

Role of insects as inhalant allergens in bronchial asthma with special reference to the clinical characteristics of patients

SAROJ GUPTA, S. JAIN, SUMAN CHAUDHRY* and M. K. AGARWAL*

Department of Medicine, Lady Hardinge Medical College, New Delhi and *Department of Allergy and Applied Immunology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India

Summary

The whole body extracts (WBEs) of 13 common insects from Delhi, namely *Musca domestica* (house fly); *Sitophilus oryzae* (rice weevil); *Callosobrochous maculatus* (pulse beetle); *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (mosquitoes); *Blattella germanica* and *Periplaneta americana* (cockroaches: male, female and nymph); *Spodoptera litura* and *Heliothis armigera* (moths), were prepared to evaluate their allergenic significance in patients with allergic bronchial asthma. Intradermal (ID) tests were performed with these WBEs on 75 patients with bronchial asthma and 20 healthy non-allergic volunteers. An ID test was considered positive when the weal diameter was more than twice that of phosphate-buffered saline control and at least 6 mm in size and an erythema of more than 15 mm. In the patients 27.7% of the ID tests performed were total positive (1+ to 4+) as compared to 7.1% in the controls. Ten point two per cent of ID responses were markedly positive in asthmatics (2+ to 4+) as against none in controls. Of the 13 bronchial provocation tests (BPTs) performed on patients giving positive cutaneous responses, eight (61.5%) were positive. Similarly, RASTs were positive in 87.5% of the limited sera tested from the patients with positive ID responses. The patients with a positive family history of allergic disorders gave higher percentages of positive ID responses as compared to the asthmatics with no family history. Similarly, the ID positivity was significantly higher in patients with associated allergic diseases like allergic rhinitis as compared to the patients with bronchial asthma alone. Interestingly, we observed significantly higher ID response to insects in seasonal asthmatics. Thus, we conclude that insects do play an important role in the aetiology of allergic bronchial asthma, and that allergy to insects has a significant bearing on the clinical characteristics of these patients.

Clinical and Experimental Allergy, Vol. 20, pp. 519–524. Submitted 28 April 1989; revised 7 February 1990; accepted 16 March 1990.

Introduction

We are surrounded by a huge population of different types of insects. The role of various insects as inhalant allergens has been studied by several investigators [1–7]. Some preliminary studies on the allergic significance of insects have also been reported from India [8,9]. The

present study was undertaken to study type I hypersensitivity to insects in the Delhi (India) area with special reference to various clinical characteristics in patients with bronchial asthma.

Material and methods

Allergens

A total number of 13 whole body extracts (WBEs) of 11 common insects in the Delhi area (see Table 1) were

Correspondence: Dr M. K. Agarwal, Department of Allergy and Applied Immunology, V.P. Chest Institute, University of Delhi, Delhi 110 007, India.

prepared by the modified method of Agarwal *et al.* [10]. In brief, the insects were killed by freezing at -20°C , lyophilized, pulverized and defatted with solvent ether. The defatted powders were extracted in 0.1 M NH_4HCO_3 , pH 7.8. These extracts were dialysed, lyophilized and stored in air-tight vials, at -20°C . The allergens were reconstituted in phosphate-buffered saline, pH 7.4 for various clinico-immunological tests.

Subjects

Seventy-five consecutive patients with allergic bronchial asthma, with or without associated allergic disorders such as allergic rhinitis, reporting to the out-patient departments and chest clinics of the Lady Hardinge Medical College and Associated Sucheta Kriplani Hospital, New Delhi and Vallabhbbhai Patel Chest Institute, Delhi, were studied. In each case a special note was made of the following points:

1. age at onset of asthmatic attacks;
2. duration of bronchial asthma when first seen;
3. season and frequency of asthmatic attacks;
4. history of associated allergic disorders, e.g. allergic rhinitis, urticaria or atopic dermatitis; and
5. history of bronchial asthma or other allergic disorders in the first degree relatives.

