

IgE-mediated acute asthma following inhalation of a powdered marine sponge

B. A. BALDO, S. KRILIS* and K. M. TAYLOR

*Roche Research Institute of Marine Pharmacology, Dee Why, N.S.W., and
*Immunology Unit, Department of Bacteriology, University of Sydney, N.S.W.,
Australia*

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Summary

IgE-antibodies reactive with extracts of a number of different marine invertebrate-species were demonstrated in the serum of a laboratory worker who experienced an acute attack of asthma following occupational exposure to powdered marine-organisms. Histamine was liberated from the patient's blood following *in vitro* challenge with sponge extract. The desirability of knowing the atopic status of employees in certain occupations is stressed.

Introduction

An unusual case of acute asthma in an adult male, following exposure to a powdered marine-sponge, is described. Radioallergosorbent test (RAST) and histamine release investigations revealed that the asthmatic attack was a true type I, IgE-mediated allergic response and that the subject's serum contained IgE antibodies that reacted with extracts from seven different species of marine sponge and two species of soft coral.

Materials and Methods

Case Report

The patient, a 30-year-old male laboratory worker, had suffered from asthma from the ages of 1-15-years-old. From 15-years-old until 3 months prior to admission to hospital he was asthma-free. While operating a hammer mill to grind a dried sponge *Dysidea herbacea*, the subject became acutely distressed with severe wheezing. Prior to the attack, he had worked for 11 months freeze-drying and then cryogenically-grinding a variety of liquid nitrogen-cooled marine invertebrates and plants. During operation of the hammer mill, finely-powdered dust was frequently released and a mask was

Correspondence: Dr B. A. Baldo, C.S.I.R.O. Wheat Research Unit, P.O. North Ryde, 2113, N.S.W., Australia.

required to be worn over the mouth and nose. When the attack occurred, the subject was operating the mill without a mask.

On admission to hospital, the patient was distressed with a respiration rate of 36 per min, an FEV₁ of 2.4 and FVC of 2.8. On the day prior to hospital admission, the subject had severe tachypnoea and obstruction, an FEV₁ of 2.2 and FVC of 4.0. After bronchodilator therapy with nebulized salbutamol, these values were 3.0 and 4.9 respectively. Chest X-ray showed some patchy consolidation in the lingula and several small densities were also seen peripherally. Blood gases on room air showed pH 7.35, pO₂ 71, pCO₂ 41 and base excess of -1. Sputum culture was normal and eosinophils were seen in the sputum. The patient also had acute urticaria. The results of the other investigations were found to be normal.

The asthma was particularly difficult to control. The patient had episodes of breathlessness requiring oxygen. He experienced bronchospasm with the FEV₁ deteriorating to approximately 1 and FVC to 2.5 (Fig. 1). It became apparent that despite intensive treatment with salbutamol and aminophylline and inhalation therapy with beclomethasone dipropionate, the patient would need systemic steroid-therapy. This was commenced, at first i.v. (Fig. 1) and then orally. After a week the patient's asthma became controllable and he was discharged.

Sera

The patient was bled on admission to hospital and the serum separated and stored at -70°C. Sera from allergic subjects were obtained from the Allergy Clinic, Royal Prince Alfred Hospital, Sydney. Sera from non-allergic, healthy adults were collected

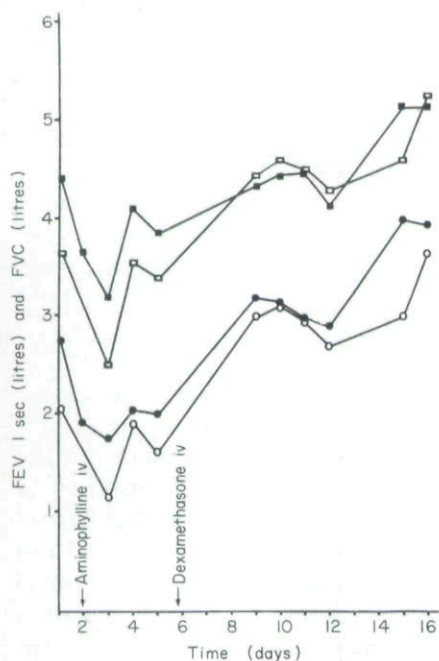


Fig. 1. Sequential changes in FEV₁ and FVC values in patient during the course of his asthmatic attack. FEV₁ before (○) and after (●) inhalation of salbutamol. FVC before (□) and after (■) inhalation of salbutamol.

