

Mealworm asthma: clinical and immunologic studies

David I. Bernstein, M.D., Joan S. Gallagher, Ph.D., and
I. Leonard Bernstein, M.D. Cincinnati, Ohio

Mealworms are larvae of the Tenibrio molitor (Tm) beetle and are widely used as fishing bait. Four of five bait handlers in a warehouse who were exposed to particulates of Tm exoskeletons reported immediate-onset asthma, rhinitis, or contact urticaria while unwrapping and packaging the larvae. Positive cutaneous prick tests to Tm extract were detected in all four symptomatic workers. Specific IgE against Tm antigens of 14.6% and 22.5% were found by the RAST method in the two workers with Tm-induced asthma. Specificity of Tm-IgE binding was confirmed by RAST inhibition of 73%, 58%, and 37% by 2×10^{-1} , 2×10^{-2} and 2×10^{-3} dilutions of Tm antigen. No significant inhibition of Tm-IgE binding by mite and cockroach antigen or by extracts of three other larval species of the Diptera and Lepidoptera orders, to which the workers were also exposed, was detected. Bronchial provocation challenge with Tm antigen performed in the two workers with asthma exhibited an immediate fall in FEV₁ of more than 20% at provocation-dose dilutions of 2×10^{-3} and 2×10^{-5} , respectively. In the latter worker, pretreatment with disodium cromoglycate inhibited the bronchial response to 2×10^{-4} dilution of Tm antigen. This study demonstrates that inhaled particulates from Tm exoskeletons are potent sensitizers and elicit IgE-mediated occupational asthma. Allergic cross-reactivity between orders of insects was not observed. This occupational setting serves as a model for the prospective study of insect allergy. (J ALLERGY CLIN IMMUNOL 72:475-480, 1983.)

Materials derived from nonhymenoptera insect species can act as potent allergens. Previous studies have demonstrated a high incidence of skin reactivity to extracts of various insect species in atopic subjects.^{1, 2} Early reports of endemic outbreaks of asthma associated with localized infestations of mayflies and caddis flies suggested that insects could act as sources of significant inhalant allergens.^{3, 4} More recently, patients with extrinsic asthma have exhibited significant cutaneous and bronchial reactivity as well as specific IgE binding to cockroach antigens.⁵ Occupational asthma and rhinitis have been described in laboratory workers handling locust,⁶ grain workers exposed to grain weevil (*Sitophilus granarius*),⁷ and in

millers and bakers exposed to insects and mites contained in flour.⁸ In 1967, Stevenson and Matthews⁹ demonstrated by bronchial provocation a case of occupational asthma in a fish bait handler associated with exposure to larvae of bee moths (*Galleria mellonella*) and documented an IgE-mediated mechanism by a positive prick test to bee moth extract and a positive Prausnitz-Küstner test. Occupational asthma caused by beetles (Coleoptera order) was first reported in a museum curator who was exposed to larvae used to digest flesh from zoological specimens.¹⁰ We report in this article clinical and immunologic studies performed on workers at a live fish bait distributing warehouse who developed allergic respiratory symptoms associated with exposure to emanations of mealworms, larval forms of the Tm beetle.

PATIENTS AND METHODS

Tm exposed workers

Five workers with regular exposure to exoskeletons and excreta produced by Tm larvae were studied. Organic dust from these larval emanations was dispersed during removal of the insects from their newspaper wrappings. Airborne dispersal of larval material also occurred when the or-

From the Department of Medicine, Division of Immunology, The University of Cincinnati Medical Center, Cincinnati, Ohio.

Supported by a grant from the National Institute of Allergy and Infectious Diseases No. AI13702-57.

Received for publication April 8, 1983.

Accepted for publication May 26, 1983.

Reprint requests: David I. Bernstein, M.D., The University of Cincinnati Medical Center, 231 Bethesda Ave., M.L. No. 563, Cincinnati, Ohio 45267.

