinensis* was determined by means of ELISA according to the method described previously.⁸ Microtiter plates (Dynatech) were first coated with 100 μ L/well of *P chinensis* (1 μ g/well) extracts and left at 4°C overnight. Each well was washed 3 times with 0.05% PBS-Tween (PBST), and the remaining binding sites were blocked by incubation with 350 μ L of 3% BSA-PBST for 1 hour at room temperature (RT). The wells were then incubated for 3 hours at RT with 50 μ L of either patient or

control sera (undiluted) from 15 patients with negative skin prick test responses to common inhalant allergens, as well as to *P chinensis*. After washing 3 times with PBST, 50 μ L of the 1:500 vol/vol biotin-labeled goat anti-human IgE or IgG4 antibody (Vector Co) was added to the wells and incubated for 1.5 hours at RT. The wells were then washed 3 times with PBST and incubated with 1:1000 vol/vol streptavidin-peroxidase (Sigma Co) for 30 minutes at RT before another washing step, which was followed by incubation with 100 μ L of *o*-phenylenediamine dihydrochloride for 10 minutes at room temperature. The positive cut-off value was decided as mean + 2 $\times$ SD of the absorbance value of control sera.

SDS-PAGE and IgE immunoblot

SDS-PAGE and immunoblot analysis were performed under reduced conditions according to the methods described previously.^{8,9} *P chinensis* antigen was mixed with sample buffer (1 mL of 0.5 mol/L TRIS-HCl [pH 6.8], 1.6 mL of glycerol, 1.5 mL of 10% [wt/vol] SDS, 0.4 mL of 0.5% 2-mercaptoethanol, and 3 mL of distilled water), which was then adjusted to 1 mg/mL and heated in boiling water for 5 minutes. Ten microliters of standard marker (Novex) and antigen solution were added to a Novex precast TRIS-glycine gel (12%) for the separation of antigens. Electrophoresis was performed with Novex Mini-cell for 90 minutes at 125 V. The gel was fixed and stained with Coomassie brilliant blue. For the immunoblot, transfer of the proteins onto nitrocellulose membranes (pore size 0.45 μ m, Bio-rad Labs) was performed on a Novex Western transfer apparatus in a transfer buffer (25 mmol/L TRIS base and 192 mmol/L glycine in 20% methanol) at 200 mmol/L for 90 minutes. The blotted nitrocellulose membrane was blocked by 5% BSA in TRIS-buffered saline with Tween (TBST) for 1 hour. The patient and control sera were diluted 1:1 vol/vol with 3% BSA-TBST. Each membrane was then incubated with patient and control sera overnight at RT and washed with TBST. Affinity-purified biotinylated goat anti-human IgE antibody (Vector Labs) was diluted to 1:500 with TBST and used as a secondary antibody solution. After washing with TBST, streptavidin-alkaline phosphatase (Sigma Chemical Co) was diluted to 1:1000 with TBST, used to incubate the membrane for 30 minutes, and then detected with a 5-bromo-4-chloro-3 indolyl phosphate/nitro blue tetrazolium system (Sigma Co) as a substrate solution.

IgE ELISA inhibition test

Competitive ELISA inhibition tests were performed to determine the specificity of the IgE binding to the *P chinensis* antigen and the allergenic cross-reactivity to bee and wasp venom and imported fire ant antigen. Fifty microliters of the pooled sera was preincubated with 4 concentrations (0.1 to 100 μ g/mL protein concentration) of *P chinensis*, imported fire ant venom, wasp venom, yellow jacket venom, and Hop Japanese pollen extracts for 1 hour at RT. Each sample was then incubated with a *P chinensis*-coated

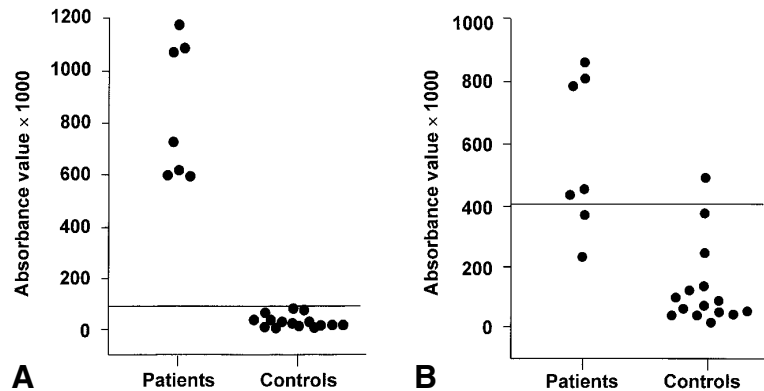


FIG 1. Specific IgE (**A**) and sIgG4 (**B**) binding to *P chinensis* antigen in study subjects and unexposed healthy control subjects.

