

Cockroach sensitization in laboratory workers

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Six laboratory workers who were exposed to American cockroaches (AC) and German cockroaches (GC) while they were performing immunologic experiments were evaluated for cockroach hypersensitivity. Prick skin testing and RAST were performed with whole body extracts (1:20 wt/vol) of AC, brown-banded (BB), and GC species as well as hemolymph and fecal (F) extracts of AC. Three of six workers reported work-related nasal and ocular symptoms associated with xenografting and bleeding of cockroaches. All three symptomatic workers exhibited cutaneous reactivity to at least one cockroach antigen. Elevated RAST binding was observed in one of the three symptomatic workers. A nasal provocation to AC was positive in the most symptomatic worker at a provocative dose of 3.2×10^{-4} mg causing a 50% decrease of nasal flow rate from baseline. After pretreatment with nasal cromolyn, the provocative dose causing a 50% decrease from baseline increased to 2.6×10^{-4} mg. Nasal provocation with the same concentrations of AC were negative in two skin test negative subjects. RAST-inhibition studies demonstrated cross inhibition of the serum-specific IgE binding to AC-hemolymph by AC, GC, and BB whole body extracts. However, specific IgE binding to AC-F was inhibited by AC-F and AC but not by GC or BB whole body extracts, suggesting there was greater specificity of the F allergens. This study demonstrated that cockroach allergens elicit IgE-dependent upper respiratory sensitization in the workplace. (*J ALLERGY CLIN IMMUNOL* 1987;80:586-90.)

Cockroach emanations have been recognized as important inhalant allergens.¹ The most common cockroach species in North America are the GC (*Blattella germanica*) and the AC (*Periplaneta americana*). In 1964, Bernton and Brown² demonstrated direct cutaneous sensitivity and positive Prausnitz-Küstner responses to cockroach allergens. Subsequent studies have also documented respiratory sensitization to cockroach allergens.³⁻¹⁰ Kang and Sulit¹⁰ noted a high prevalence of cutaneous reactivity to cockroach allergens among urban patients with asthma. The same investigators subsequently documented the presence of both immediate- and dual-phase bronchoconstrictive responses in patients with extrinsic asthma after bronchial challenge with cockroach allergen.^{3, 5}

This article presents clinical and immunologic stud-

Abbreviations used

- AC: American cockroaches
- H: Hemolymph
- BB: Brown-banded cockroaches
- GC: German cockroaches
- F: Fecal
- PD₅₀: Provocative dose of allergen required to cause a decrease of 50% of nasal flow rate from baseline
- Tm: *Tenebrio molitor*

ies on cockroach-laboratory workers, some of whom developed nasal and ocular symptoms associated with work-related exposure to cockroaches and IgE-mediated sensitization to cockroach allergens.

METHODS

Clinical evaluation

The study group consisted of six laboratory workers from the Department of Biological Sciences at the University of Cincinnati who were performing experimental studies on cockroaches and represented all the workers in the laboratory who were regularly exposed to AC and GC. The workers reported no other significant exposure to cockroaches outside the workplace or at home. Exposure to fecal material

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occurred in those workers who also participated in feeding and maintenance of cockroach colonies located in a separate storage room. There was further exposure to AC-H when animals were exsanguinated for in vitro cellular and humoral studies. In addition, xeno- and allografting experiments were performed with BB and GC. Because this laboratory was primarily involved with the investigation of cockroaches, none of the six workers had an opportunity for exposure to other laboratory animals. An informed consent was obtained from all subjects.

An occupational history was obtained on all six workers by physicians. The duration of laboratory exposure and latency period before onset of symptoms was recorded. Information was elicited regarding possible work-related ocular, nasal, and bronchial symptoms. A complete personal and family atopic history was also obtained.

Immunologic studies

Cutaneous prick testing was performed in all six exposed and eight laboratory workers not exposed to cockroaches. Pure culture extracts of AC, GC, and BB whole bodies were prepared by Dr. Larry Arlian, Wright State University, Dayton, Ohio. Whole insect bodies were dried in a dessicator with anhydrous calcium sulfate for 48 hours and then extracted in phosphate-buffered saline, followed by sterilization through a Millipore filter (Millipore Corp., Bedford, Mass.).¹⁶ All skin test reagents were prepared at a final concentration of 1:20 wt/vol. H and F extracts were prepared in a similar manner. Protein content was determined by the Bio-Rad (Bio-Rad Laboratories, New York, N. Y.) method with bovine gamma globulin as a reference protein. Prick testing was also performed with commercial extracts of dust mite, ragweed, grass, and cat allergens (Hollister-Stier Laboratories, Spokane, Wash.) to define atopic status. A prick test wheal that measured 3 mm greater than the saline control wheal accompanied by flare was considered a positive skin test.