Abbreviations used

Tm:	<i>Tenibrio molitor</i>
RAST:	Radioallergosorbent test
FEV ₁ :	Forced expiratory volume in 1 sec

ganisms were agitated in a sifting device to separate the shed exoskeletons from the larvae. The Tm larvae were transferred into paper cups containing wheat bran before they were shipped to local bait shops. Of the five exposed workers, four directly handled the mealworms while the other worker, a supervisor, sustained only occasional direct exposure. Complete occupational and allergic histories were performed by physicians from the University of Cincinnati Allergy Laboratory.

Immunologic studies

Skin testing. A mixture of Tm exoskeleton and excreta was extracted in phosphate-buffered saline to make a concentrated solution of 1:20 w/v. Serial dilutions of 2×10^{-2} , 2×10^{-3} , 2×10^{-4} , and 2×10^{-5} were prepared for testing. An extract and dilutions of wheat bran were prepared in a similar manner. Skin tests with the cutaneous prick test titration method were performed on all five workers and six nonexposed control subjects with both wheat bran and Tm larval extracts.

Radioimmunoassay. Specific IgE to Tm larval extract was measured by a modification of the RAST as previously described with Tm extract (1:20) coupled to methylcellulose disks activated by cyanogen bromide.¹¹ Results were expressed as percent binding, which was calculated by dividing counts per minute bound by the worker's serum by counts per minute of total added ¹²⁵I-labeled anti-IgE multiplied by 100.

RAST inhibition studies were performed on the serum that exhibited the highest Tm RAST binding.¹² Equal volumes of the worker's serum were preincubated at 37° C for 2 hr with 2×10^{-1} , 2×10^{-2} , and 2×10^{-3} gm of Tm larval extracts and with the same concentrations of wheat bran extract and were centrifuged at $1500 \times g$ for 15 min. The upper two thirds of the supernatant was removed and incubated with Tm-coated methylcellulose disks followed by the addition of ¹²⁵I-labeled anti-IgE. The percent of RAST inhibition was calculated as follows:

$$1 - \frac{\text{cpm } ^{125}\text{I-labeled anti-IgE bound by preabsorbed sample}}{\text{cpm } ^{125}\text{I-labeled anti-IgE bound by nonabsorbed sample}} \times 100$$

Bronchial provocation. Bronchial provocation tests to aerosolized Tm larval extracts were performed in two exposed symptomatic Tm workers, three nonexposed extrinsic asthmatic patients, and three nonasthmatic controls. Bronchial provocation tests were performed with wheat bran extracts in those workers who had positive bronchial responses to Tm extracts. Before each provocation, a saline control

bronchial challenge was performed. Sequential provocation doses of 0.5 ml of solution delivered via a No. 40 DeVilbiss nebulizer were administered with decreasing tenfold dilutions of Tm extract or wheat bran extract beginning with a concentration of 2×10^{-5} . Baseline pulmonary function measures of forced vital capacity, peak expiratory flow, and FEV₁ were performed and repeated at 5, 10, 15, 30, 60, 90, 120, and 180 min after each challenge. A positive bronchial response to Tm was defined as a 20% or more fall in FEV₁ after the challenge dose. Hourly peak flow measurements were performed for 12 hr after completion of each challenge.

RESULTS

Clinical evaluation

All four bait handlers described an immediate onset of respiratory symptoms associated with exposure to Tm larval dust (Table I). Two workers (workers 1 and 2) reported immediate-onset asthma and rhinitis symptoms, and workers 3 and 4 noted rhinitis symptoms only. Worker 4 reported contact urticaria while handling Tm larval material. Mean duration of exposure before the onset of symptoms in these four workers was 5.4 yr. Only worker 3 had a prior atopic history consistent with tree and grass pollenosis. Worker 5, a supervisor who had only occasional exposure to the larval dust, was asymptomatic. Case histories of workers 1 and 2 are presented below.

Worker 1

A 34-yr-old white man had worked at the live bait company for 5 yr. Exposure to Tm larval materials was greatest during the winter months when he was directly involved with handling and packaging live bait. For 2 yr he described an immediate onset of ocular itching, rhinorrhea, wheezing, and dyspnea shortly after beginning work with Tm larvae and continuing for up to 4 hr after coming home. Symptoms resolved on weekends and vacations. There was no prior atopic history. On chest examination, faint expiratory wheezes were present. The white blood count was 7800 with 12% eosinophils. Total serum IgE was 230 IU/ml. Skin testing to common inhalant allergens revealed positive intradermal tests to trees, grass, ragweed, dust mites (10^{-3}), and wheat bran (10^{-3}). RAST binding was 15% to timothy and 14% to ragweed.