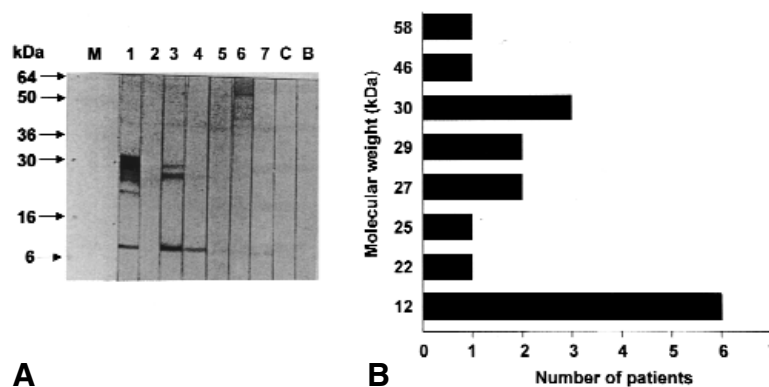


FIG 2. IgE immunoblot analysis and number of reactive sera of the study subjects (1-7), control sera (C), and buffer (B). M, Marker protein.

microtiter plate for 2 hours. The same steps were followed as in the ELISA. After studying control samples in which equal volumes of PBS were preincubated instead of inhibitors, we found that the inhibition of the sIgE binding could be expressed as follows:

$$100 - \left(\frac{\text{Absorbance of sample preincubated with allergen}}{\text{Absorbance of sample preincubated with PBS}} \right) \times 100(\%)$$

RESULTS

Clinical characteristics

Table I shows the clinical characteristics of 7 patients with anaphylaxis. Four subjects had associated allergic diseases, such as urticaria, allergic rhinitis, or bronchial hyperreactivity. All the patients undergoing skin prick tests with *P chinensis* had positive responses to *P chinensis* extract. High total IgE levels were noted in all but one patient. Fig 1 demonstrates sIgE and sIgG4 binding in patients and control subjects. Specific IgE to *P chinensis* was considered positive if the absorbance value was higher than the cutoff value (0.092), which was derived from mean + 2× SD absorbance values from 15 unexposed control subjects; all the subjects had positive serum sIgE antibody, as shown in Fig 1, A. When the cutoff value for sIgG4 antibody (0.41) was derived from mean + 2× SD of absorbance values of 15 control subjects, 5 subjects had positive sIgG4 antibody (Fig 1, B).

SDS-PAGE and IgE immunoblot analysis

Twelve percent SDS-PAGE analysis showed that *P chinensis* extract was resolved into bands ranging from 6 to 64 kD. Fig 2, A, shows the patterns of IgE-binding components in 7 individual patients, pooled sera from control patients, and buffer control. The control serum pool was derived from 5 patients' sera showing negative responses to *P chinensis* on skin prick tests. There were 8 bands (58, 46, 30, 29, 27, 25, 22, and 12 kD) bound to IgE antibody, with one (12 kD) bound to the IgE in 85% of the sera tested (Fig 2, B). A combination of 2 allergens (12 and 30 kD bands) would be positive in 100% of the patients.

P chinensis IgE ELISA inhibition test

Fig 3 shows the results of *P chinensis* IgE ELISA inhibition tests with 2 groups of sera: group A from patients with anaphylaxis induced by both *P chinensis* ant and bee venom and group B from patients with *P chinensis*-induced anaphylaxis alone. Fig 3, A, shows the results of a *P chinensis* ELISA inhibition testing with serum from group A. Significant inhibitions were noted with *P chinensis* antigen, and a lesser degree of inhibition was noted with the addition of yellow jacket antigen. Minimal inhibitions were noted with imported fire ant, wasp, honeybee, and Hop Japanese antigens. Fig 3, B, shows the results of

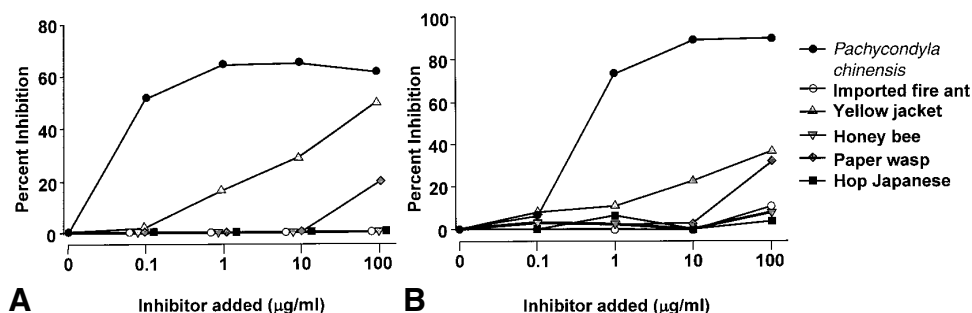


FIG 3. Comparison of inhibition results of *P. chinensis* ELISA with addition of *P. chinensis*, yellow jacket, wasp, honeybee, imported fire ant, and Hop Japanese pollen antigens by using a serum pool from patients with anaphylaxis induced by both *P. chinensis* and bee and wasp venom (A) and from patients with anaphylaxis induced by *P. chinensis* alone (B).

