

Inhalant allergy following occupational exposure to blowflies

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(Received in original form 3 July 1985 and in revised form 29 September 1985;
accepted for publication 3 October 1985)

Summary

An allergic reaction, provoked by exposure to the blowfly *Lucilia cuprina* and shown to be IgE-mediated, occurred in a subject employed in an entomological research laboratory. The subject's serum, and sera from three other asthmatic patients with IgE antibodies to blowfly extracts, also reacted with extracts from the screw-worm fly (*Chrysomya bezziana*). Results suggested that antigens from the two species share immunological cross-reactivity. Cross-reactions also exist between the different developmental stages of both species. Allergic reactions to inhaled insect allergens may not be uncommon in the Australian community.

Introduction

There is a steadily increasing body of literature suggesting that insects can be an important source of allergens that promote inhalant allergy in Man. Bellas, for example, quotes over 300 references implicating insect species from at least twelve orders [1]. Many of these reports are case histories and thus provide little information on the nature of the sensitizing insect components or on the mechanism(s) underlying the adverse reactions described.

A number of the published reports deal with insect species that were being reared and studied because they are pests [1]. Sensitization following occupational exposure has provided clear evidence of the involvement of insects in inhalant allergies and has demonstrated that insects may be the source of some extremely potent allergens that affect a high proportion of workers [1-5]. Severe allergic manifestations were observed among persons involved in the distribution of adults of the American screw-worm fly,

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Cochliomyia hominivorax. In this programme sterilized flies were released from aeroplanes and pilots and the persons whose job it was to eject the flies from the plane were affected. Seventy percent of the individuals involved in the aerial dissemination of the flies experienced symptoms of nasal congestion, eye and nose irritation, wheezing, coughing and shortness of breath [6–8].

This report describes a case of IgE-mediated allergic asthma and rhinitis that occurred in an employee of an entomological research laboratory following exposure to the blowfly *L. cuprina* during a fly rearing programme. This species, also known as the Australian sheep blowfly, occurs widely in Australia where it is a pest for both man and livestock. Evidence for possible allergenic cross-reactivity between adult and immature forms of the blowfly and Old World screw-worm fly, (*C. bezziana*), a related species, is presented.

Materials and methods

Case report

The subject was a 24-year old male employee of an entomology research unit with no personal or family history of atopy and no previous respiratory illness. His work included rearing of sheep blowflies. For this purpose caged flies were kept on shelves above his work bench exposing him to insect debris which was sufficient to be obvious on the shelves within 2–3 days after cleaning. Contact also occurred in the course of sorting live and dead flies on trays.

Early in the second year of employment the subject noticed dryness and irritation of the pharynx. This was intermittent at first but within 4 months was recurring daily within 15 min of starting work. At this time sneezing, rhinorrhoea, conjunctival irritation and a hot flushed feeling gradually developed. Chest tightness was noted for the first time about 20 months after he began work in the unit. All these later symptoms occurred about 30 min after exposure to fly dust and recurred daily. The sore throat lasted a few minutes, to be followed by rhinitis and chest tightness which progressed to reach a plateau and began to improve about 1 hr after ceasing work. Particularly heavy insect dust exposure was related to persistence of rhinitis through the night and weekends, otherwise remission at these times was complete. Symptomatic relief was best achieved with antihistamines but the hot flush was eased by washing hands and face in cold water and standing in a cool room. Sympathetic bronchodilators were never used but a nasal decongestant was helpful in clearing the nose.

After 2 years in the laboratory, the subject was transferred to field work in an attempt to reduce his overall exposure. Duties included the trapping and release of flies from porous cloth cages and the sieving of pupae. The level of exposure needed to produce allergic symptoms now appeared to be less and some of the more severe reactions occurred at this time. He then developed an urticarial rash on his arms where they had been in contact with the cloth cages. Three years and 8 months after commencing work on the blowfly programme, the subject was transferred to Papua New Guinea for 3 months to mass rear the screw-worm fly (*C. bezziana*). He had daily exposure of a few minutes to a fly population one-tenth the size kept in his Australian laboratory. Throughout this period he remained free of symptoms. On resumption of his usual work in Australia with sheep blowfly, rhinitis and chest tightness recurred within 1 hr.

Protective equipment was not worn by the subject until he sought medical advice

approximately 21 months after commencing work with the insects. After that he did wear a paper mask conscientiously. His symptoms were at their worst when he did not wear the mask.

The subject was examined within 6 weeks of returning to Australia. Examination included skin-prick testing to insect extracts and to a panel of common allergen extracts and the measurement of FVC and FEV, before and after exposure to nebulized histamine. These tests were performed approximately 24 hr after work exposure to blowflies.

