

Prevalence of insect allergen-specific IgE in allergic asthmatic children in Cincinnati, Ohio

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Airborne insect antigens can represent significant outdoor aeroallergens. In this pilot study, allergic asthmatic children were screened by RAST for IgE directed against common outdoor insects. Insects studied included ant, cricket, grasshopper, housefly, and moth. Spiders were also tested. Three different extraction procedures were compared to determine which method(s) best preserves the allergenic proteins. RAST discs were made for each insect extract and sera from allergic, asthmatic and nonallergic, nonasthmatic children were tested. The allergic asthmatic sera had a higher percentage of isotope binding than the nonallergic nonasthmatic sera for all insects tested. The number of subjects with a Phadebas RAST score of 2 or greater was significantly higher in the allergic asthmatic group for moth (16 of 37, 43%), housefly (7 of 30, 23%), cricket (8 of 41, 19%), and grasshopper (7 of 36, 19%). Only one of the 25 control sera had a score of 2 to moth; none of the control sera reacted to any of the other insects. Most positive sera tested showed cross-reactivity between insects. A few sera, despite showing positive RAST results to two different extracts, showed no cross-reactivity between the two extracts, suggesting that these sera contained species-specific IgE.

These results indicate that a significant proportion of allergic asthmatic children have IgE that binds to extracts of moth, cricket, grasshopper, and housefly. Further studies are needed to determine whether these insect aeroallergens can produce clinically significant symptoms in sensitized atopic individuals.

INTRODUCTION

The substances generally recognized as important inhalant allergens include pollens, mold spores, dust mite, and animal proteins. Insects are a large and ubiquitous source of protein antigens that may be an unrecognized cause of allergic disease. Airborne insect-derived particles, such as shed hairs, scales, excreta, and bits of disintegrated insect bodies, have been implicated as a cause of respiratory allergy. The insects that have been studied most extensively include caddis fly,¹⁻⁴ May fly,⁵⁻⁷ moth and butterfly,⁸⁻¹³ chironomid midges,¹⁴⁻¹⁹ and cockroaches.²⁰⁻²⁴ Various other insects (eg, mushroom flies, aphids, bedbugs, locusts, bees, house flies, beetles, moth flies, blowflies, and silverfish) have also been reported to cause sensitization.^{7,25-31}

While certain allergic individuals are exposed to high concentrations

of cockroach and other indoor insect allergens, exposure to outdoor insect-derived particles is universal, similar to pollen and mold spore exposure. In this pilot study, asthmatic children were screened by RAST for IgE-mediated sensitivity to several outdoor insects prevalent in the midwestern United States. The goal of the study was to target potentially allergenic insects for future in-depth evaluation.

Several different extraction methods for insects have been described in the literature.^{9,32,33} In this study, three of these methods, and a commercially available extract, were compared to determine which method(s) best preserve the allergenic proteins.

METHODS

Specimen Collection

The insects studied included mixed species of moth (Lepidoptera species), grasshopper (Acrididae), cricket (Gryllidae), house fly (*Musca domestica*), and ant (Formicidae species). Spiders, like insects, are members of the phylum Arthropoda but fall into the class Arachnida rather than Hexapoda.

Spiders were also studied. Specimens were collected by hand, by cordless vacuum, or by light trap, and immediately frozen at -20 °C. Within 24 hours the insects were transferred to a -70 °C-freezer where they were stored until the extraction procedure was performed.

Extraction Procedure

Three extraction methods were tested (Table 1). The lyophilized extracts were stored at -20 °C. The commercially available whole body extract of moth (Hollister-Stier) was also obtained.

Subjects

Two groups of subjects were studied: an "allergic asthmatic" group and a "nonallergic nonasthmatic" group. The allergic asthmatic group was composed of children older than 1 year of age, who presented with symptoms of cough, dyspnea, and/or wheezing responsive to bronchodilators and antiinflammatory medications. All of the allergic asthmatic subjects had typical symptoms of allergy: seasonal or exposure-related flares of symptoms, and associated rhinoconjunctivitis and/or eczema. Sera from 50

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Table 1. Extraction Methods

Method A. (7,25)

1. The frozen whole insects are coarsely ground with mortar and pestle.
2. The ground insect material is defatted with ether, then dessicated and finely ground.
3. The dessicated powder is mixed with sterile buffered saline in a 1:10 wt/vol ratio; this solution is kept at 4 °C for three days, with intermittent agitation.
4. The solution is centrifuged for 15 minutes at 12,000 rpm and the supernatant removed.
5. The supernatant is dialyzed in cellulose tubing (Spectrapore 3; Spectrum Medical Industries, Inc., Los Angeles, CA) against sterile distilled water at 4 °C, for 4 days with two changes of water per day.
6. The dialysate is filter sterilized using Millex-GV 0.22- μ m filter units (Millipore Corp, Bedford MA) and then lyophilized using a Thermovac (FDC-8) lyophilizer.