Steroid-dependent asthmatics and those with associated cardio-pulmonary disorders were not included in the present study. Patients in acute exacerbation and those who could not stop antihistamines and/or bronchodilators for at least 24 hr without developing symptoms were also excluded from the study. As a control 20 healthy individuals with no personal or family history of allergic disorders were also studied.

Intradermal tests

The allergenic significance of the 13 insect WBEs was screened by performing intradermal (ID) tests with these extracts (1:500 w/v) on the volar surface of the forearm and lateral aspect of the arm of 75 patients with bronchial asthma and 20 non-allergic healthy individuals. In addition, buffered saline and histamine (100 $\mu\text{g}/\text{ml}$) were also tested as negative and positive controls, respectively.

The techniques and criteria of Shivpuri and Agarwal [11] were followed for performing and grading the ID tests. Averages of the diameters of weal and erythema taken at right angles were used. The tests were graded according to:

Grade of reaction	Weal diameter (times that of negative control)	Erythema (mm)
1+	Over twice and 6 mm	15–20
2+	3–3.5 times	> 20
3+	3.5–4 times with 1 or 2 pseudopodia	> 30
4+	4–6 times with several pseudopodia	> 35

The markedly positive intradermal reactions were confirmed the same day by repeating the test on an identical site of the other arm. The tests were performed by one of the co-authors (SJ) throughout the course of the study.

Bronchial provocation tests

In order to evaluate the specificity of ID response, BPTs were performed on 23 patients, of whom 13 elicited different grades of positive skin reactions with various insect WBEs and 10 were negative on skin testing. An open method of inhalation challenge was employed [12]. A fall in forced expiratory volume in the first second (FEV_1) of 15% or more over the basal level up to 30 min after the allergen inhalation was considered as a positive test.

Radio-allergosorbent test (RAST)

Allergen-specific IgE levels to cockroach (*Periplaneta americana*) WBE were estimated in the serum of 10 patients according to the method described by Gleich and Yunginger [13]. RASTs were graded on the basis of RAST ratios obtained by comparing the binding with patients' serum to that of a healthy non-allergic volunteer. In brief, the *P. americana* male WBE-coupled discs were separately incubated with the patients' serum (50 μl) as well as that for healthy non-allergic volunteers (NHS). After washing, these discs were incubated with 50 μl of anti-human-IgE labelled with ^{125}I (Pharmacia, Uppsala, Sweden). After repeated washings, these discs were analysed for radioactivity in a gamma-counter, and the counts per minute (c.p.m.) obtained were expressed as per cent of the total counts added. Subsequently, a RAST ratio with a test serum was calculated by the following formula:

$$\text{RAST ratio} = \frac{\% \text{ c.p.m. with test serum}}{\% \text{ c.p.m. with NHS}}$$

A RAST ratio of five or more was considered as positive.

Results

Intradermal tests

In the present study 75 patients with bronchial asthma aged from 12 to 64 yr (mean 28.6 yr, 17 males and 58

Table 1. Results of intradermal tests on 75 patients with bronchial asthma and 20 control subjects with 13 insect whole body extracts