from laboratory-staff members. Cord sera were provided by St. John of God Hospital, Perth, Australia.

Marine extracts

Sponges, corals, ascidians, anemones, molluscs and algae were collected by diving teams at various localities on the Australian coastline. Organisms were classified taxonomically as far as possible. Extracts for testing were prepared by freeze-drying, cryogenic-grinding and subsequent extraction with water. The organisms tested were: (1) Sponges—*Ancorina alata* (and one other unidentified species of *Ancorina*), *Dysidea herbacea*, *D. fragilis*, *Ianthella basta*, *Ircinia* sp., *Phyllospongia dendyi*, *P. foliascens* (and two other unidentified species from this genus), two unidentified species of the genus *Spongia*, *Stelletta conulosa* and *Tedania digitata*;

(2) Soft corals—*Lemnalia africana*, *Litophyton* sp., *Lobophytum denticulatum* and *Simularia* sp.; and

(3) Algae—*Codium adhaerens*, *Cystophora moniliformis*, *Laurencia elata*, *Plocamium costatum*, *Sargassum globulariaefolium* and the blue-green alga, *Synechococcus* sp.

Other organisms examined were an unidentified species of black coral, the sea pen *Scytalium tentaculatum* and an unidentified simple ascidian.

Allergen discs

Water soluble extracts of marine organisms were coupled to Whatman grade 44 6 mm paper discs using cyanogen bromide as described by Ceska, Eriksson and Varga (1972).

RAST and histamine-release assays

RAST examinations were carried out as previously described (Wide, Bennich & Johansson, 1967; Baldo and Uhlenbruck, 1977).

Histamine released from human leucocytes, following antigen challenge, was determined by the enzymatic-isotopic micro-assay using whole human-blood, S-adenosyl-L-[methyl-³H] methionine and mouse brain as a source of histamine methyltransferase (Taylor, Krilis & Baldo, 1980).

Skin tests

The method and materials employed for prick testing have been described (Baldo, Krilis & Wrigley, 1979).

Results

Prick test studies

Clear, positive prick test responses were obtained from the patient to house dust, house-dust mite (*Dermatophagoides pteronyssinus*) and *Lolium perenne* and *Phleum pratense* pollens. The strongest reactions occurred with the mite and pollen extracts. Weak reactivity was observed with cat-hair extract (Table 1).

Examinations for antibodies that react with marine organisms

Immunodiffusion experiments in agarose gel showed that serum from the patient contained a precipitin that reacted with *D. herbacea* water-soluble extract when the

Table 1. Prick test responses by the subject to some commonly-encountered allergens.