Method B. (24)

Steps 1, 2, and 3 are followed as in Method A.

4. The crude aqueous extract is passed through a Buchner filter.
5. Four parts of cold acetone are added to the extract. This precipitates the protein.
6. The material is centrifuged at 12,000 rpm for 15 minutes. The supernatant is discarded.
7. The residue is air dried, then dissolved in buffered saline in a 1:10 wt/vol ratio.
8. Dialysis, filter sterilization, and lyophilization are performed as in Method A.

Method C. (25)

Steps 1 and 2 are followed as in Method A.

3. Approximately 1 g of ground insect particles are placed in a Ten Broeck tissue grinder with 10 mL of cold sterile buffered saline.
4. The insect material is ground for six 1-minute intervals.
5. The suspension is centrifuged for 15 minutes at 12,000 rpm.
6. The supernatant is filter sterilized/lyophilized as in Methods A and B.

allergic asthmatic patients were tested. Of these, 26 patients had previously had positive skin tests to dust mite (73%), ragweed pollen (85%), grass pollen (65%), weed pollen (65%), tree pollen (62%), molds (62%), and/or animal allergens (19%). The remaining 24 subjects had not had allergy skin testing.

Excess sera obtained from children admitted to Children's Hospital for surgical treatment, and having no history of allergy, asthma, or other chronic disease, were included in the "nonallergic, nonasthmatic" group.

Evaluation of Extraction Methods and Extract Concentrations

The first set of allergic asthmatic sera was assayed using moth extracts prepared by all three extraction procedures and the commercial extract. Method C and commercial extracts were tested in various concentrations to determine the optimal concentration for coating the RAST disc. The ant RASTs and the majority of the cricket and grasshopper RASTs were performed using ex-

tracts A, B, and C in concentrations of 10 mg/mL. The remainder of the moth, cricket and grasshopper RASTs, and all of the housefly and spider RASTs, were performed using extraction method C and a concentration of 10 mg/mL. Because of limitations in the volume of serum available, not all allergic asthmatic sera were tested to all species of insect. A total of 42 allergic asthmatic sera were tested to spider; 41 to cricket; 37 to moth; 36 to grasshopper, 31 to ant and 30 to housefly. All the nonallergic nonasthmatic sera were tested using extraction method C and a concentration of 10 mg/mL. Twenty-five nonallergic nonasthmatic sera were tested with moth, cricket, grasshopper, and spider; only 19 were tested with ant and housefly.

RAST Testing

In preparation for RAST, 5-mm paper discs were coated with each insect extract after priming with cyanogen bromide. Each disc was incubated with 50 μ L of serum for three hours at room temperature;

the discs were then washed with RAST buffer three times. Fifty microliters of ^{125} I-labeled rabbit anti-human IgE was added to each disc and incubated at room temperature for 16 hours. After washing three times with RAST buffer, the samples were tested for bound radioactivity using a gamma counter (Micromedex model 28026). All assays were performed in duplicate. Two negative controls, using RAST buffer rather than serum, were included in each assay. Two 50- μ L aliquots of the anti-IgE solution were also included in each gamma counter run to determine the total activity in counts per minute (cpm) available for each assay. Pharmacia reference discs were also included in each run. The reference discs are coated with a reference allergen (birch). Reference serum A is prepared from a standardized human serum pool with a high content of anti-birch IgE. Sera B, C, and D are standardized dilutions (1:5, 1:25, and 1:50) of serum A.

Data Analysis

The data were evaluated by two methods. The first method is percentage of radioisotope binding. The radioactivity bound to each RAST disc was measured; the cpm of the negative control discs, representing nonspecific binding, was subtracted from the cpm of each specimen. The remaining cpm was expressed as the percentage of the total activity available. The mean percentage of bound radioisotope was compared between the allergic asthmatic and nonallergic nonasthmatic groups for each insect species by two-tailed Wilcoxon two-sample rank test. It was necessary to use this nonparametric analysis because of the large differences in standard deviation between the two groups. The second method is number of subjects with Phadebas RAST score of 2 or greater. RAST scores were assigned based on the Pharmacia reference discs. A RAST disc with activity lower than that of reference disc D was assigned a score of 0;

activity between D and C received a score of 1; between C and B, the score was 2; between B and A the score was 3; and activity greater than that of reference disc A was scored as 4. The number of subjects in the allergic asthmatic versus the nonallergic nonasthmatic group having RAST scores of 2 or greater to each insect species was compared by Fisher's exact test.