Name of the antigen	Weal diameter (mean $\pm$ s.d.)					Patients				Controls		
	n	1+	2+	3+	4+	1+ to 4+		2+ to 4+		n	1+	%
		7.4	10.5	12.5	16.8							
		$\pm$	$\pm$	$\pm$	$\pm$							
		1.2	1.9	2.3	1.8	n	%	n	%			
<i>Musca domestica</i> (house fly)	44	7	5	4	3	19	43.2	12	27.3	20	2	10
<i>Sitophilus oryzae</i> (rice weevil)	23	3	5	1	0	9	39.1	6	26.1	0	0	0
<i>Callosobrochous maculatus</i> (pulse beetle)	75	11	10	4	4	29	38.7	18	24.0	20	1	5
<i>Anopheles stephensi</i> (mosquito)	75	18	6	2	0	26	34.7	8	10.7	20	2	10
<i>Periplaneta americana</i> (cockroach—male)	75	10	6	3	1	20	26.7	9	13.3	20	1	5
<i>Periplaneta americana</i> (cockroach—female)	75	19	1	4	1	25	33.3	6	8.0	20	1	5
<i>Blattella germanica</i> (cockroach—male)	75	18	4	2	0	24	32.0	6	8.0	20	2	10
<i>Blattella germanica</i> (cockroach—female)	75	10	0	1	0	11	14.7	1	1.3	20	2	10
<i>Blattella germanica</i> (cockroach—nymph)	75	7	2	0	0	9	12.0	2	2.7	20	0	0
<i>Culex quinquefasciatus</i> (mosquito)	75	16	5	1	0	22	29.3	6	8.0	20	3	15
<i>Aedes aegypti</i> (mosquito)	75	13	3	4	0	20	26.7	7	9.3	20	0	0
<i>Spodoptera litura</i> (moth)	75	17	2	0	0	19	25.3	2	2.7	20	3	15
<i>Heliothis armigera</i> (moth)	75	7	4	3	0	14	18.7	7	9.3	20	0	0
Total	892	156	53	29	9	247	27.7	91	10.2	240	17	7.1

Average weal diameter of (1) phosphate-buffered saline = 3.3 ± 1.2 ; (2) negative ID response to insect WBEs = 4.0 ± 1.8 ; (3) histamine = 9.7 ± 1.9 .

A total of seventeen 1+ reactions were obtained on 12 of the 20 healthy controls skin tested with the 13 insect WBEs.

females) and 20 healthy non-allergic controls varying in age from 17 to 58 yr (mean 29.5 yr; 10 males and 10 females) were studied. The results of the 892 intradermal tests conducted on patients and those of 240 tests conducted on non-allergic healthy controls are presented in Table 1.

Of the 75 patients skin tested, 27 (36%) elicited markedly positive (2+ to 4+) reactions to at least one insect WBE. The distribution of these patients according to the number of insect extracts eliciting 2+ to 4+ intradermal reactions is given in Table 2.

Bronchial provocation tests

The BPTs were uniformly negative in all the 10 patients eliciting a negative skin test, while eight (61.5 per cent) of the 13 tests conducted on patients with positive cutaneous responses to different insect WBEs were positive (Table 3).

RAST

RASTs were positive in 87.5% of the limited sera tested from the patients eliciting positive intradermal responses to the cockroach male allergen (Table 4). None of the patients with a negative skin test gave a positive RAST.

The average level of allergen-specific IgE antibodies as indicated by the RAST ratios was found to increase with the increase in the intensity of the skin test response.

The results of skin tests with insect WBEs on 75 patients with bronchial asthma have been analysed according to their clinical characteristics in Table 5. The skin positivity (1+ to 4+) of 35% to insect WBEs in asthmatics with an early onset (age 20 yr) was significantly higher ($P < 0.01$) as compared to 21.9% in those with a late onset of asthma (age 20 yr). The duration of the disease did not, however, significantly affect the cutaneous sensitivity to insects.

In this study the asthmatics were considered as seasonal, irregular and perennial according to the classification of Shivpuri [14]. Patients were considered as seasonal when symptoms of asthma occur and re-occur during the same months of consecutive years and the patients remain symptom-free during the rest of the year. The two major seasons in Delhi are February–April (Spring) and September–November (Autumn) when the insect concentration in the environment of Delhi area is quite high.

In the present study 44 (58.7%) patients had a seasonal periodicity of asthma and these patients gave 33.7% positive intradermal reactions to insect extracts which

Table 2. Distribution of the 27 asthmatics according to the number of positive cutaneous responses (2+ to 4+) to the 13 insects whole body extracts

No. of patients*	No. of WBEs giving positive ID test
11	1
3	2
4	3
3	4
1	5
1	7
1	8
2	9
1	12

* Of the 75 patients tested, only 27 (36%) showed 2+ to 4+ ID response to different insect WBEs.