Insects

The following species or insect materials used, and their sources were: sheep blowfly (*L. cuprina*) adults, larvae and eggs and silverfish (*Ctenolepisma longicaduta*) from C.S.I.R.O. Division of Entomology, Canberra; *Drosophila melanogaster* from the Department of Zoology, University of Sydney; Australian plague locust (*Chortoicetes terminifera*) adults and faeces from the Biological and Chemical Research Institute, N.S.W. Department of Agriculture, Rydalmere; common clothes moth (*Tineola bisselliella*), case-bearing clothes moth (*Tinea translucens*), variegated carpet beetle (*Anthrenus verbasci*) and furniture carpet beetle (*Anthrenus flavipes*) from C.S.I.R.O. Division of Textile Industry, Belmont, Victoria; screw-worm fly (*C. bezziana*) adults, pupae and larvae from the Australian Screw-worm Fly Unit, Laloki, Papua-New Guinea; German cockroaches (*Blattella germanica*), collected locally from a Sydney house.

The sheep blowfly and the screw-worm fly are both members of the Dipteran family Calliphoridae. *L. cuprina* is placed in the sub-family Calliphorinae as are the many species of large brown blowflies of the genus *Calliphora* which are common throughout southern Australia while *C. bezziana* and *Cochliomyia hominivorax* are placed in the sub-family Chrysomyinae.

Preparation of insect extracts

Adult insects, pupae, larvae, eggs or faeces were weighed, ground in a pestle and mortar, triturated to a paste and diluted with distilled water. The extract was transferred to a beaker, cooled in an ice bath and stirred for 2 hr in a cold room. The suspension was centrifuged (27 000 g) at 4°C for 30 min and the supernatant filtered throughout 0.22 µ Millipore filters before freeze drying.

Skin tests

For prick testing, insect extracts were formulated at a concentration of 10 mg/ml in a diluent of 50% glycerin in sterile saline. Diluent was added to the dry extracts immediately before use and the reconstituted extracts stored frozen. Other extracts used for prick testing (mite, pollens, fungi etc) were obtained from Hollister-Stier. Histamine 1 mg/ml was used in every patient as a standard. All extracts were tested for non-specific irritant effects by skin-prick testing ten non-allergic, healthy staff members of the Prince of Wales and North Shore Hospitals. Only extracts that gave negative skin reactions were used in the study.

Radioallergosorbent test (RAST)

This was carried out with paper [9] or nitrocellulose [10] discs using Pharmacia ¹²⁵I-anti-human IgE. Insect allergen discs were prepared by spotting 6 mm discs punched

from Millipore nitrocellulose, pore size $0.45\ \mu$ with $10\ \mu\text{l}$ of extract $10\ \text{mg/ml}$ in 1% potassium hydroxide. This solvent has been found to significantly enhance the reactivity of nitrocellulose-adsorbed proteins detected by immunoassay [11]. After drying, discs were blocked with 0.1% Tween 20 in phosphate buffered saline. RASTs for IgE antibodies to house dust mite (*Dermatophagoides pteronyssinus*), ryegrass pollen (*Lolium perenne*), couch grass pollen (*Cynodon dactylon*), the mould *Alternaria alternata* and cat epithelia were carried out with Pharmacia allergen discs.

Results

Lung function tests

The patient's FEV₁ was 4.6 l and FVC was 5.8 l (predicted values were 4.3 l and 5.1 l respectively). Histamine 1 mg/ml nebulized for 60 sec produced a 17% decrease in FEV at 90 sec. At a dose of 2 mg/ml the FEV₁ fell by 35% indicating bronchial hyperreactivity.

Skin-prick tests

No immediate reactivity was present on prick testing (on the volar aspect of each arm) to a range of local grasses, pollens, animal dander and house dust mite. The histamine standard produced a weal which measured $3 \times 3\ \text{mm}$. Tests to twelve insect extracts showed reactions to sheep blowfly adults ($2 \times 3\ \text{mm}$), sheep blowfly larvae ($3 \times 5\ \text{mm}$), cockroach ($2 \times 2\ \text{mm}$), vinegar fly ($2 \times 2\ \text{mm}$) and screw-worm fly adults ($2 \times 4\ \text{mm}$). A smaller reaction was seen to locust extract. No reactions were seen to any of the other insect extracts. The predominance of the patient's skin test responses to the blowfly and screw-worm fly extracts is emphasized by comparing potencies relative to the histamine standard which was used at a concentration of 1 mg/ml and allotted a potency of 1 [12,13]. The histamine equivalents of the insect extracts were sheep blowfly adults 0.8, blowfly larvae 1.3, cockroach 0.7, vinegar fly 0.7, screw-worm fly adults 1.0 and locust 0.3.

Extracts of ryegrass, couch grass, plantain and Timothy grass pollens, house dust, house dust mite, epidermal mix, mould mix, *Mucor racemosus* and control diluent (all from Hollister-Stier) did not produce positive skin test reactions.

Examinations for IgE antibodies

The subject's serum was used in the RAST with insect allergen discs to determine the presence or absence of IgE antibodies to the ten different insect species. Results are shown in Table 1. A positive value in the RAST was taken as a radioactive uptake of greater than 2.5 times the cord/normal serum values [14-16]. Strong positive reactions to each of the blowfly and screw-worm fly extracts were detected but tests to all other insect extracts were negative.

