

A Case of Anaphylaxis by Ant (Ectomomyrmex SPP.) Venom and Measurements of Specific IgE and IgG Subclasses

Si-Chan Kim and Chein-Soo Hong

Hypersensitivity to the stings of the Hymenoptera has been described since antiquity. The hypersensitive reactions to insect stings vary from minor skin reactions to severe and sometimes fatal anaphylaxis. Concerns about sting hypersensitivity have been increasing because of many incidents of allergic reactions of patients to the fire ant in the southern area of the United States as well as the harvester ant in some areas. We experienced one unique case with severe allergic reactions by ant of the Ectomomyrmex spp. of the subfamily Ponerinae, which is not a harvester ant. For three years the patient had been suffering from generalized allergic reactions such as anaphylaxis after the ant stings four-five attacks in a year. We determined that her reactions were due to specific IgE mediated type I hypersensitivity by the detection of a high level of specific IgE to the ant venom in her serum. The high level of specific IgG₄ to the ant venom was also noted in her serum, however, the role of ant venom IgG₄ was not clearly determined.

Key Words: Ant venom, anaphylaxis, specific IgE, IgE subclasses

Insects can induce allergic reactions in human-beings by the stinging of bees or ants, by the biting of fleas, bedbugs and lice and by the inhalation of fragments of caddis fly and midges. The order of Hymenoptera includes the largest number of insects in the stinging category. Reactions from sting vary from minor skin rashes to severe systemic allergic reactions and sometimes fatal anaphylaxis (Lockey 1974; Valentine 1984; Rhoades et al. 1989). Since the first description of an insect allergy by Benson and Semenov in 1930, the stinging insect allergy has been learned by a specific IgE mediated hypersensitivity to the venom. These days, interest in the ant sting allergies is increasing be-

cause of the many allergic reactions to fire ants (including sometimes fatal anaphylaxis) in the southern part of the United States (James 1976; James et al. 1976; Hensel et al. 1983; Paull 1984; Bahana et al. 1988; Staffore et al. 1989). Another stinging ant that has been reported is the harvester ant *Pogonomyrmex* sp. (Pinna et al. 1977).

In Korea, there has been only one report before this of systemic allergy reaction (Kang et al. 1985) to an ant's sting which was not caused by fire ant or harvester ant. The authors experienced a case of an ant sting allergy from a species of *Ectomomyrmex*, a subfamily of Ponerinae. A 40 year old housewife had had severe systemic reactions to the ant stings for 3 years. We confirmed her reactions to the ant stings (*Ectomomyrmex* spp.) were allergic reactions by a positive allergy skin test of the ant venom and determination of increased amounts of specific IgE in the patient's serum.

CASE SUMMARY

Patient: Female, 40 years old, housewife

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C.C.: Urticaria, dizziness, abdominal cramps, shortness of breath and hypotension immediate after ant stings

Duration: Several attacks per year for 3 years

Family History: No known atopy or asthma

Past Illness: No known allergies

P.I.: For 3 years previous to her visit to our clinic, she had had the anaphylactic episodes 4-5 times in a year after ant stings received while working in her garden. She was given emergency care several times at hospitals near her home due to anaphylaxis. She visited the Allergy Clinic of Severance Hospital for further evaluation on August 1990.

Physical findings: Unspecific abnormalities.

Laboratory test: CBC and liver function test results were normal, total eosinophilic count was $80/\text{mm}^3$, and total IgE was 201.7 IU/mL .

Allergy Skin Test: The prick tests for 50 inhalant allergens (Bencard Co. England) including ant and household insects (Torii Co. Japan) were all negative.