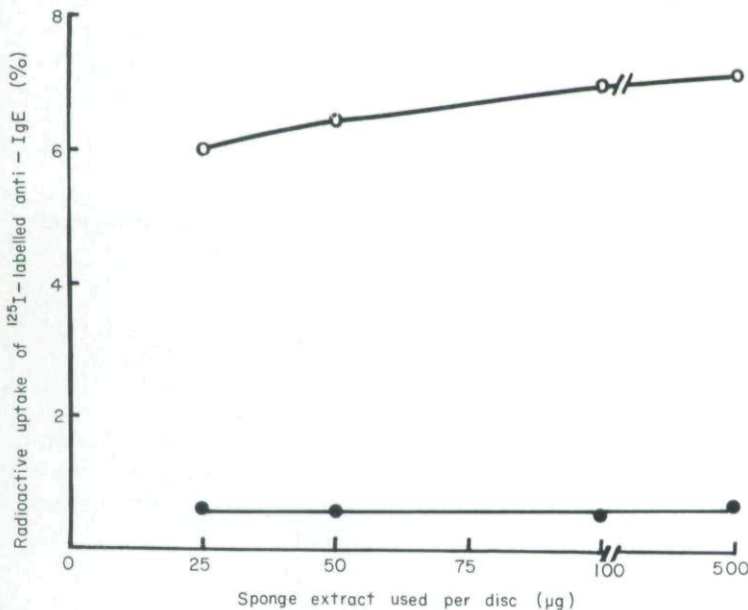
Allergen extract	Relative potencies of prick test solutions*
House dust	1.8
<i>Dermatophagoides pteronyssinus</i>	1.8
Cat hair	0.4
Dog dander	N.R.
<i>Alternaria alternata</i>	N.R.
<i>Aspergillus</i> mix	N.R.
<i>Lolium perenne</i> pollen	3.3
<i>Phleum pratense</i> pollen	2.2

* Relative to histamine acid-phosphate standard used as reference and allotted a potency of 1.

N.R. = No reaction.

latter was tested at 50 and 100 mg/ml. Sera from five normal subjects and from seven different patients, allergic to one or more of the mite and pollen extracts, did not precipitate with the sponge extract.

Dysidea herbacea water-soluble extract was coupled to cyanogen bromide-activated paper discs and examined in direct RAST experiments with sera from the patient, normal and other allergic individuals and cord blood. Serum from the patient reacted with the coupled discs producing a maximum radioactive uptake of 7.0–7.3% when discs reacted with 100–500 μ g of extract were used (Fig. 2). Similar uptakes were obtained when discs coupled with *D. fragilis* were used. By contrast, radioactive

**Fig. 2.** Uptakes of ¹²⁵I-labelled anti-IgE after incubating patient's serum (O) and cord serum (●) with paper discs coupled to increasing quantities of *Dysidea herbacea* water-soluble extract.

uptakes with sera from five normal and seven allergic subjects and from a sample of cord blood were in the range 0.6–1.5%

Although the patient's asthmatic attack appeared to be precipitated by the inhalation of powdered *D. herbacea*, the possibility that another marine extract(s) was the sensitizing agent could not be excluded. A number of species that had previously been subjected to cryogenic-grinding by the patient and other species, selected at random, were therefore also examined in RAST experiments with the patient's serum. This study revealed that discs, coupled with seven different sponge-extracts and two species of soft coral, clearly reacted with IgE antibodies present in the patient's serum. (Fig. 3).

Many marine invertebrates are known to contain lectins (Baldo, Uhlenbruck & Steinhausen, 1977; Goldstein & Hayes, 1978). It seemed possible therefore, that the sponge and soft-coral extracts coupled to discs might bind IgE antibodies via the carbohydrate portion of the immunoglobulin (Uhlenbruck *et al.* 1978). The failure of the other sera, from mite- and pollen-allergic subjects, to produce positive radioactive uptakes in RAST experiments and the absence of lectin reactivity in the *Spongia* and *Lobophytum* spp. which reacted in the RAST with serum from the patient, suggested that lectins were not involved in the binding of the patient's serum to the discs. To further investigate this point however twenty different marine extracts with known