Cross Reactivity Studies

Cross-reactivity between insect allergens was evaluated by RAST inhibition, as follows: sera that showed positive RAST results to two different insects were tested. A 50- μ L aliquot of serum was preincubated with a RAST disc coated with extract of insect A. After 24 hours, the disc was removed, a RAST disc coated with extract of insect B was added, and a standard RAST assay was performed. Control sera were RAST tested to insect B after preincubation with a blank disc. The amount of anti-insect B IgE removed from the serum by preincubation with insect A was expressed as the percent decrease in isotope binding, compared with the control: $C - A/C \times 100$, where C is cpm measured for the control sera, and A is cpm measured for the sera preincubated with insect A. Each RAST inhibition assay was performed in quadruplicate. Sera with positive RAST results to both moth and cockroach or dust mite were tested for RAST inhibition by these antigens, using commercial extracts (Hollister-Stier) of cockroach and dust mite. RAST inhibition of 50% or more was considered to represent significant cross-reactivity. To evaluate the validity of the method, RAST inhibition was also performed using cat, milk, and egg allergens as negative controls.

RESULTS

Comparison of extraction methods A, B, and C revealed that for moths, method C extracts had the highest IgE-binding capacity (Table 2). For ants, crickets, and grasshoppers,

Table 2. Allergenic Potency of Extracts Produced by Methods A, B, and C and a Commercial Extract (HS)

Extraction Method and Concentration*	Mean cps†/Mean % Binding			Grasshopper (n = 24)
	Moth (n = 27)	Ant (n = 31)	Cricket (n = 18)	
A; 10 mg/mL	2731/5.8	974/2.1	1150/3.3	1656/5.9
B; 10 mg/mL	2618/5.6	699/1.5	934/2.7	1605/5.7
C; 10 mg/mL	4201/8.9	1115/2.4	1367/3.9	1632/5.8
C; 50 mg/mL	4193/8.9			
C; 100 mg/mL	4341/9.2			
HS; 1 mg/mL	1413/3.0			
HS; 10 mg/mL	3523/7.5			
HS; 100 mg/mL	3653/7.8			

* Concentration of extract used to coat the RAST discs.

† Mean cps = mean counts per second measured after RAST testing each group of sera with the extract indicated. Mean % binding refers to the percentage of the total gamma radiation in 50 μ L of isotope that bound to each set of RAST discs.

methods A and C were roughly equivalent. A few individual sera, however, reacted more strongly with the method A or B extracts of each insect studied. No significant increase in IgE binding was found when the concentration of moth extract used to coat the RAST discs was increased above 10 mg/mL.

The commercial moth extract was comparable in allergenic potency to the method C extract; again, the 10-mg/mL solution appeared to coat the RAST disc maximally.

The age range in the allergic asthmatic group was 2.5 years to 17.2 years with a mean age of 10.5 years. The ages in the nonallergic nonasthmatic group ranged from 1.5 to 18.0 years with a mean age of 8.5 years.

The mean percentage of radioisotope binding was significantly higher in the allergic asthmatic group than in the nonallergic nonasthmatic group for all insect species studied (Fig 1). The number of subjects with Phadebas RAST score of 2 or greater was significantly higher in the allergic asthmatic group for moth, cricket, grasshopper, and housefly (Table 3).

Results of cross-reactivity studies are shown in Table 4. Significant cross-reactivity was found among the insects studied; however, two of

the 30 sera that were RAST-positive to two insects showed no cross-reactivity between the two.

In the few sera tested, there was a wide range of cross-reactivity between moth and dust mite. As expected, cat and food antigens showed little cross-reactivity with the insect allergens.

DISCUSSION

In order to determine whether outdoor insects are significant respiratory allergens, three criteria must be met: (1) a significant proportion of the allergic population must be shown to have IgE directed against the insect antigens. (2) The insect antigens must be shown to be present in the outdoor air in sufficient quantity to cause sensitization of susceptible individuals (ie, concentrations comparable to those of known allergens such as pollens). (3) The insect antigens must be shown to produce symptoms of allergic rhinitis and/or asthma upon inhalation challenge.