Worker 2

A 23-yr-old white man had worked at the live bait company for 5 yr during the fall and winter months. After 3 yr, he first noted ocular itching, lacrimation, rhinorrhea, wheezing, and dyspnea that occurred immediately on exposure to Tm larvae. There was no prior history of allergic rhinitis or asthmatic symptoms. Symptoms at work were partially controlled with inhaled metaproterenol sulfate and antihistamines and improved at home and on vacation. He had mild expiratory wheezes on chest auscultation. The total serum IgE was 520 IU/ml. Intradermal skin tests were positive to dust (10^{-4}) and mold (10^{-3}).

TABLE I. Clinical and immunologic data*

Worker	Tm-induced symptoms	Atopic history	Skin test	RAST (% binding)
1	Asthma/rhinitis	—	2×10^{-2}	14.6
2	Asthma/rhinitis	—	2×10^{-4}	22.5
3	Rhinitis	Present	2×10^{-2}	4.5
4	Rhinitis/contact Urticaria	—	2×10^{-3}	3.5
5	None	—	Negative	2.9
Control (6 subjects)			Negative	2.68 (SD $\pm$ 0.7)

*Presented are workers' symptoms associated with Tm dust exposure; atopic history; skin prick test titration results (expressed as log threshold of Tm extract dilution that elicited a positive skin test); specific IgE to Tm larval extract expressed as percent RAST binding. RAST and skin test results of six negative control subjects in which bronchial provocation to Tm was performed.

Immunologic studies

All four symptomatic workers exhibited positive cutaneous prick tests to Tm larval extracts with dilutions ranging from 2×10^{-2} to 2×10^{-4} (Table I). Prick tests performed in all workers with the same dilutions of wheat bran extracts were negative. Prick tests to Tm and wheat bran extract were negative in worker 5 who was asymptomatic as well as all nonexposed asthmatic and nonasthmatic control subjects.

Significant RAST bindings of 14.6% and 22.5% were demonstrated in only workers 1 and 2, respectively, who had experienced asthmatic symptoms on exposure to Tm larvae.

Results of RAST inhibition studies performed with the serum of worker 2 are presented in Table II. Significant inhibition of more than 50% by Tm extract was found at dilutions of 2×10^{-1} and 2×10^{-2} . No inhibition by wheat bran extract of IgE binding to Tm was demonstrated. To further examine antigenic cross-reactivity of Tm with acarid or other insect allergens, RAST inhibition studies were performed with mite extract supplied by Dr. Larry Arlian, Wright State University, cockroach extract (Hollister-Stier Laboratories, San Leandro, Calif.), and exoskeleton extracts of three other insect larval species to which the workers were also exposed. Two of the latter three species are members of the Diptera order and the other, the wax worm, of the Lepidoptera order. Fig. 1 shows that there was no significant inhibition of ^{125}I -labeled anti-IgE binding to Tm-coated methylcellulose disks by cockroach extract or by the aforementioned larval antigens.

Bronchial provocation

Bronchial provocation tests performed on the two asthmatic workers (workers 1 and 2) are shown in Fig. 2. The bronchial provocation dose that elicited a positive response of a fall in FEV_1 of more than 20%

(PD_{20}) to Tm extract in worker 1 was 2×10^{-3} . Worker 1 also had a positive bronchial challenge to wheat bran at a PD_{20} of 2×10^{-3} . Both the latter responses began at 5 min, peaked at 15 min after challenge, and returned to baseline after 1 hr. Worker 2 had a positive bronchial challenge to Tm at a PD_{20} of 2×10^{-5} and a negative response to wheat bran. The decrease in FEV_1 of a fall of 36% from baseline peaked at 15 min and reversed without treatment 2 hr after challenge. Bronchial provocation testing with Tm at dilutions of 2×10^{-5} and 2×10^{-4} in worker 2 30 min after pretreatment with 40 mg of cromolyn sodium revealed no significant decrease in pulmonary function. There were no late asthmatic responses as measured by hourly peak flow measurements 3 to 12 hr after provocation in workers 1 and 2. Bronchial provocation tests were performed on three extrinsic nonexposed asthmatic patients and three nonexposed normal subjects to Tm extracts at 2×10^{-5} , 2×10^{-4} , and 2×10^{-3} dilutions; no positive bronchial responses of the immediate or late type were observed in these subjects.

