



Letter

Bee moth (*Galleria mellonella*) allergy New insight from a case of monosensitization

We present a case of bee moth (*Galleria mellonella*) allergy with new clinical and immunologic features. While taking part in trout fishing games during the past year, a 34-year-old amateur fisherman with no personal history of allergic diseases experienced several attacks of rhinoconjunctivitis and late asthmatic reactions. Symptoms of rhinoconjunctivitis began when starting to fish and persisted during the day. This was followed by mild wheezing and dyspnea during the following night. Symptoms worsened during the most recent fishing excursion, and mild breathing difficulty tends to last until the night of the next day. In the latest episode, he had a strong asthma attack during the night, which required treatment in the emergency department with intravenous steroids and salbutamol spray. The results of skin prick tests (SPTs) and IgE dosing for a large panel of airborne allergens were negative.

We later performed a prick-by-prick test with living bee moth larvae, the bait the patient used for trout fishing. The test produced a strong positive reaction (irregularly outlined wheal area, 34 × 9 mm). Although the histamine skin reaction faded gradually, the *G mellonella* site had a progressive increase of edema and erythema, which peaked at 6 to 8 hours, when it broadened to approximately half of the forearm, lasting with no significant changes up to 12 hours. Eighteen hours after the test, the skin reaction had gradually decreased, but at 24 hours a red papule surrounded by a relatively small area of pale erythema and edema was clearly evident. After 2½ days, a small papule on a little palpable area of indurate skin was still visible.

Because a previous study found no reactivity to heat-treated *G mellonella* larvae (mummies),¹ we performed a prick-by-prick test with a mummified, uncolored larva. Surprisingly, the test elicited a strong immediate response (wheal area, 13 × 10 mm) followed by a late-phase reaction similar to that seen with the living larva, which disappeared after approximately 30 hours. Prick-by-prick tests performed with both live and mummified larvae in 2 non-allergic controls had negative results.

A challenge test was performed simply by opening a commercial package that contained 35 live baits in the presence of the patient. A honey-like smell spread out in the room, causing immediate symptoms of rhinoconjunctivitis in the patient. No symptoms were triggered with a box of mummified larvae, and fishing with mummies did not elicit any symptoms.

To study the allergens responsible for the reaction, proteins were extracted from both raw and heat-treated larvae with phosphate-buffered saline (soluble proteins) and with a denaturing buffer (total proteins). Sodium dodecyl sulfate–polyacrylamide gel

electrophoresis in reducing conditions and immunoblotting analyses were performed as previously described.² IgE and IgG (subclasses 1, 3, and 4) binding was revealed by specific secondary antibodies. The serum of a healthy individual was used as the control (not shown). Immunoblotting analysis of raw *G mellonella* revealed a strong IgE-binding band with a mobility of approximately 27 kDa in both the soluble and total protein extracts, which could correspond to the hemolymph glycoprotein, previously identified as a bee moth allergen.³ Another band was detected in both the soluble and total protein extracts, where found mobilities of 45 and 40 kDa, respectively. In contrast, the analysis of heat-treated *G mellonella* revealed a single IgE-binding band of approximately 20 kDa, suggesting that cooking the larvae changes protein extractability and pattern (Fig 1). This latter band was found in the total protein extract but not in that obtained with phosphate-buffered saline (soluble protein). This could be why no thermostable allergens were found in previous study.¹ Despite the presence of IgE, it is not surprising that handling and fishing with bee moth mummies did not challenge respiratory symptoms because they seem to be caused by inhalation of living insect emanations.

Skin late-phase reaction (LPR) to bee moth and others insects used as fish bait has never previously been reported. In our case, the development of the skin LPR was continuous with the immediate reaction (not biphasic) and elicited by prick tests with both alive and heat-treated larvae. Skin LPRs are usually induced by intradermal injection (not by SPT) of sufficiently high concentrations of allergen. The inflammatory process demonstrates itself to be uninterrupted, but skin and respiratory responses can appear biphasic because early and late reactions have different thresholds.^{4,5} Depending on individual sensitivity, by increasing the allergen doses, the flare and wheal reaction proceeds without discontinuity into LPR.^{4,5} Provocation by SPT (instead of intradermal test) entails a very high degree of hyperactivity and a strong sensitizing capability of the *G mellonella* allergens.