MATERIALS AND METHODS

Ant venom extract

The ants which provoked the allergy reaction were captured in the patient's garden for the making of venom extract. The species of ant was verified as *Ectomomyrmex* spp. of the subfamily Ponerinae by an entomologist (Fig. 1). The posterior parts of the abdomen, containing the venom sacs,

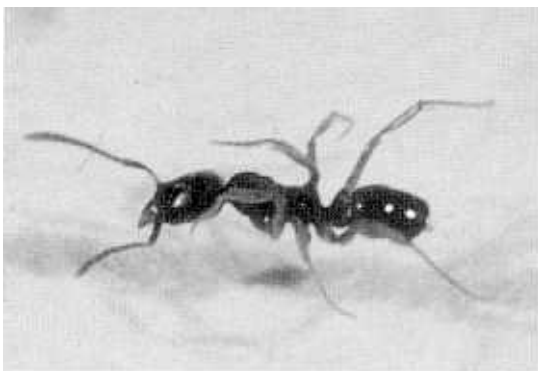


Fig. 1. The picture of *Ectomomyrmex* spp. Note large head, manible, and antennae, thorax with legs, petiole, postpetiole and gaster (the posterior abdominal segment).

were taken from about 200 ants and were homogenized with 1 mL of PBS. This solution was stored in a refrigerator for 3 days and then centrifuged at $20,000 \text{ g}$ for 60 minutes. The supernatant (ant venom extract) was dialysed against PBS (twice: 1000 mL each for 24 hours in a cold room) and stored at 4°C for further studies.

Determination of specific IgE and IgG subclass antibodies of ant venom by ELISA (ant venom ELISA)

Each well of EIA plate (Flat bottom, Costar Corporation, One Alewife Center, Cambridge, MA 02140) was incubated with one μL of the ant venom extract and $100 \mu\text{L}$ of 0.1 M carbonate buffer (pH 9.6) overnight in a cold room (4°C). The plate was washed with PBS-Tween 20 three times and then the wells were blocked with $300 \mu\text{L}$ of 1% BSA-PBS-T for one hour at room temperature. $100 \mu\text{L}$ of sera of the patient and control were incubated for one hour. The sera were diluted to $1:50$ for IgG₁, IgG₂ and IgG₃ but not diluted for IgG₄ and IgE. And the plate was washed with PBS-T three times. $100 \mu\text{L}$ of $1:1,000$ diluted solution of biotin-labelled monoclonal antibodies of IgG subclasses (SIGMA) for measurement of IgG subclasses and of biotin-labelled polyclonal antihuman IgE (Vector Lab) for measurement of IgE were incubated for one hour. After washing of the plate three times, $100 \mu\text{L}$ of $1:1,000$ diluted streptavidin-peroxidase solution (SIGMA, reconstructed in 1 mL distilled water) was incubated for 30 minutes. The plate was washed five times with PBS-T. And $100 \mu\text{L}$ of ABTS solution [55 mg of ABTS in 100 mL of 700 mM citrate phosphate buffer (pH 4.2)] was put into each well. After five minutes the enzyme reaction was stopped by $100 \mu\text{L}$ of 2 mM NaN_3 . The wells of the plate were read the absorbance at 410 nm in an ELISA microtiter reader.

ELISA inhibition test

For evaluation of the specificity of the ant venom ELISA we tested ELISA inhibition. Each aliquot portions ($500 \mu\text{L}$) of the patients's serum were incubated with $50 \mu\text{L}$ of different dilutions of the ant venom extract (*Ectomomyrmex* spp.) and other antigens such as the venom extract of another species of ant (*Formica japonica*), whole body extract of house dust mite (*Dermatophagoides farinae*, 10 mg/mL) and ovalbumin (10 mg/mL) for 1 hour. The patient's serum was inhibited with diluted solutions of the ant venom extract ($1:100$, $1:25$, $1:10$, $1:4$, $1:2$, $1:1$ solution). With inhibited serum sam-

ples the ant venom ELISA for specific IgE and IgG₄ was performed.