DISCUSSION

Occupational asthma among bait handlers was first identified in a worker handling bee moth larvae or wax worms (*Galleria mellonella*).⁹ The worker who presented with IgE-mediated asthma was the only symptomatic individual among 18 other exposed workers. Recently, Stockley et al.¹³ demonstrated late-onset asthma in a fisherman associated with handling *Calliphora* maggots used as fish bait. In this article, we have described four live fish bait handlers who developed skin test reactivity to larval antigens of the Tm mealworm. Although only one worker had an atopic history, all four of these individuals reported an immediate onset of respiratory symptoms in association with exposure to Tm larvae only. These findings,

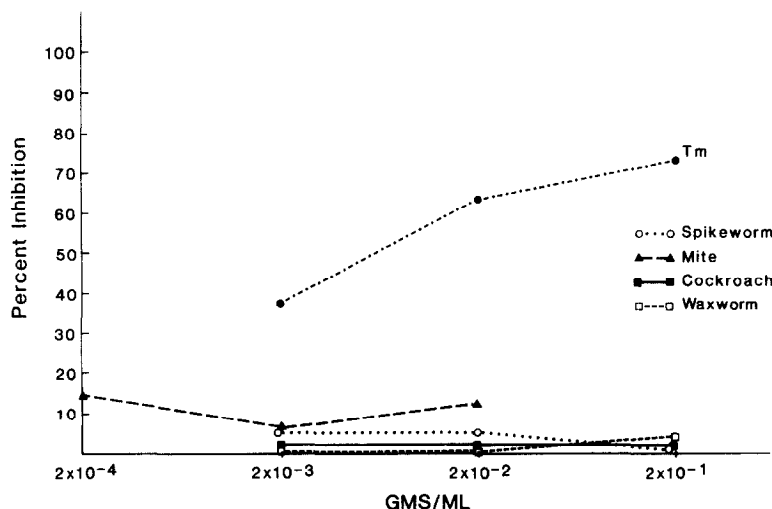


FIG. 1. RAST inhibition of ^{125}I -labeled anti-IgE binding to Tm-coated disks after preabsorption with various dilutions of mealworm (Tm), spikeworm, mite, cockroach, and wax worm extracts expressed as percent inhibition.

TABLE II. RAST inhibition studies on worker 2 with serial dilutions, Tm, and wheat bran extracts

		cpm of ^{125}I -labeled anti-IgE bound to Tm disks	I % Inhibition
Saline		15,101	0
Tm extract	2×10^{-1}	4,017	73
Tm extract	2×10^{-2}	6,295	58
Tm extract	2×10^{-3}	9,585	37
Wheat bran	2×10^{-1}	15,200	0
Wheat bran	2×10^{-2}	15,132	0
Wheat bran	2×10^{-3}	15,053	0

reflecting a high prevalence of sensitization to Tm antigens, suggest that Tm larval emanations may contain unusually potent allergens.

Positive bronchial provocation tests in response to Tm larval antigen were demonstrated in the two workers with asthma associated with mealworm exposure. In addition to a positive bronchial challenge to Tm extract, worker 1 also exhibited a positive bronchial challenge to wheat bran extract as well. This finding in worker 1 may be explained by his cutaneous sensitivity to grass allergens, which are known to be cross-reactive with wheat allergen.¹⁴ Cromolyn sodium completely inhibited the Tm-induced bronchial response in worker 2. Since specific IgE against Tm extract was detectable in only the two workers with asthma, it appears that the prick test was more sensitive than RAST for the purpose of identifying the total number of workers sensitized to Tm antigens.