This study demonstrates that a significant proportion of allergic asthmatic children have IgE that binds to moth, cricket, grasshopper, housefly, and ant antigens. There is some cross-reactivity among these insect extracts, implying that there are shared insect allergens. The find-

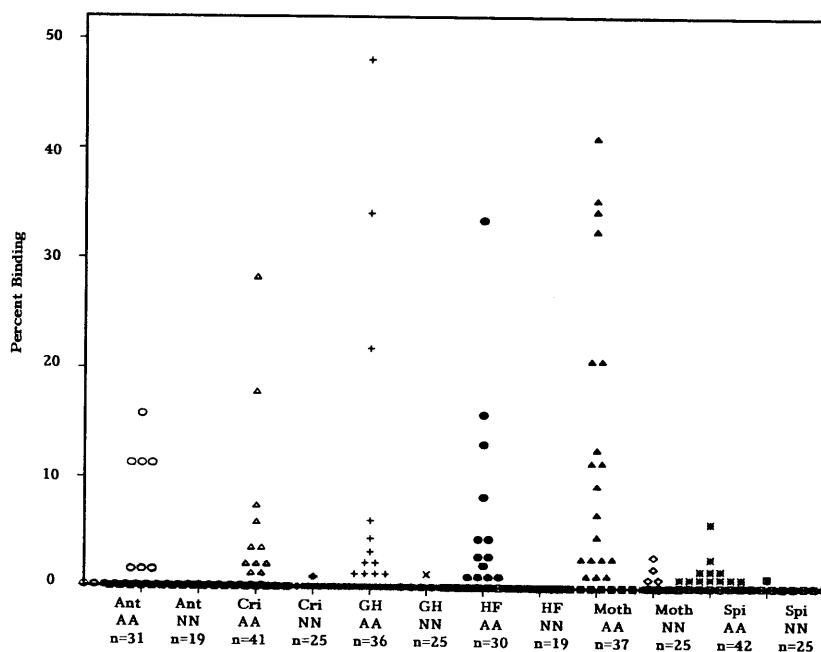


Figure 1. Percentage of radioisotope bound by each RAST assay was calculated as follows: counts per minute (CPM) of test disc/total cpm available $\times 100$, where total cpm available is the count obtained by placing 50 μ L of the isotope solution in the gamma counter.

AA = allergic asthmatic sera; NN = nonallergic nonasthmatic sera; Cri = cricket; GH = grasshopper; HF = housefly; Spi = spider.

Probability levels yielded by two-tailed Mann-Whitney U test, comparing AA with NN group for each insect species: ant and housefly: $P < .0001$, grasshopper and spider: $P < .005$, moth: $P < .01$, and cricket: $P < 0.05$.

Table 3. Percentage Bound Radioisotope Found by RAST to Each Insect

	Allergic Asthmatic			Nonallergic Nonasthmatic			P^*
	n	Median/Mean % Binding	Range	n	Median/Mean % Binding	Range	
Ant	31	0.40/2.06	0-15.8	19	0.00/0.04	0-0.4	$<.0001$
Cricket	41	0.30/2.05	0-28.2	25	0.20/0.21	0-0.2	$<.05$
Grasshopper	36	0.30/3.67	0-48.1	25	0.00/0.12	0-1.2	$<.005$
Housefly	30	0.55/3.30	0-33.5	19	0.10/0.09	0-0.5	$<.0001$
Moth	37	1.10/6.58	0-35.3	25	0.10/0.39	0-2.9	$<.01$
Spider	42	0.20/0.63	0-5.9	25	0.10/0.10	0-0.9	$<.005$

* Probability levels yielded by two-tailed Mann-Whitney U test.

ing that some of the individual sera showed no cross-reactivity between insect extracts indicates that these sera reacted with different allergens peculiar to each species. In previous studies, several insect allergens have been identified. Baur et al,¹⁵ studying chironomid midges prevalent in the Sudan, found that the major

allergens reside in the hemoglobin molecule and that these allergens are cross-reactive among several midge species. Similar allergens have also been reported in a midge species found in Wisconsin.¹⁸ Koshte et al³⁴ identified a prominent caddis fly allergen that appeared to be located on the hemo-

globin molecule and cross-reacted with a homologous protein in extracts of mussel, oyster, shrimp, crab, honeybee, and yellow jacket venom. Cockroach allergens have also been studied^{24,35-40}; several different allergens have been identified, some of which are species-specific and some of which are shared among several species of cockroach. Species-specific allergens have also been identified for house fly, blow fly, moth, grain borer, and silverfish.²⁹