RESULTS

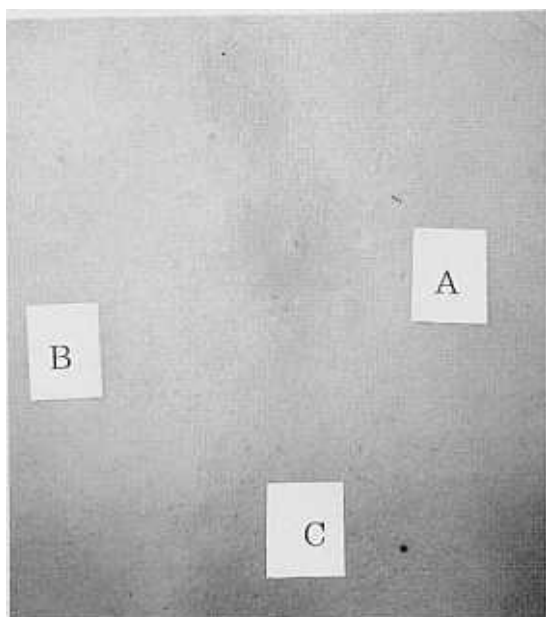


Fig. 2. Skin prick test on patient's back with the ant venom extract showed a strong reaction (A) when compared with the histamine control (C, 1 mg/mL). There was no reaction to the skin prick test with commercial ant extract (B) from Torii Co. (Japan).

To the skin prick test with the ant venom extract from the *Ectomomyrmex* spp. the patient showed strong positive reaction (4+, Fig. 2) but seven subjects without histories of allergic reactions of ant sting were all negative. She also described mild systemic reactions during the prick test with the ant venom extract.

The O.D.s of the ant venom ELISA for specific IgE and IgG₄ with the patient's serum were significantly higher than those in the sera of controls (Table 1). There was a linear dose-response curve on the ant venom ELISA for specific IgE and IgG₄ according to the various dilutions of the patient's serum (Table 2 and Fig. 3).

In the inhibition test, the venom extract made from the *Ectomomyrmex* species showed significant inhibition only on ant venom ELISA for specific IgE and IgG₄ (Table 3). And there was no cross-reactivity of venom extracts between the two different species of the ants, *Ectomomyrmex* spp. and *Formica japonica*.

Ant venom ELISA for specific IgE and IgG₄ showed gradually reduced inhibition with the dilution of the ant venom extract (Table 4 and Fig. 4).

From the above results, we could suggest that the patient's reactions to the ant stings was IgE

Table 1. Determination of specific IgE and IgG subclass antibodies to ant (*Ectomomyrmex* spp.) venom by ELISA

	IgE	IgG ₁	IgG ₂	IgG ₃	IgG ₄
Patient	0.227	0.014	0.014	0.002	0.218
Control mean	0.009	0.008	0.002	0.000	0.003
(n=17) SD	±0.012	±0.003	±0.006	±0.006	±0.005

Table 2. The dilution tests of patient's serum to determine the specific IgE and IgG₄ of ant venom (*Ectomomyrmex* spp.)

	Dilution of patient's serum*				
	1 : 100	1 : 25	1 : 10	1 : 4	1 : 2
IgE	0.021	0.054	0.097	0.180	0.190
IgG ₄	0.009	0.024	0.050	0.140	0.164
					0.273
					0.227

O.D. at 410 nm

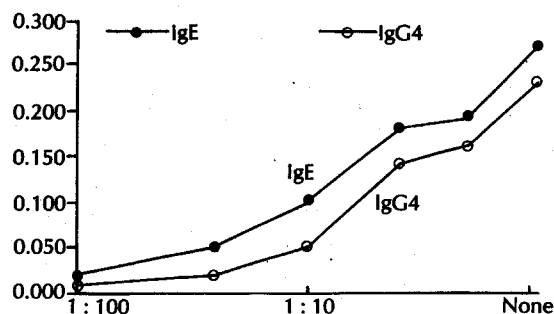


Fig. 3. Dilution test of patient's serum.

% inhibition

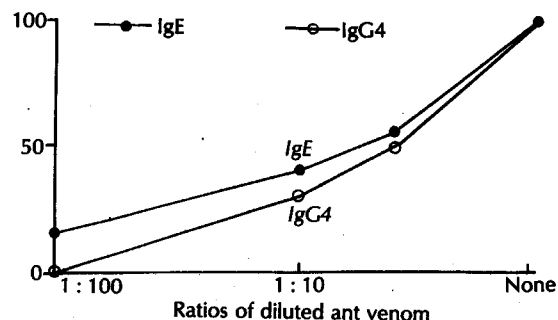


Fig. 4. ELISA inhibition test with diluted ant venom.