Specific IgE to cockroach allergens was measured by a modified RAST assay.¹¹ RAST results were expressed as percent binding calculated by dividing counts per minute bound by the worker's serum by counts per minute of total ¹²⁵I-labeled anti-IgE added multiplied by 100.

RAST-inhibition studies were performed with serum that exhibited the highest RAST binding to cockroach.¹² Equal volumes of test serum were incubated at 37° C for 2 hours with successive tenfold dilutions of each cockroach extract and centrifuged at 1500 × g for 15 minutes. The upper two thirds of the supernatant was removed and incubated with AC-H coated and AC-F coated methylcellulose disks followed by the addition of ¹²⁵I-labeled anti-IgE. The percent of RAST inhibition was calculated as follows:

$$\left[1 - \frac{\text{cpm bound by preabsorbed sample}}{\text{cpm bound by nonabsorbed sample}} \right] \times 100$$

Nasal-challenge study

Dose-response nasal-provocation studies were performed with anterior rhinometry. A saline control challenge was performed before allergen challenge. The beginning dose

TABLE I. Results of symptoms, skin tests, and RAST in cockroach-exposed and nonexposed laboratory workers

Worker No.	Symptoms	Skin test	RAST
1	Nasal, ocular, and palatal	Pos	Pos
2	Nasal, ocular	Pos	Neg
3	Nasal, ocular	Pos*	NA
4	None	Pos*	Pos
5	None	Neg	Neg
6	None	Neg*	NA
Control subjects (N = 8)	None	Neg	Neg

NA - not available because subjects refused venipuncture; pos = positive; neg = negative.

*Cutaneous reactivity to inhalant aeroallergens.

was delivered by nasal instillation of 0.05 ml of solution containing 5×10^{-3} mg based on weight per volume of the AC whole body extract. Nasal flow rate was measured before and after challenge with a Connell (Nasal Diseases Study Center, Englewood, N. J.) anterior rhinometer and expressed in milliliters per second at a constant inspiratory pressure of -1.5 cm H₂O. The same nostril was instilled every 5 minutes with increasing tenfold concentrations of AC until a positive response or a final concentration of 1.0 mg/ml wt/vol was achieved. The best flow rate of three inspiratory maneuvers was recorded, and a fall in nasal patency of 50% from baseline saline control was considered a positive challenge. The cumulative PD₅₀ was determined by extrapolation from a dose-response curve. Nasal challenges were also performed on two control subjects with no cutaneous reactivity to cockroach allergens.

RESULTS

Clinical evaluation

Three of the six laboratory workers described the immediate onset of nasal stuffiness, runny nose, and sneezing accompanied by ocular symptoms and itching associated with cockroach exposure (Table I). These symptoms primarily occurred during bleeding and xenografting experiments. None of the workers described wheezing, chest tightness, cough, or shortness of breath. Of the three symptomatic workers, only one (No. 3) gave a prior atopic history consistent with ragweed and grass pollenosis and exhibited cutaneous reactivity to at least one inhalant aeroallergen. The mean latency period before onset of work-related symptoms was 6 months. The history of the most symptomatic worker (No. 1) is presented below.

C. M., a 24-year-old white female research assistant, had worked in the laboratory with cockroaches for 1½ years. Approximately 1 month after beginning work, she noted the

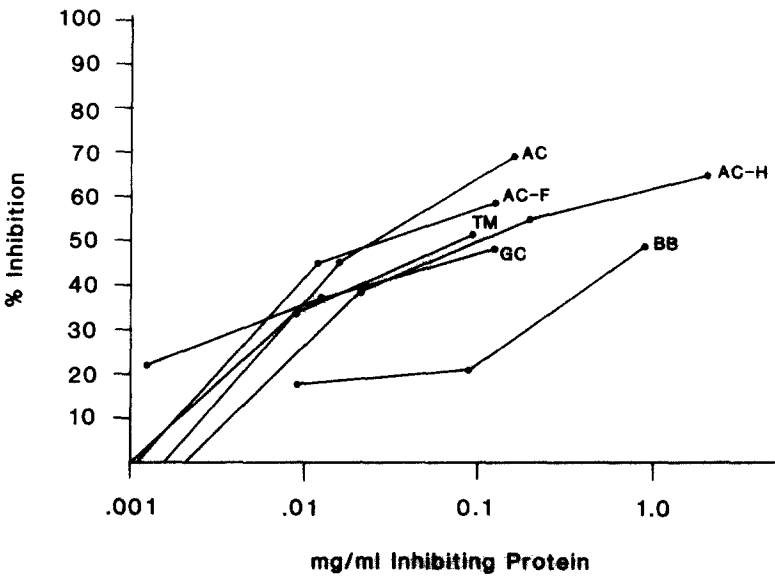


FIG. 1. RAST inhibition studies (worker No. 4) of specific IgE binding to AC-H disks. Inhibition was achieved by AC whole body and AC-H as well as whole body extracts of GC, BB cockroach, and a beetle species, Tm.