RAST inhibition demonstrated specificity of IgE binding to Tm larval antigen and also lack of in vitro cross-reactivity by RAST inhibition with mite antigen. Interestingly, RAST inhibition studies demonstrated no significant allergenic cross-reactivity between and among cockroach (Dictyoptera order), wax worms (Lepidoptera order), larvae of the Diptera order, and meal worm (Coleoptera order) allergens. In previous studies, observations of the high incidence of cutaneous reactivity to a wide variety of insect antigens have been explained by the possible existence of cross-reactivity between and among orders.¹⁵ Langlois et al.¹⁶ demonstrated antigenic cross-reactivity among and between orders of insects by hemagglutination and gel diffusion studies that used rabbit immune sera produced against five different insects. Because the allergenic fractions were not isolated, these in vitro studies failed to demonstrate the existence of allergenic cross-reactivity between orders.

This study, which demonstrates that allergenic determinants may not be cross-reactive between orders of insects, is in agreement with passive transfer studies performed by Stevenson and Matthews⁹ on an individual with occupational asthma caused by emanations of bee moth larvae. In this case, cross-reactivity was demonstrated among species within but not outside the Lepidoptera order. RAST inhibition is a useful and appropriate in vitro method for characterization of insect allergens.

This is the first article that reports IgE-mediated disease caused by exposure to Tm larvae. The extent of this clinical problem among fishermen, bait retailers, or producers handling mealworms is unknown. However, a careful history of allergies should