Table 3. Inhibition test of ant venom ELISA for specific IgE and IgG₄ with specific and nonspecific antigens

	Ant Venom Extract*			
	Ectomomyrmex	Formica	D. farinae*	Ovalbumin*
IgE	92.2%	18.6%	9.4%	17.3%
IgG ₄	97.7%	-46.6%	-29.9%	13.8%

*inhibition%

Table 4. Inhibition test of ant venom ELISA for specific IgE and IgG₄ with dilution specific ant venom

	Dilution of Ant Venom Extract*				
	None	1:4	1:10	1:100	PBS
IgE	0.008	0.088	0.129	0.173	0.206
IgG ₄	0.001	0.079	0.111	0.155	0.155

*O.D. at 410 nm

mediated anaphylaxis to the ant venom (*Ectomomyrmex* spp.). The role of the specific IgG₄ of the ant venom which was at a high level in this case has not been determined.

DISCUSSION

Ants are found nearly everywhere in the world. There are 5,000-10,000 species of ant, usually classified according to 8-10 subfamilies. Ants in Korea are classified into four subfamilies, 33 genera and

104 species (Choi 1985).

Systemic allergic reactions caused by ant sting have been reported with increased frequency. The so called fire ant is so named because of the typical immediate reactions of burning, itching and pain at the sites of its sting. There are two famous species in fire ants, *Solenopsis invicta* and *Solenopsis richteri*, which entered the Gulf Coast area of the United States from South America approximately 50-70 years ago (James et al. 1976; Rhoades et al. 1975). The incidence of insect sting anaphylaxis has been reported about 0.5-5% in allergic reactions caused by the stings of these ants (Golden et al. 1982; Val-

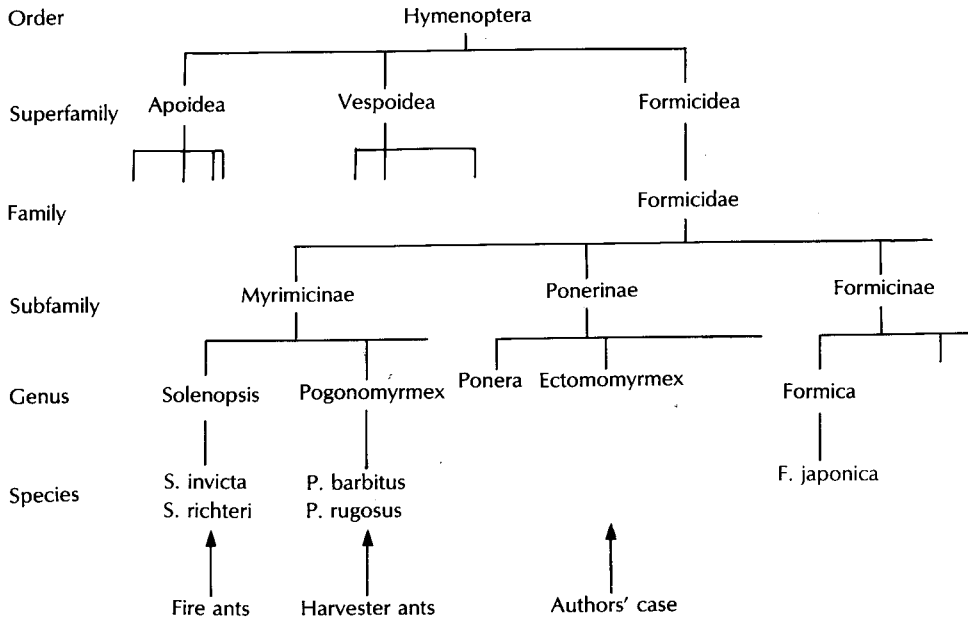


Fig. 5. Taxonomy of ants whose stings sometimes cause allergic reactions.

entine and Lichtenstein 1987). The peak yearly incidences of fire ant allergy are in July according to several reports (Hannan *et al.* 1986; Bahana *et al.* 1988). This may reflect the concomitant seasonal increase of the population in this area, or it is possible that there may be a seasonal increase in allergenic potency of the fire ant venom (Hannan *et al.* 1986). Another ant species, harvester ants (*Pogonomyrmex* spp.), are also implicated in venom hypersensitivity in southern parts of the United States and in Mexico (Pinna *et al.* 1977; Lockey 1974).