TABLE II. RAST binding results to various cockroach allergens in two workers and eight nonexposed workers

Worker No.	RAST (% binding)				
	AC whole body	AC-H	AC-F	GC whole body	BB whole body
1	6.0*	2.8	5.1*	2.6	2.5
4	5.6*	8.6*	10.1*	6.2*	1.7
Control subjects (N = 8)	2.5 ± 0.35	2.1 ± 0.40	2.4 ± 0.60	2.4 ± 0.60	2.4 ± 0.40

*Significant RAST binding is 3 SD above the $\bar{X}$ of nonexposed control subjects; results presented in $\pm$ SEM.

immediate onset of ocular itching, nasal stuffiness, sneezing, and palatal itching while she was feeding and transporting cockroaches. These symptoms were also noted in the laboratory while she was performing experiments on animals and resolved completely later at home, on weekends, and on vacation. Subsequent pretreatment with intranasal cromolyn sodium attenuated her work-related nasal and ocular symptoms.

Immunologic studies

There were four workers with cutaneous reactivity to cockroach antigens. All three symptomatic workers exhibited positive skin tests to one or more of the cockroach reagents (Table I). Although worker No. 4 was asymptomatic, he exhibited cutaneous reactivity to four of five cockroach allergens as well as common inhalant aeroallergens. One of three symptomatic ver-

sus two of three asymptomatic workers had positive skin tests to common inhalant aeroallergens consistent with their personal atopic histories.

Two or more cutaneous responses to the panel of cockroach allergens were observed in the four cutaneous reactors. Worker No. 1 exhibited positive skin tests to all cockroach allergens. Prick tests to cockroach allergens were negative in eight laboratory workers not exposed to cockroaches.

Two of four individuals with cutaneous reactivity to cockroach exhibited significant RAST binding (Table II) to cockroach antigens defined as a value >3 SD above the mean of eight laboratory control subjects. As presented in Table II, worker No. 1 exhibited elevated RAST binding to AC (6.0%) and to AC-F (5.1%). Worker No. 4, who was asymptomatic, exhibited significant RAST binding to four of five

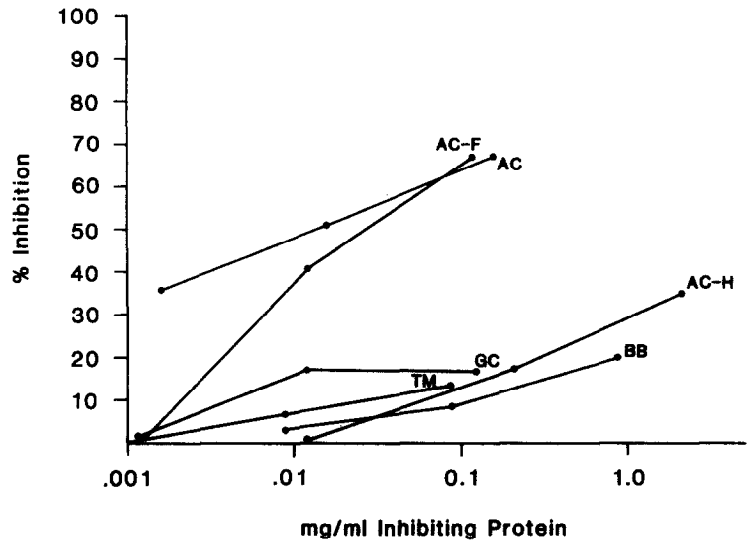


FIG. 2. RAST inhibition studies (worker No. 4) of specific IgE binding to AC-F disks. Significant inhibition was achieved by AC whole body and AC-F but not by whole body extracts of GC, BB cockroach, and a beetle species, Tm.