were significantly higher ($P < 0.01$) than the 19.4% given by patients with irregular or perennial asthma (Table 5). Twenty point nine per cent of the patients with asthma alone showed intradermal reactivity to insects, while those with an associated allergic disorder like allergic rhinitis showed a significantly higher positivity rate of 33.1% ($P < 0.01$). The cutaneous responses to insect allergens were also significantly higher ($P < 0.01$) in

Table 3. Results of bronchial provocation tests with insect whole body extracts on bronchial asthma patients showing different grades of intradermal responses

Skin test reaction	Number of BPT	Positive responses	
		<i>n</i>	%
Negative	10	0	0
Positive (+)	8	5	62.5
Positive (2+)	5	3	60.0
Total	13	8	61.5

The WBEs used for BPT were *Heliothis* ($n=3$), *Callosobrochus* ($n=7$), *Periplaneta* male ($n=6$), *Periplaneta*, female ($n=1$), *Blattella* male ($n=2$), *Aedes* ($n=2$) and *Culex* ($n=2$).

Table 4. Results of RASTs with the sera of patients eliciting different grades of intradermal response to *P. americana* (male) whole body extract

Skin test results	Weal diameter (mm)	c.p.m.*	RAST ratio†	RAST result‡	Average RAST ratio
—	4	0.52	1.21	Negative	1.76
—	5	0.99	2.30	Negative	
1+	8	1.34	3.12	Negative	11.91
1+	9	8.90	20.70	Positive	
2+	10	2.54	5.91	Positive	
2+	10.5	3.09	7.19	Positive	12.38
2+	12.0	5.26	12.23	Positive	
2+	12.0	10.40	24.19	Positive	
3+	13.0	5.55	12.91	Positive	19.76
3+	15.0	11.44	26.90	Positive	

Of the eight sera from patients showing a positive cutaneous response, seven (87.5%) gave a positive RAST.

* Percentage counts per minute of total added.

† Serum from a healthy non-allergic volunteer (NHS), produced a % c.p.m. of 0.43.

‡ RAST ratio of five or more was considered positive.

patients with a positive family history of allergic disorders (Table 5).

Discussion

The aetiological significance of insects as inhalant allergens has been widely debated, as the presence of insect-related particles of respirable size could not be demonstrated in the air. An immunochemical technique has now been developed to measure various air-borne allergens irrespective of their morphological features [15]. Using this technique insect-related particles of less than 10 μ m in size have been demonstrated in the air by different workers [16–18]. These reports support the concept of allergenic significance of insects in respiratory allergic disorders.

In the present study 892 ID tests were performed on 75 bronchial asthma patients with 13 insect WBEs. The total positive reactions (1+ to 4+) ranged from 12% with *Blattella germanica* (nymph) to 43.2% with *Musca domestica* (house-fly) giving an average positivity of 27.7%. In controls, the positivity varied from 0 to 15% (average of 7.1%). Interestingly, 10.2% of the tests were markedly positive (2+ to 4+) in patients, as against none in controls, establishing the specificity of these reactions. A few 1+ reactions elicited by healthy controls could be attributed to latent or subclinical allergy and/or due to the presence of some non-specific irritants in the test

Table 5. Analysis of skin test results with insect WBEs according to the clinical characteristics of the 75 patients with bronchial asthma

Patient groups	No. of patients	No. of tests done	ID positivity	
			1+ to 4+	%
Age of onset, ≤ 20 yr	33	394	138 (35.0)**	
Age of onset, > 20 yr	42	498	109 (21.9)	
Duration of disease, ≤ 5 yr	43	526	140 (26.6)	
Duration of disease, > 5 yr	32	366	107 (27.7)	
Seasonal asthmatics	44	516	174 (33.7)**	
Perennial and irregular asthmatics	31	376	73 (19.4)	
Asthma alone	33	393	82 (20.9)**	
Asthma with associated allergies	42	499	165 (33.1)	
Patients with positive F/H* of allergic disorders	34	379	119 (31.4)**	
Patients without positive F/H* of allergic disorders	41	513	128 (25.0)	

* Family history.