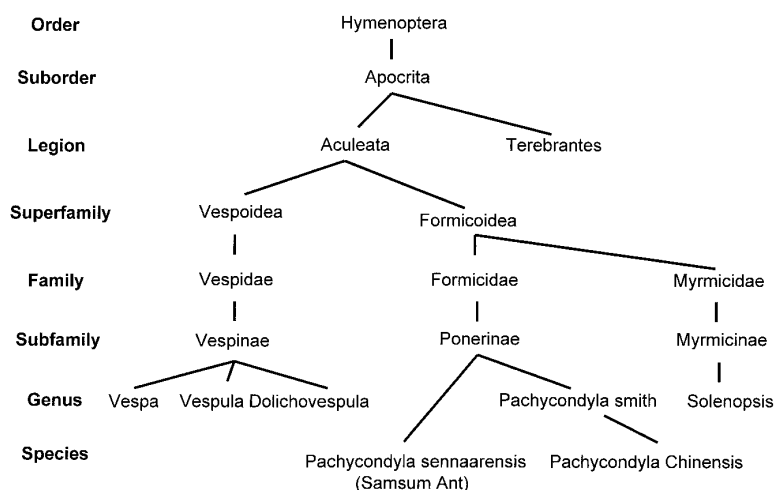


FIG 4. Taxonomy of Hymenoptera.

a *P. chinensis* ELISA inhibition testing with serum from group B. Significant inhibitions were noted with additions of *P. chinensis* extract in a dose-dependent manner, whereas minimal inhibitions were noted with imported fire ant venom, wasp venom, honeybee venom, yellow jacket venom, and Hop Japanese pollen antigens.

DISCUSSION

This study demonstrated a clinical summary of 7 patients with *P. chinensis*-induced anaphylaxis. Although there has been only one case report of *P. chinensis*-induced anaphylaxis in this country, this is the first study to confirm the presence of IgE in 7 patients by means of detecting sIgE and sIgG4 antibodies and identifying 8 IgE-binding components and a major allergen within the *P. chinensis* extract. IgE-mediated reaction may be the major mechanism. In the present study, all the patients had high serum *P. chinensis* sIgE antibody levels, as determined with ELISA, and ELISA inhibition tests confirmed the specificity of IgE binding to *P. chinensis* antigen. Furthermore, immunoblot analysis revealed 8 IgE-binding components and one component that might be considered a

major allergen because 85% of the subjects had positive bindings to this allergenic band. These findings confirmed that *P. chinensis* venom induced IgE-mediated anaphylaxis in exposed patients. Because *P. chinensis* immunotherapy extracts have not been developed until now, these results will serve as a basis for developing diagnostic and therapeutic extracts of *P. chinensis*.

Regarding the role of sIgG4 in patients with insect venom allergy, previous studies^{6,7} demonstrated that sIgE levels decreased and sIgG4 levels increased after allergen immunotherapy, which has been known to contribute to symptomatic improvements. In this study 5 patients had positive serum sIgG4 antibody levels, although they had anaphylaxis symptoms and had never received any immunologic treatment with *P. chinensis* antigen. Follow-up studies will be needed to clarify the role of sIgG4 after treatment.

There is agreement on the suggestion that the closer the taxonomic relationship between allergens, the greater the level of cross-inhibition seen, and this has been demonstrated in house dust mite^{10,11} and insect¹² antigen studies. *P. chinensis* belongs to the order of Hymenoptera, Formicidae family, Ponerinae subfamily, and *Pachy-*

condyla Smith genus and *P chinensis* species, whereas imported fire ant (*Solenopsis invicta* and *Solenopsis richteri*) belongs to the Myrmicinae subfamily and Formicidae family (Fig 4). Four IgE-binding components, Sol i I (38 kd), Sol i II (26 kd), Sol i III (24 kd), and Sol i IV (13 kd), were found within fire ant extract.¹³ It is known that Sol i I had similar structure to phospholipase allergen in wasps.¹⁴ In this study 5 subjects had bee venom-induced anaphylaxis with positive sIgE levels. In ELISA inhibition tests with serum from group A, significant inhibitions were noted with additions of *P chinensis* antigen, whereas a lesser degree of inhibition was noted with addition of yellow jacket antigen in a dose-dependent manner, suggesting that cross-reactivity could be greater if higher concentrations of yellow jacket antigen (>1 mg/mL) were added. However, in ELISA inhibition tests with serum from group B, minimal inhibitions were noted with additions of yellow jacket antigen or other insect antigens. These results suggest that there may be a possibility of cross-allergenicity between *P chinensis* and yellow jacket antigen in some patients, which may result in the presentation of concurrent anaphylaxis induced by both *P chinensis* and yellow jacket venom. The possibility of cross-reactivity between imported fire ant and *P chinensis* seems low because ELISA inhibition testing with 2 kinds of serum showed minimal inhibitions caused by imported fire ant. Further studies with a larger number of patients will be needed to clarify the allergenic relationship with bee venom or other insect allergens.

In conclusion, *P chinensis* venom can induce anaphylaxis by means of an IgE-mediated reaction. Eight IgE-binding components and one major allergen (12 kd) were identified in *P chinensis* extract.

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