

Short communication

Bee moth (*Galleria mellonella*) allergic reactions are caused by several thermolabile antigens

Background: Exposure and contact with bee moth (*Galleria mellonella*) larvae (Gm) can cause an allergic reaction both in anglers and breeders. We described the case of an amateur fisherman who experienced an allergic reaction using Gm but not using heat-treated Gm (h-Gm) (mummies). The aim of this study was to demonstrate by immunoblotting and radioallergosorbent test (RAST)-inhibition experiments the loss of allergenic epitopes in h-Gm extracts.

Methods: *Galleria mellonella* larvae and h-Gm were homogenized and extracted at 10% (w/v) in 0.5 M phosphate-buffered saline, pH 7.4 containing 0.5% NaN₃ for 16 h at 4°C. Gm and h-Gm extracts were electrophoresed in a 10% polyacrylamide precast Nupage Bis–Tris gel at 180 mA for 1 h and the resolved proteins stained with 0.1% Coomassie brilliant blue and the molecular weight calculated. For the immunoblotting detection of allergenic components the resolved extracts were transferred onto a nitrocellulose membrane and incubated with the patient's serum. Bound specific-IgE was detected by peroxidase-conjugated anti-human IgE. RAST inhibition experiments were performed according to the Ceska method.

Results: The protein profile of Gm and h-Gm extracts resulted markedly different in number, intensity and the position of bands, indicating that heat-treatment modifies the chemical–physical characteristics of the protein contents. The Gm extract showed a strong-coloured band at 73 kDa and more than 20 components ranging from 12 to 133 kDa; h-Gm showed two main band at 77 and 38 kDa and about 15 faint bands between 20 and 133 kDa apparently without any correspondence to the bands present in the Gm extract. Immunoblotting with the patient's serum demonstrated several bands of reactivity with the Gm extract ranging from 20 to 100 kDa and no recognizable bands, but only a diffuse smear with h-Gm. When used in a RAST inhibition experiment the h-Gm extract demonstrated an inability to compete with the Gm one for the binding to patient's IgE serum.

Conclusions: The h-Gm seems to lose the allergenic epitopes and has two advantages for anglers: to avoid new possible sensitizations as well as allergic symptoms in sensitized people, without interfering with their skills and satisfaction in their fishing performance.

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Exposure and contact with live fish baits can cause allergic reactions both in anglers and breeders. Indeed, angioedema, urticaria, rhinitis, asthma and contact dermatitis associated with exposure to the bluebottle (*Calliphora vomitoria*) (1), greenbottle (*Lucilia caesar*) (2), housefly (*Musca domestica*) (3), flesh fly (*Sarcophaga carnaria*) (4), mealworm (*Tenebrio molitor*) (5), earthworms such as *Eisenia foetida* (6) and marine worms such as *Marphysa sanguinea* (7) were previously described.

Bee moth (*Galleria mellonella*) larvae (Gm) are frequently used in river and lake fishing, and recent reports

demonstrated that they can cause allergic sensitization, especially among amateur fishermen (8, 9). Recently, heat-treated *Galleria mellonella* larvae (h-Gm) (mummies) were released on the market. These larvae produce similar fishing results to those achieved with live bait and can be stored for a considerable length of time with no special storage measures.

In this study, we reported the case of an angler who experienced allergic reactions when he used Gm as fishing bait but not when he used h-Gm and we were able to demonstrate the loss of the majority of the allergenic proteins in h-Gm extract in respect to Gm extract by

immunoblotting and radioallergosorbent test (RAST)-inhibition experiments.

Materials and methods

Case report

A 40-year-old man, with no personal history of atopic disorders, was studied in our outpatient clinic. In the last 2 months he had complained of pronounced symptoms with nasal itching, sneezing, rhinorrhea dyspnoea and wheezing immediately after the exposure to the bee moth larvae used as fish bait for angling in rivers and lakes. Symptoms persisted for 1–2 h after the exposure and in the last attack he experienced a late asthmatic reaction during the night, for which he had required emergency room treatment, where he was treated with systemic steroids and β_2 stimulating drugs. Ten days later he came to our outpatient clinic for the aetiological study.

Physical examination was normal as well as was a basal spirometry. Skin prick tests carried out with the common inhalant allergens and the detection of serum IgE (Pharmacia CAP System, Uppsala, Sweden) resulted negative. The prick-by-prick test with fresh Gm minced with a lancet and diluted 1 : 2 with saline was clearly positive with a wheal of 2.2 cm and pseudopodes, while four healthy volunteers tested with the same procedure were negative. The patient was diagnosed as allergic to Gm and he was advised not to use the Gm as fishing bait in the future. Two weeks later, without a previous consultation with the allergist he used h-Gm as fishing bait and he did not experience any allergic reaction. The same happened on other occasions. When he went back for a check-up he reported what had happened and immediately a blood sample was collected for immunoblotting and RAST inhibition testing.