** Significant at $P < 0.01$.

allergens. The high prevalence of markedly positive reactions which were seen exclusively in patients gave evidence for the allergenic significance of insects in Indian asthmatics. Interestingly, of the 27 patients eliciting a 2+ to 4+ ID reaction, 11 were positive to a single insect WBE, while five patients showed such reaction to more than six insect WBEs. The elicitation of markedly positive reaction to only one WBE in some patients and to multiple WBEs in some others may suggest that various insect extracts may possess unique as well as some crossreacting allergens. In a recent report from this laboratory the presence of shared as well as specific allergenic and antigenic components in male and female cockroach (*P. americana*) WBEs has been reported [19]. However, the positivity to more than one extract could also be due to the chance occurrence of coexisting sensitivity to more than one insect.

It is known that skin tests, although a very convenient and cheap procedure, may produce false-positive reactions [20]. In order to substantiate the cutaneous sensitivity to insect allergens further, specific bronchial challenge and RAST tests were conducted in selected cases. More than 60% of the skin-test positive patients gave a positive bronchial response and 87.5% a positive RAST response. None of the patients with a negative cutaneous test gave a positive BPT or RAST indicating the absence of any false negative reactions on skin testing. However, we did have some patients showing negative BPT and RAST even though they gave a positive ID response to the homologous insect WBEs. This may have been due to false positive reactions on ID testing when graded according to

the criteria followed in our study. The high frequency of skin reactivity in our patients along with the BPT and RAST results with various insect WBEs suggest that the insect particles and detritus possess potent allergenic components and are present in the air in sufficient quantities and for a sufficient time to cause sensitization. Our results therefore clearly establish the role of insects as inhalant allergens in asthmatics in India.

Further investigations on the relationship of the presence of insect hypersensitivity in relation to the clinical characteristics of these patients revealed interesting features (Table 5).

It has been believed that allergic factors play an important role in patients who have the onset of asthma at a younger age. This fact was noted as early as 1917 by Walker [21] and has also been observed in the present study. The familial incidence of allergic diseases as indicated by Cooke and Vanderveer [22] has also been observed in this study. The patients with a positive family history of allergic disorders reacted significantly more often to insect allergens on intradermal testing. The patients with associated allergic diseases such as allergic rhinitis showed significantly increased cutaneous reactivity as compared to the patients with bronchial asthma alone. One of the possible reasons may be the presence of an extra 'shock organ' (e.g. the nasal mucosa) in these patients.

It is common knowledge that most insects have seasonal growth and reproductive spurts. Kino *et al.* [16] have also demonstrated seasonal variations in the quantity of airborne moth allergens which correlated well with

the clinical symptoms of moth-allergic patients. These findings suggest that insect-sensitive patients are more likely to have seasonal insect symptoms as well as concomitant allergies to some other allergens. The results of this study showing significantly higher reactivity to insects in the seasonal asthmatics validate these observations. Hence, our results have shown that insects play an important role as inhalant allergens in patients with bronchial asthma in India and that insect allergy has a significant bearing on the clinical characteristics of these patients.

Acknowledgments

The authors are grateful to Professor S. K. Jain, Department of Cardiorespiratory Physiology, V.P. Chest Institute for his help and suggestions during the course of this study. We are also thankful to Dr U.P.S. Chauhan for providing facilities for conducting RAST experiments in his laboratory, and to Dr C. K. Gupta of the V.P. Chest Institute for statistical analysis of the data.