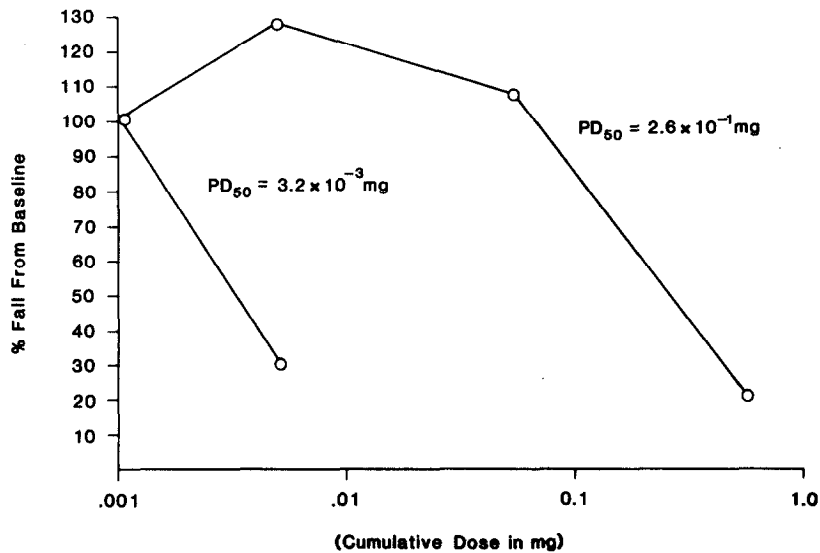


Fig. 3. Nasal provocation challenge to AC whole body extract in worker No. 1. Graph shows the PD₅₀ expressed as the cumulative dose of antigen delivered to achieve a 50% reduction in nasal airflow and the percent fall of nasal airflow from prechallenge baseline before treatment (●—●) and after treatment (○—○) with cromolyn.

cockroach allergens (AC, AC-H, AC-F, and GC). These responses in worker No. 4 could represent immunologic reactivity before onset of clinical sensitivity.

RAST inhibition was performed on the serum of worker No. 4 with AC-F- and AC-H-coated allergen disks. As illustrated in Fig. 1, inhibition of RAST binding to H was achieved by all three cockroach-body extracts (AC, GC, and BB) as well as AC-F and

AC-H allergens. Inhibition was also obtained with a larval extract from the beetle Tm which belongs to a different insect order. Fig. 2 illustrates inhibition experiments on the same serum with AC-F-coated disks. There was no significant inhibition by the body extracts of GC, BB, or Tm. By contrast, there was good inhibition of RAST binding to F allergen by the homologous AC-F allergens and AC whole body but significantly less by AC-H.

Nasal challenge

Nasal provocation with AC body extract was performed on worker No. 1 (C. M.) (Fig. 3). After instillation of AC extract, nasal patency fell 69.2% from baseline, at which time the subject noted palatal itching and sneezing. The PD_{50} was 3.2×10^{-3} mg on the initial challenge. Two weeks later, the challenge was repeated after pretreatment with intranasal cromolyn. The PD_{50} increased to 2.6×10^{-1} mg. Thus, cromolyn inhibited the nasal response to cockroach allergen. There was no significant variation from baseline after nasal provocation in two nonexposed subjects who lacked cutaneous reactivity to cockroach allergens.

DISCUSSION

Previously, Bernton and Brown² reported occupational sensitization to cockroaches among entomologists and a laboratory worker. We have demonstrated work-related allergic rhinitis among invertebrate immunologists who were regularly exposed to cockroaches.

Richman et al.⁸ reported that whole body, cast skin, egg casing, and fecal materials of the GC were allergically distinct by RAST-inhibition analysis. In this study, the least potent of these allergens was fecal material with whole bodies and cast skins sharing relatively higher potencies. The same investigator found little cross-reactivity between the AC and GC extracts. In the present study, we have demonstrated that AC-H possess a broad range of cross-reactivity not only with AC-F allergens but with a whole range of whole body allergens of different cockroach species. In addition, cross-reactivity was demonstrated between H of AC and a beetle larval antigen, Tm, of a different insect order. This finding could be due to a multiplicity of potential allergens contained in H that are common to various insect orders and species. The finding of RAST inhibition of AC-H by AC-F but lack of inhibition of AC-F by AC-H could also reflect nonspecific binding of multiple proteins to the complex AC-H. By contrast, the AC-F antigen system exhibited a more specific inhibition profile. Although AC-F allergens exhibited cross-reactivity with AC whole body allergens, there was no cross-reactivity

with allergens of different species, thereby demonstrating a high specificity for F allergen.

Laboratory workers have developed respiratory allergy to insects in other occupational settings.^{13, 14} This study demonstrated the development of clinical sensitivity to whole body, F, and H cockroach allergens in the workplace. The clinical significance of these allergens in cockroach sensitization requires further investigation.

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