Preparations of extracts

Galleria mellonella larvae were homogenized and extracted at 10% (w/v) in 0.15 M phosphate-buffered saline (PBS), pH 7.4 containing 0.5% NaN_3 for 16 h at 4°C. An analogous extract was prepared starting from the h-Gm of the same commercial kind used by the patient as fishing bait. After removing debris and insoluble material by centrifugation at 10 000 g for 20 min supernatants were dialyzed against PBS and filtered through a 0.22 μm membrane. The protein content of Gm and h-Gm extracts, as determined by the total protein test kit (Bio-Rad, Hercules, CA, USA), was 5.2 and 0.5 mg/ml, respectively.

SDS-PAGE and immunoblotting

Thirty microlitres of Gm and h-Gm extracts were electrophoresed in a 10% polyacrylamide precast Nupage Bis-Tris gel, according to the manufacturer's instructions (Novex; Prodotti Gianni, Milan, Italy), at 180 mA for 1 h. The resolved proteins were stained with 0.1% Coomassie brilliant blue and their molecular weight was calculated using the ImageMaster (Amersham Pharmacia-Biotech, Milan, Italy) software. For immunoblotting detection of allergenic components the resolved extracts were transferred onto a nitrocellulose membrane according to Towbin et al. (10). The membrane was saturated in TBS buffer containing 2% defatted dry milk (saturating buffer) for 1 h, before being incubated with the patient's serum or with a pool of sera from nonallergic subjects, both diluted 1 : 10 in a saturating buffer. Bound specific-IgE was detected by peroxidase-conjugated anti-human IgE, using an ECL western blotting kit (Amersham Pharmacia-Biotech) as substrate.

Preparation of solid phase for RAST inhibition

Polystyrene beads (6.4 mm diameter; Precision Plastic Ball, Chicago, IL, USA) were coated with Gm extract according to the following procedure. Beads were pretreated with glutaraldehyde 0.2 M in PBS for 5 h at room temperature before being incubated overnight in end-over agitation with 150 μl /bead of Gm extract (at a concentration of 10 $\mu\text{g}/\text{ml}$ in PBS). After several washings with PBS the beads were stabilized with NaHBO_3 0.37% in PBS for 1 h and finally saturated with 5% BSA in PBS for 24 h and stored at 4°C until use.

RAST inhibition

RAST inhibition experiments were performed according to Ceska (11), in order to verify the effect of heat-treatment on Gm allergenic activity.

Briefly, a serial twofold dilutions (as triplicate) of both Gm and h-Gm extracts in 1% BSA in PBS was preincubated for 3 h with the patient's serum. Subsequently, one Gm extract-coated bead, prepared as previously described, was added to each tube and incubated overnight. Bound IgE were detected by ^{125}I -labelled anti-human IgE goat (KLH; Celbio, Milan, Italy). The residual radioactivity was measured in a gamma scintillation counter and data was expressed as a percentage of inhibition with respect to a maximum binding obtained without an inhibitor.

Results

SDS-PAGE and immunoblotting

The protein profile of the two extracts examined (Gm and h-Gm) resulted markedly different in number, intensity and position of bands indicating that heat-treatment modifies the chemical-physical characteristics of the h-Gm extract (Fig. 1, lanes A and B). While Gm extract showed a strong-coloured band at 73 kDa and more than 20 components ranging from 12 to 133 kDa, the h-Gm extract, besides a lowered quantity of extractable proteins (0.5 vs 5.2 mg/ml), showed a different pattern with two main bands at 77 and 38 kDa and about 15 faint bands between 12 and 133 kDa, but apparently with no correspondence to the bands present in the Gm extract. In the same way, the patient's serum demonstrated a different IgE reactivity to the Gm and h-Gm extract by immunoblotting, showing a strong reactivity with several Gm extract components above 20 kDa, in the first case (Fig. 1, lane C) and only a diffuse smear without any recognizable band in the second one (Fig. 1, lane D).

RAST inhibition

When used in RAST inhibition experiments, the h-Gm extract demonstrated an inability to compete with the Gm one for the binding to patient's IgE serum. In fact no reduction in the IgE reactivity of the serum against Gm-coated solid phases was found, when the h-Gm extract was used as inhibitor. In contrast, the use of the Gm extract itself as inhibitor resulted in a dose-response