The ants which caused anaphylaxis in this patient were identified as *Ectomomyrmex* spp., a subfamily of Ponerinae, which is not rare in Korea (Fig. 5). There have been no reports of severe toxic or allergic reactions to the stings from ant of this kind of species.

For confirmation that the patient's symptoms would be due to IgE mediated reactions, we evaluated allergy skin test with the ant venom extract and tried to detect specific IgE in the serum (Hunt *et al.* 1976; Sobotka *et al.* 1978). When we performed the skin prick test with the ant venom extract, the patient showed strong reactivity (4+). The skin tests of 7 healthy subjects for control were all negative with the ant venom extract. Her skin tests

with other common allergens were all negative. Interestingly, during skin test with the ant venom, the patient had mild systemic symptoms such as abdominal cramps, chest tightness, and dyspnea as well as a local skin rash and urticaria.

Specific IgE and the IgG subclasses to the venom of the ant (*Ectomomyrmex* spp.) which induced the sting allergy in the patient were measured by ELISA (Table 1). For the control, we used sera of 17 patients with the respiratory allergic diseases. The levels of the specific IgE and IgG₄ antibodies to the ant venom in the patient's serum were significantly higher than those in control sera. But the level of other subclasses of IgG (IgG₁, IgG₂, IgG₃) of the ant venom were negligible in the patient's serum and control sera. We also demonstrated the specificity of ant venom IgE and IgG₄ by the ELISA inhibition test with the ant venom and other antigens including ant extract of *Formica japonica*, whole body extract of house dust mites (*Dermatophagoides farinae*), and ovalbumin (Table 3). The ant venom of *Ectomomyrmex* spp. noted maximal inhibition (92% in IgE ELISA and 98% in IgG₄ ELISA). However, an abdominal extract of different species of ant, *Formica japonica*, showed no specific inhibition as 18.6% in IgE and -46.7% in IgG₄; *D. farinae* (50 μ L of

10 mg/mL solution) as 9.4% in IgE and -29.9% in IgG₄; ovalbumin (50 µL of 10 mg/mL solution) as 17.3% in IgE and 13.8% in IgG₄. The ELISA inhibition test with diluted ant venoms showed that higher diluted venom noted higher O.D. (Table 4) or less suppression in ant venom IgE and IgG₄ (Fig. 4). From these results we suggest that this case was IgE mediated anaphylaxis to the ant venom (*Ectomomyrmex* spp.).

We also noted the high level of specific IgG₄ of the ant venom in the serum of patient who did not have immunotherapy. Allergen specific IgG₄ antibodies have been found to increase in atopic dermatitis and asthma (Shakib *et al.* 1977; Gwynn *et al.* 1978, 1982) and anaphylaxis (Shakib and Stanworth 1979). There have been considerable controversies concerning the possible roles of allergen specific IgG₄ antibodies in allergic diseases such as the mediator releasing effect (Fagan *et al.* 1982), the development of food allergies (Bjorsten *et al.* 1983), the blocking effects of anaphylaxis (Van der Giessen *et al.* 1975; Cheung *et al.* 1983; Nakagawa 1986) and feedback regulation of IgE synthesis (Cheung *et al.* 1983). These IgG₄ antibody responses are also associated with chronic antigenic stimulation (Aalberse *et al.* 1983). The role of IgG₄ as allergen-blocking antibody is currently under further investigation. Considering the above possible reasons of high levels of IgG₄ to the ant venom in our patient could include: one, intermittent stimuli of ant stings with low doses of venom which didn't produced high enough levels of IgG₄ to prevent as a blocking antibody her anaphylaxis, and two the possible role of IgG₄ as well as IgE in provoking anaphylaxis. However the exact roles of IgG₄ remain to be studied.

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