References

- 1 Bagenstose AH, Mathews KP, Homburger HA, Saaveard-Delgado AP: Inhalant allergy due to crickets. *J Allergy Clin Immunol* 1980; 65:71-4.
- 2 Bernton HS, McMahon TF, Brown H: Cockroach asthma. *Br J Dis Chest* 1972; 66:61-6.
- 3 Kang B, Vellody D, Homburger H, Yunginger JW: Cockroach cause of allergic asthma. Its specificity and immunological profile. *J Allergy Clin Immunol* 1979; 63:80-6.
- 4 Kino T, Oshima S: Allergy to insects in Japan. I. The reaginic sensitivity to moth and butterfly in patients with bronchial asthma. *J Allergy Clin Immunol* 1978; 61:10-16.
- 5 Kino T, Oshima S: Allergy to insects in Japan. II. The reaginic sensitivity to silkworm moth in patients with bronchial asthma. *J Allergy Clin Immunol* 1979; 64:131-8.
- 6 Osgood H: Allergy to caddis fly (Trichoptera). II. Clinical aspects. *J Allergy* 1957; 28:292-300.
- 7 Pepys J, Chan M, Hongreare FE: Mites and housefly allergy. *Lancet* 1968; i:1270-2.
- 8 Shivpuri DN, Shali PL, Agarwal MK, Prakash D, Bhatnagar PL: Insect allergy in India. *Ann Allergy* 1971; 29:588-97.
- 9 Shivpuri DN, Shali PL, Bhatnagar PL, Agarwal MK: Studies on insect allergy in Delhi. *Aspects Allergy Appl Immunol* 1969; 2:81-90.
- 10 Agarwal MK, Chaudhry S, Jhamb S, Bansal S: Current concepts on the etiologic significance of insects in respiratory allergy. *Proc Int Symp Recent Adv Allergy Immunol*, 1990. in press.
- 11 Shivpuri DN, Agarwal MK: Studies on the allergenic fungal spores of Delhi, India, Metropolitan area—clinical aspects. *J Allergy* 1969; 44:204-13.
- 12 Jain S: Studies on the etiologic significance of some common insects as inhalant allergens in bronchial asthma. PhD Thesis, University of Delhi. 1988.
- 13 Gleich GJ, Yunginger JW: Standardisation of allergens. In: Rose NR, Friedman H, eds. *Manual of clinical immunology*. Washington DC: American Society for Microbiology, 1976:575.
- 14 Shivpuri DN: Studies on the methods of clinical research in bronchial asthma and allied conditions. *Aspects Allergy Appl Immunol* 1974; 7:15-19.
- 15 Agarwal MK, Yunginger JW, Swanson MC, Reed CE: An immunochemical method to measure atmospheric allergens. *J Allergy Clin Immunol* 1981; 68:194-200.
- 16 Kino T, Chihara J, Fukuda K, Sasaki Y, Shogaki Y, Oshima S: Allergy to insects in Japan. III. High frequency of IgE antibody responses to insects (moth, butterfly, caddis fly and chironomid) in patients with bronchial asthma and immunochemical quantitation of insect related airborne particles smaller than 10 μ in diameter. *J Allergy Clin Immunol* 1987; 79:857-66.
- 17 Swanson MC, Agarwal MK, Reed CE: An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air sampler: studies with mite, roach, cat, mouse and guinea pig antigens. *J Allergy Clin Immunol* 1985; 76:724-9.
- 18 Wynn SR, Swanson MC, Reed CE, Penny ND, Showers WB, Smith JM: Immunochemical quantitation, size distribution and cross-reactivity of Lepidoptera (moth) aeroallergens in south-eastern Minnesota. *J Allergy Clin Immunol* 1988; 82:47-54.
- 19 Chaudhry S, Jhamb S, Chauhan UPS, Gaur SN, Agarwal HC, Agarwal MK: Shared and specific allergenic and antigenic components in the two sexes of American cockroach—*Periplaneta americana*. *Clin Exp Allergy* 1990; 20:59-65.
- 20 Agarwal MK, Gupta CK, Shali PL, Shivpuri DN: Comparative evaluation of various diagnostic tests in the patients of bronchial asthma. *Aspects Allergy Appl Immunol* 1969; 3:95-100.
- 21 Walker C: Studies on the causes and treatment of bronchial asthma. *J Am Med Assoc* 1917; 69:363.
- 22 Cooke RA, Vanderveer A, Jr: Human sensitization. *J Immunol* 1916; 1:201.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.