The pattern of progressive development of skin response from immediate to long-lasting LPR, clearly matched with the evolution of symptoms in our patient. Specific IgE plays a pivotal role in the induction of both early- and late-phase pathophysiologic responses, and skin LPR generally represents a suitable model of chronic allergic inflammation and as a whole reflects the development of clinical disease. However, LPR proved to be a complex pathogenetic process not completely explained^{5,6} in which skin infiltration of eosinophils, basophils histamine release, and T lymphocytes seem to play a key role, although other cells, cytokines, and chemokines are involved.⁶ On the basis of the similar features and course of LPR and the immune complex–mediated Arthus reaction and assuming as a model the allergic bronchopulmonary

Disclosures: Authors have nothing to disclose.

<https://doi.org/10.1016/j.anai.2017.09.057>

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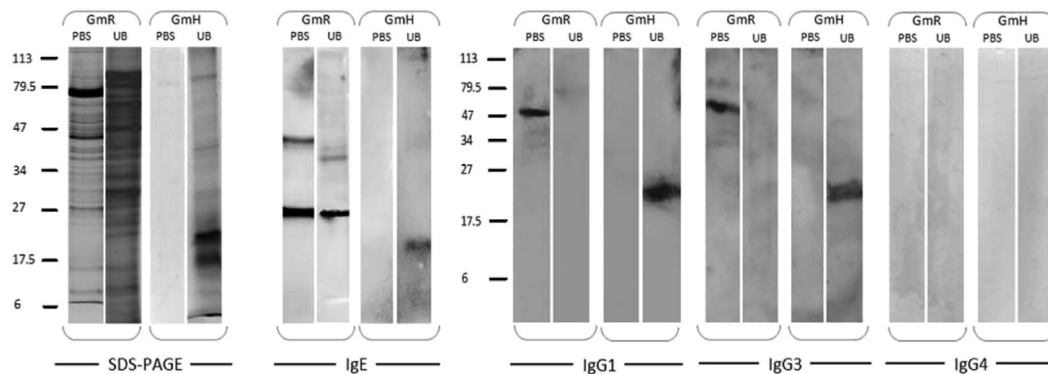


Figure 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting analyses of *Galleria mellonella* soluble (phosphate-buffered saline [PBS]) and total proteins (urea buffer [UB]) obtained from raw (GmR) and heat-treated (GmH) *G. mellonella* larvae. Proteins were revealed with Coomassie Brilliant Blue (SDS–PAGE) and patient's serum IgE IgG subclasses 1, 3, and 4 binding by using the corresponding anti-antibodies.

aspergillosis (a condition in which allergen specific IgE and IgG coexist), a role of precipitating IgG antibodies was previously suggested. However, other studies failed to detect deposition of complement and immunoglobulins in allergen-induced skin LPR in atopic individuals.⁷

To discover whether specific IgG antibodies could be involved in the development of skin reactions and clinical symptoms, anti-human IgG1, IgG3, and IgG4 antibodies were used to detect the presence of patient's IgG binding to the larvae proteins in immunoblotting. A thin band of approximately 47 kDa in the soluble protein extracts of raw *G. mellonella* and a well-defined band of approximately 20 kDa in the total protein extract of heat-treated *G. mellonella* were detected by anti-IgG1 and anti-IgG3 antibodies. These results were confirmed by 2 independent laboratories. It is reasonable that the 20-kDa protein corresponds to the same hemolymph allergen³ found in living larvae and recognized by both IgE and IgG antibodies. No IgG4 antibodies were found (Fig 1).

The IgG4 immune response in general indicates that tolerance mechanisms have been activated. Conversely, IgG1 and especially IgG3 are potent proinflammatory factors because they can efficiently trigger the classic complement route and are highly effective in the induction of effector mechanisms of the immune response. For the first time to our knowledge, we found in the present case study serum IgG specific for the same *G. mellonella* proteins recognized by IgE antibodies. Unfortunately, because of the patient's refusal, we could not obtain skin biopsy specimens to detect deposition of complement and immunoglobulins into the skin. However, a number of studies found that both specific IgE and IgG are usually involved in the pathophysiologic mechanisms of the hypersensitivity reactions to some insects.⁸

Allergic sensitization to bee moth seems to be uncommon, but the matter is of great interest as the practice of eating insects starts to spread to Western countries. Consumption of edible insects (which include bee moth) for human nutrition has been fostered by the Food and Agriculture Organization and approved by regulatory authorities in some European countries.^{9,10} Atopy has been indicated as a possible risk factor,⁹ and the risk of allergic reactions to insect proteins, by cross-reactivity or primary sensitization, has been stressed in advisory reports.¹⁰

In the present case study, we have observed for the first time, to our knowledge, immediate and late skin reactions to heat-treated *G. mellonella* larvae and IgE binding to thermostable allergens. With the introduction of insects as foods for humans, we should expect

and take into consideration the risk of sensitization and allergy systemic reactions from the consumption of bee moth and other edible insects.

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