Evaluation of the role of insects as sources of outdoor and indoor aeroallergens has been hampered by the inability to identify visually insect-derived debris on standard air sampling media. Quantitation of airborne insect antigens and their correlation with symptom patterns of insect-sensitized patients has been difficult. In recent years, however, the development of immunochemical assays has allowed reasonable estimation of the concentration of insect antigens in outdoor and indoor air. Kino et al¹¹ used a volumetric air sampler and a RAST inhibition assay to measure the concentration of moth, butterfly, and caddis fly antigens in the outdoor air in Japan. They found concentrations of insect allergens of $<10 \mu$ diameter to peak at 2 to 5 ng/m^3 during the period of May to June, and noted a second peak of 3 to 18 ng/m^3 during September to November. These concentrations are in the same range as those reported by Agarwal et al⁴¹ for ragweed antigen E (1 to 4 ng/m^3) and Alternaria Alt-1 (1 to 8 ng/m^3), using a similar technique. Wynn and colleagues utilized RIA inhibition to quantify outdoor moth antigens in Minnesota.¹² They detected peaks of moth allergen concentration during June through October ranging from 2 to 22 ng/m^3 . Significant concentrations of insect allergens have also been measured in indoor air⁴² and in dairy barns⁴³ using immunochemical assays.

The role of insects as inhalant

Table 4. Crossreactivity of Insect Extracts with Other Extracts

Extract A	Extract B	No. of Sera Tested	Mean % RI	Range of RI, %
Grasshopper	Moth	9	23%	0-40
Moth	Grasshopper	9	59%	42-72
Moth	Fly	6	59%	47-66
Cricket	Grasshopper	6	55%	0-93
Dust Mite	Moth	7	30%	0-81
Cockroach	Moth	4	65%	52-84
Cat	Moth	4	11%	0-22
Milk	Moth	4	11%	8-15
Egg	Moth	4	0%	0

F = Rast inhibition calculated as $C - A/C \times 100$ (A = counts per minute of the RAST to insect E after the serum was preincubated with a RAST disc coated with extract A; C = counts per minute of the RAST to insect B, after the serum was preincubated with a blank disc). RAST inhibition of 50% or more was considered to represent significant cross-reactivity.

allergens is further supported by data showing positive bronchial or nasal challenges with various insects. Several studies have documented positive inhalation challenge results with cockroach.^{22,23,44,45} Gupta et al found that 61.5% of asthmatic subjects with positive skin tests to moth, beetle, cockroach or mosquito whole body extracts had positive bronchial challenges with these insects.⁴⁶ Fruit fly extract has also resulted in positive nasobronchial challenges in six of seven laboratory workers with respiratory allergic symptoms associated with fruit fly exposure.⁴⁷

Increasing evidence indicates that insects are potentially a significant source of both indoor and outdoor inhalant allergens. Further study is needed to determine which outdoor insects are significant causes of respiratory allergic disease. The data presented here suggest that moth, cricket, grasshopper, housefly, and cat antigens may be important allergens; their possible role as aeroallergens requires further study.

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7. Owner (If owned by a corporation, its name and address must be stated and also immediately thereunder the names and addresses of stockholders owning or holding 1 percent or more of total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given. If owned by a partnership or other unincorporated firm, its name and address, as well as that of each individual must be given. If the publication is published by a nonprofit organization, its name and address must be stated.) (Item must be completed.)				
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8. Known Bondholders, Mortgagees, and Other Security Holders Owning or Holding 1 Percent or More of Total Amount of Bonds, Mortgages or Other Securities (If there are none, so state)				
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NONE				
9. For Completion by Nonprofit Organizations Authorized to Mail at Special Rates (DMM Section 424.12 only) The purpose, function, and nonprofit status of this organization and the exempt status for Federal income tax purposes (Check one) (1) ☒ Has Not Changed During Preceding 12 Months (2) ☐ Has Changed During Preceding 12 Months (If changed, publisher must submit explanation of change with this statement.)				
10. Extent and Nature of Circulation (See instructions on reverse side)		Average No. Copies Each Issue During Preceding 12 Months		Actual No. Copies of Single Issue Published Nearest to Filing Date
A. Total No. Copies (Net Press Run)		6,000		5,889
B. Paid and/or Requested Circulation 1. Sales through dealers and carriers, street vendors and counter sales				
2. Mail Subscription (Paid and/or requested)		5,430		5,369
C. Total Paid and/or Requested Circulation (Sum of 10B1 and 10B2)		5,430		5,369
D. Free Distribution by Mail, Carrier or Other Means Samples, Complimentary, and Other Free Copies		200		150
E. Total Distribution (Sum of C and D)		5,630		5,519
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G. TOTAL (Sum of E, F1 and 2—should equal net press run shown in A)		6,000		5,889
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(See instructions on reverse)