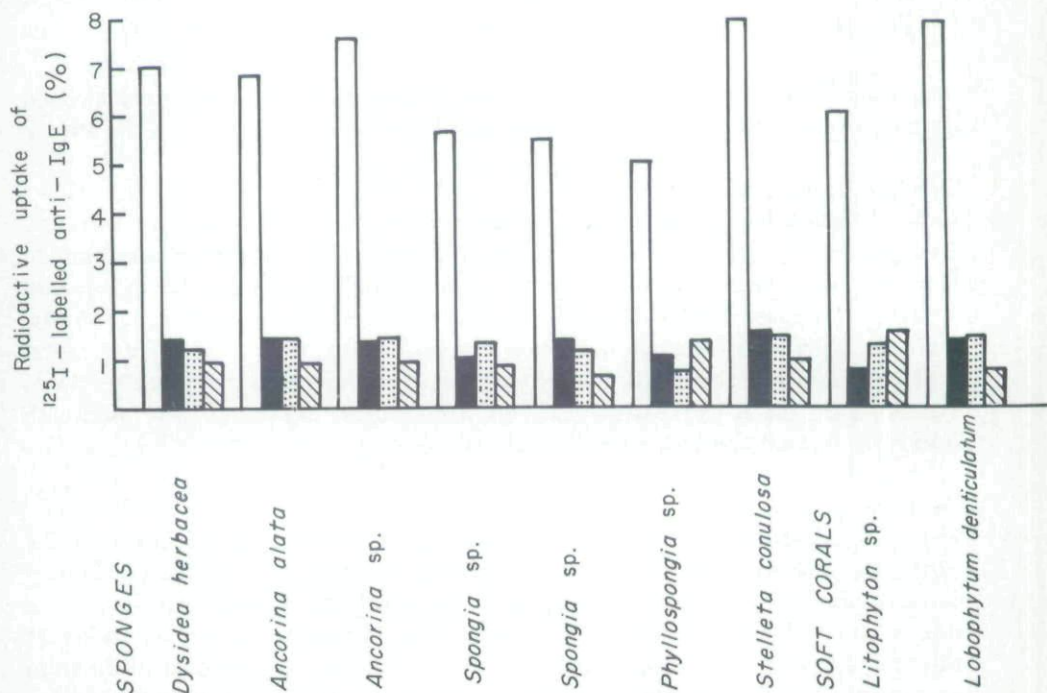


Fig. 3. Estimation by RAST of the presence of IgE antibodies reactive with marine extracts. Patient's serum, serum from cord blood (IgE-free control) and sera from normal and mite- and/or pollen-allergic subjects (50 μ l) were used with cyanogen bromide-activated paper discs coupled to various marine extracts (see Materials and Methods). Only those extracts that gave a positive reaction are shown. The presence of allergen-specific IgE in sera was determined from the percentage of radioactive uptake by coupled discs of ^{125} I-labelled anti-IgE. □, Subject; ■, allergic subject; ▨, normal subject; ▩, umbilical cord.