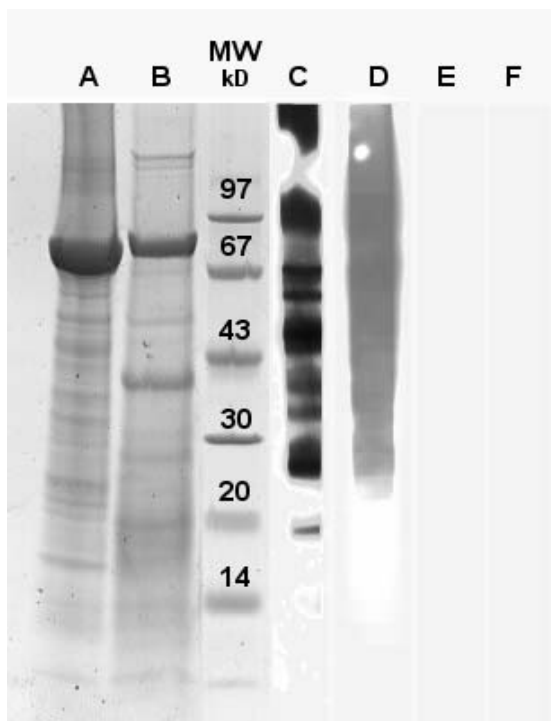


Figure 1. SDS-PAGE and immunoblotting experiments with *Galleria mellonella* (Gm) and heat-treated *Galleria mellonella* (h-Gm) extracts. Lane A, Gm extract; lane B, h-Gm extract; lanes C and D, IgE reactivity of patient's serum against Gm and h-Gm extracts, respectively; lanes E and F, IgE reactivity of normal serum against Gm and h-Gm extract, respectively.

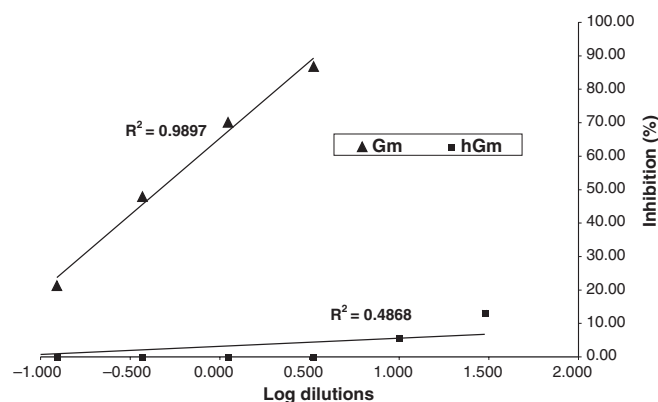


Figure 2. RAST inhibition experiments using Gm-coated solid phase (▲, inhibition by Gm extract; ■, inhibition by h-Gm extract).

inhibition curve with a good correlation coefficient ($R^2 = 0.98$) (Fig. 2).

Discussion

Many cases of sensitization to insect larvae used as fishing bait have been reported in the literature. *Galleria*

mellonella larvae are frequently used in particular in trout fishing in European countries and represent one of the possible sources of allergy in anglers and occupationally exposed workers (8). In the latter category, the frequency of sensitization appears to be relatively high (12). Up to the present day, all the major Gm allergens are still unknown, it is most likely that they are represented by one or several of the numerous proteins to be found in the haemolymph. Some of these proteins such as the ferritin, composed of two major polypeptides of 26 and 32 kDa and one minor polypeptide of 30 kDa, the haemoagglutinating agent (18 kDa), the apolipoprotein III, the male-specific protein (55 kDa), the storage proteins (74, 76 and 81/82 kDa, respectively), the low molecular mass silk proteins (24 and 30 kDa), the epidermis-origin haemolymph protein (44 kDa), the juvenile hormone-binding protein, and the inducible antimicrobial peptides have been detected, purified and characterized. The presence of a few of these proteins and their number, however, depends on the stage of their larval development, as well as the temperature at which the larvae have been kept, thus the proteic profiles can change according to the variables formerly described.

Recently, Gm larvae treated to prolong their conservation and known as 'mummies', have been released on the market. Even if such procedures are subject to strict manufacturing secrecy, and probably vary from manufacturer to manufacturer, at the basis, the larvae always undergo a heat-treatment. We were able to demonstrate that this procedure modifies the chemical-physical characteristics of the proteic components and consequently their antigenicity. The SDS-PAGE, in fact demonstrated that the bands obtained with the GM extract do not appear to match those of the h-Gm extract and the immunoblotting performed on the patient's serum revealed several reactivity bands ranging from 20 to 100 kDa with the Gm extract. However, for the h-Gm extract there was only a diffuse signal, which was probably a result of an aspecific effect. This data, together with the inability of the h-Gm extract to prevent the bonding of the patient's IgE to a Gm-coated solid phase, confirms the loss of antigenicity of the h-Gm extract and explains the lack of allergy symptoms reported by the patient under our care when he fishes using the bee moth 'mummies'. However, seeing that at the moment the production processes of the 'mummies' are not standardized, and cannot only vary from manufacturer to manufacturer, but also from batch to batch of the same manufacturer, their lack of allergenicity cannot be definitively asserted by the data we have obtained. To be able to give a definitive answer, both immunoblotting and inhibition RAST studies will have to be performed on several products on the market and on different batches of the same products.

In conclusion, by studying the serum of a patient with a bee moth allergy, we have demonstrated that he reacts with different proteic components ranging from 20 to

100 kDa of a Gm extract, but does not show any reactivity if combined with h-Gm extract, revealing that the main allergenic epitopes are thermolabile. From this data we can conclude that the use of the bee moth

'mummies' gives anglers two advantages, in that they avoid new sensitizations as well as allergic symptoms in hypersensitized people without interfering with their fishing performance.

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