The explanation for the presence of IgE antibodies to screw-worm fly extracts was sought by examining sera from 33 Sydney asthmatics with no occupational or other known abnormal exposure to blowflies, and who had never been exposed to screw-worm flies but who had serum IgE antibodies to blowfly extracts. Sera from two patients, Ec and Pe, with high levels of such antibodies and one patient, Mi, with weaker serum reactivity, were subjected to further RAST studies in which blowfly and screw-worm fly extracts were examined side-by-side. With each serum, positive

Table 1. Results of tests on the subject's serum for IgE antibodies specific for insect allergens

Insect extract	Radioactive uptake (%) of ¹²⁵ I-anti-human IgE with:	
	Subject's serum	Cord serum
Blowfly	20.7	0.8
Blowfly larvae	25.4	1.3
Screw-worm fly adult	17.3	1.0
Screw-worm fly pupae	23.1	1.3
Screw-worm fly larvae	25.0	2.3
Locust adult	1.0	0.4
Locust faeces	0.4	0.5
Common clothes moth	0.7	0.5
Case-bearing clothes moth	1.5	0.8
Furniture carpet beetle	2.4	1.0
Variegated carpet beetle	0.5	0.5
Cockroach	1.2	1.0
Silverfish	0.5	0.4
Vinegar fly	1.9	1.0

reactions were obtained with screw-worm fly adult, pupae and larvae extracts (Table 2).

RAST uptakes for twenty sera from normal, non-allergic subjects did not exceed 2% and were never more than twice the radioactive uptakes recorded for cord sera when used with any of the insect extracts. With serum from the subject employed rearing blowflies, RASTs on some environmental inhalant allergens commonly implicated in allergy in Eastern Australia proved negative; no IgE antibodies were

Table 2. Binding of IgE antibodies to blowfly and screw-worm fly extracts. Results with sera from other asthmatic patients* with IgE antibodies to blowfly allergens

Insect extract	Radioactive uptake (%) of ¹²⁵ I-anti-human IgE with serum			
	Ec†	Mi†	Pe†	Cord
Blowfly adult	8.8	2.5	19.3	0.8
Blowfly larvae	19.0	4.8	13.8	1.0
Screw-worm fly adult	14.1	3.0	21.3	0.9
Screw-worm fly pupae	24.8	3.5	17.6	1.1
Screw-worm fly larvae	21.5	4.6	11.0	1.8

* Patients attending chest clinic, Prince of Wales Hospital with no occupational or other known exposure to blowflies.

† Skin-prick test histamine equivalents for blowfly adult and blowfly larvae extracts respectively: For Ec 2.2 and 2.8; for Mi 1.0 and not done; for Pe 0.7 and 0.6.

detected to house dust mite, ryegrass and couch grass pollens, cat epithelia and the mould *Alternaria*.

Discussion

Experiments described in this communication clearly implicate IgE antibodies in the asthmatic response observed in a laboratory employee engaged in the rearing of large numbers of blowflies. Such reactions are known to occur in individuals involved in the breeding of some other insect species [1–5, 17] and allergic reactions to the American screw-worm fly (*C. hominivorax*), a species in the same sub-family as *C. bezziana*, the screw-worm fly studies here, have been described.

Apart from some notable exceptions [17, 18], little is known of the nature of insect allergens and this is also true for the blowflies. We presume that the allergens are 'emanations' of some sort that are inhaled by humans. According to Bellas [19], such material may be proteinaceous products from hairs, setae and scales, old cuticle (exuviae), faeces or the dried disintegrating bodies of dead flies.

RAST studies with sera from patients who had IgE antibodies to blowfly extracts but who had never been exposed to screw-worm fly demonstrated high levels of antibodies to extracts from both species. It can be safely concluded from their histories that patients Ec, Mi and Pe have never been in contact with screw-worm flies at any stage of its life cycle since the species is not found in Australia. Hence, the IgE antibodies found to these two species appear to have been induced by blowfly antigens and these antigens apparently cross-react with screw-worm fly antigens. Cross-reactivity also exists between the different developmental stages of both species. High uptakes of IgE antibodies occurred with pupae and larvae as well as with adult flies.

Although IgE antibodies in the subject's sera reacted strongly with screw-worm fly extracts, the subject experienced no allergic symptoms while working for a short time in New Guinea with this species. We would not be surprised, however, if an allergic response appeared following a longer period of time in contact with screw-worm flies and/or after handling greater numbers of the insects.

High levels of IgE antibodies that react with blowfly antigens found in three asthmatic subjects who apparently had no abnormal exposure to blowflies suggests that allergic sensitivity to this species may not be as rare as one would imagine. This finding, together with the detection of a high incidence of IgE antibodies to other insect species in fifty asthmatics in whom insect hypersensitivity was not suspected (Kaufman, Baldo, Tovey and Gandevia, unpublished), suggests that allergic reactions to inhaled insect 'emanations' may not be uncommon in the Australian community. Production of insect-reactive IgE antibodies may not, of course, be initiated by exposure to insect antigens but to antigens from apparently unrelated sources. Without further investigations we cannot say whether or not such cross-reactions exist.

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