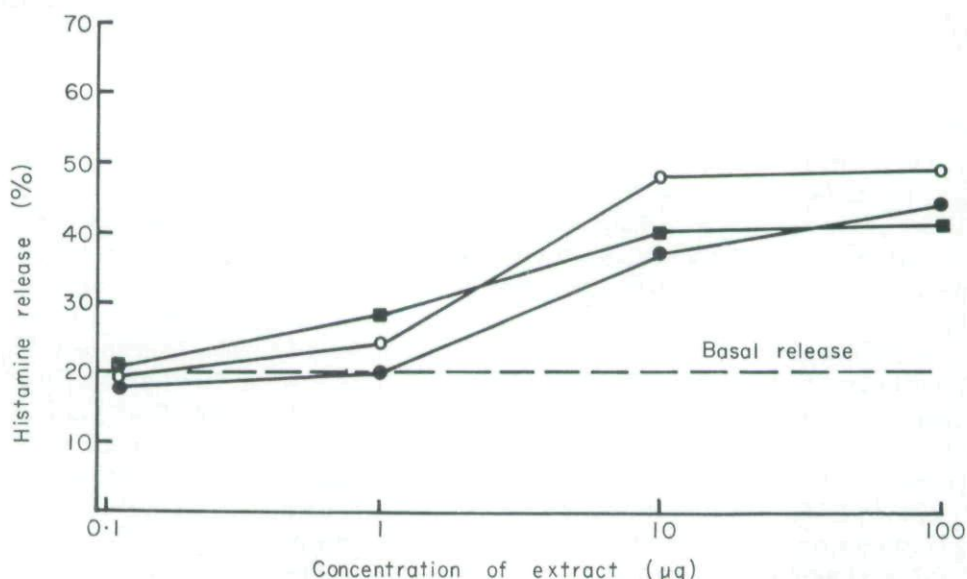


Fig. 4. Allergic histamine-release following challenge of aliquots of patient's whole blood (200 μ l) with house-dust mite extracts (Bencard, U.K.) *Dermatophagoides farinae* (○) and *Dermatophagoides pteronyssinus* (●) and sponge *Dysidea herbacea* extract (■). Concentrations of extracts shown on the abscissa are μ g/ml. Histamine was estimated using the enzymatic-isotopic micro-assay (Taylor *et al.*, 1980).

lectin content were used in RAST experiments with serum. This study revealed no correlation between the presence or absence of lectins and reactivity with the serum.

Histamine-release studies

Aliquots of whole blood from the patient were challenged with *D. herbacea* extract and the amount of histamine liberated was measured and compared with results obtained when house-dust mite extract was used for *in vitro* challenges. Both the sponge and mite extracts released 40.0–50.0% of the total histamine when used in the range 10–100 μ g of extract per tube. Even without the addition of allergen, up to 20.0% of the total blood histamine was released (Fig. 4). No release of histamine above the basal level was observed when the RAST-positive extracts from marine organisms were used with blood from normal and mite or pollen-allergic subjects.

Discussion

Experiments described in this communication clearly implicate IgE antibodies in the severe asthmatic response observed in a laboratory assistant, working with powdered marine-organisms, following inhalation of a powdered sponge. However, the demonstration of IgE antibodies reactive with other sponge species and two species of soft coral, suggests that the patient had become sensitized to a number of different invertebrate species or that the IgE antibodies involved cross-reacted with the various species. An answer as to which of these two possibilities is correct could only be provided by absorption and inhibition studies and, preferably, by the isolation and comparison of the IgE-binding components from the sponges and soft corals.

In addition to a type I reaction, the involvement of a type III reaction in the patient's asthma was indicated by the prolonged nature and severity of the reaction,

failure of aminophylline and salbutamol to control the attack, the X-ray picture and the presence in the serum of precipitating antibodies specific for *D. herbacea* (Pepys, 1971).

Reports of immediate hypersensitivity reactions following inhalation of marine-invertebrate materials are, not surprisingly, rare. Immediate respiratory hypersensitivity following contact with abalone (*Haliotis ruber* and *Schismotis laevegata*) has been reported by Clarke (1979) and Karlin (1979) has described a case of occupational asthma following exposure of a woman to powdered clam-liver extract.

Although there is no evidence available to indicate that the powdered marine-organisms used are potent allergen-sources likely to provoke allergic reactions in many people, the allergic asthmatic-attack experienced by the patient was sufficiently severe to warrant systemic steroid-therapy. However, the patient had suffered from asthma since infancy and this may be the most relevant information in understanding and learning from this unique case. There are several occupations in which employees are exposed to allergenic materials. In at least one of these, the baking industry, it has been suggested that a person with a previous or current atopic illness should not enter the baking trade where he will be exposed to flour dust (Jarvinen *et al.*, 1979).

The present case illustrates the desirability of knowing the atopic status of workers likely to be exposed to materials which may be potentially allergenic if inhaled in sufficient quantities. It follows therefore that one should be wary of placing an asthmatic individual in a job where large amounts of dust are generated. Of course, for this to occur, one would need to know the atopic status of the prospective employee and although in the present case the subject was aware of his asthma, his employer was not. As a result of this unusual example of allergic asthma and in an effort to avoid similar sensitizations, the employer involved in this case had dust extractors installed and now requires that both old and new employees be subjected to prick testing with a panel of common allergen-extracts. These are positive occupational health measures that may be increasingly and more widely used as recognition of potential allergen-sources in the work place increases.

Acknowledgments

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