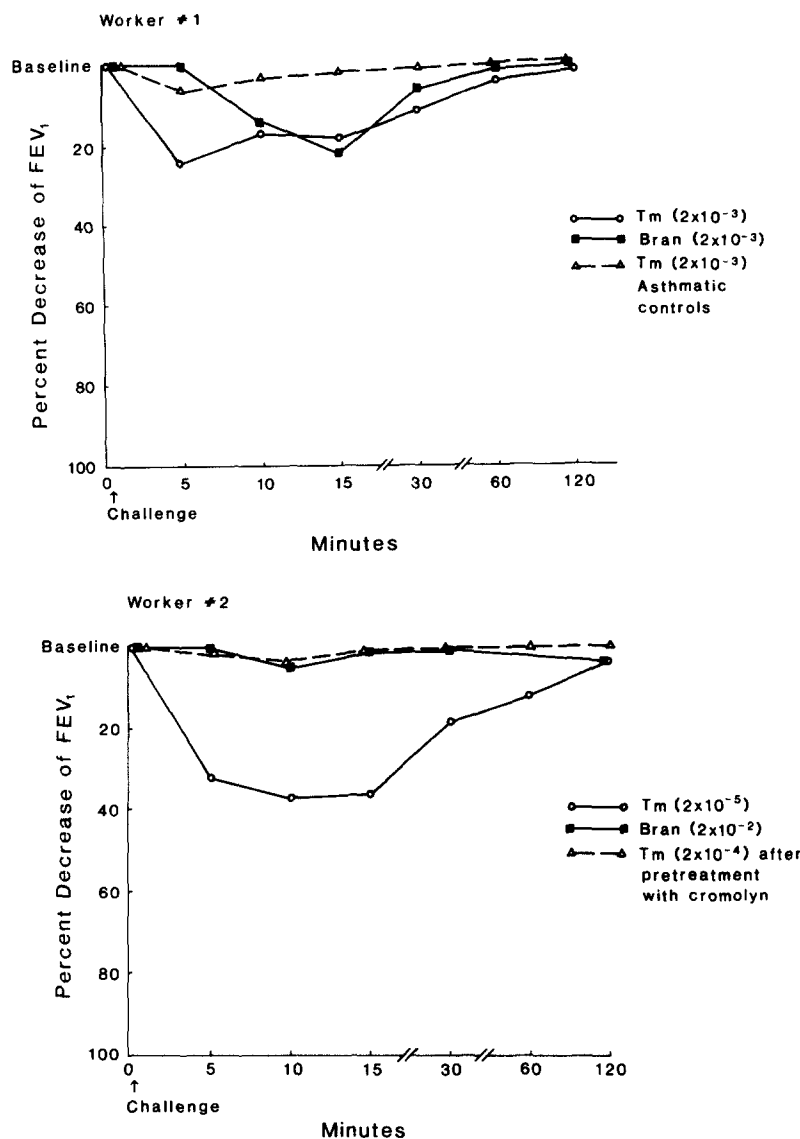


FIG. 2. Bronchial provocation studies. *Top*, Worker 1 exhibited a PD_{20} to Tm and wheat bran extract of 2×10^{-3} gm/ml. Also shown is a control challenge to Tm extract performed on 1 of 6 control subjects. *Bottom*, Worker 2 found to have a PD_{20} to Tm of 2×10^{-5} gm/ml; bronchial responses to Tm at 2×10^{-4} gm/ml were inhibited after pretreatment with cromolyn sodium.

be obtained from all individuals with significant occupational exposure to insect larval emanations. Based on our study, in vitro immunoassays for measurement of specific IgE to Tm larval antigens in combination with skin testing are suitable surveillance methods for identifying those workers at most risk to develop allergic respiratory symptoms. A longitudinal study will be carried out over the next 3 yr in this group of Tm exposed workers. This setting may serve as an excellent model for the study of the natural history of insect allergy. Isolation of the allergenic fractions contained in Tm larval extracts is currently being performed. If the pharmacologic management of these patients with cromolyn sodium and bron-

chodilators is inadequate for controlling symptoms, immunotherapy that uses the fractionated allergenic components of Tm extract should be considered as a suitable treatment alternative.

REFERENCES

1. Feinberg AR, Feinberg SM, Benaim-Pinto C: Asthma and rhinitis from insect allergens. I. Clinical importance. *J ALLERGY* 27:437, 1956.
2. Wiseman RD, Woodin WG, Miller HC, et al: Insect allergy as a possible cause of inhalant sensitivity. *J ALLERGY* 30:191, 1959.
3. Figley KD: Asthma due to mayfly. *Am J Med Sci* 178:388, 1929.
4. Parlato SJ: The sand fly (caddis fly) as an exciting cause of

- allergic coryza and asthma. II. Its relative frequency. *J ALLERGY* **1**:307, 1920.
5. Kang B, Vellody D, Homburger H, Yunginger JW: Cockroach cause of allergic asthma. *J ALLERGY CLIN IMMUNOL* **63**:80, 1979.
 6. Burge PS, Edge G, O'Brien IM, et al: Occupational asthma in a research center breeding locusts. *Clin Allergy* **10**:355, 1980.
 7. Frankland AW, Lunn JA: Asthma caused by the grain weevil. *Br J Ind Med* **22**:157, 1965.
 8. Popa V, George SA, Gavanescu O: Occupational and nonoccupational respiratory allergy in bakers. *Acta Allergol* **25**:159, 1970.
 9. Stevenson DD, Matthews KP: Occupational asthma following inhalation of moth particles. *J ALLERGY* **39**:274, 1967.
 10. Sheldon JM, Johnston JH: Hypersensitivity to beetles (Coleoptera). *J ALLERGY* **12**:493, 1941.
 11. Wide L, Bennich H, Johansson SGO: Diagnosis of allergy by an *in vitro* test for allergen antibodies. *Lancet* **2**:1105, 1967.
 12. Bernstein IL, Perara M, Gallagher JS, Michael S, Johansson SGO: In vitro cross-allergenicity of major aeroallergenic pollens by the radioallergosorbent technique. *J ALLERGY CLIN IMMUNOL* **56**:323, 1975.
 13. Stockley RA, Hill SL, Drew R: Asthma associated with a circulating IgG antibody to calliphora maggots. *Clin Allergy* **12**:15, 1982.
 14. Hoffman DR: The specificities of human IgE antibodies combining with cereal grains. *Immunochemistry* **12**:535, 1975.
 15. Perlman F: Insect allergens: their interrelationship and differences. *J ALLERGY* **32**:2, 1961.
 16. Langlois C, Shulman S, Arbesman C: Immunologic studies of caddis fly. *J ALLERGY* **34**:385, 1963.