

Propolis, a new bee-related allergen

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● PROPOLIS, or “bee glue”, is the generic name for the resinous substance collected by honeybees from various plant sources. Contact dermatitis to propolis in beekeepers has been known for years (1). Nevertheless, in the literature, there are no studies reporting immediate hypersensitivity to propolis.

We report the case of a 10-year-old boy, resident in a rural environment, who helped his father (a beekeeper) in the manipulation of beehive products (honey, propolis, beeswax, and pollen). He had suffered several attacks of angioedema for some years, but these symptoms appeared only in association with the manipulation of these products. He was free of symptoms when not working with his father.

Two years before, he had experienced an immediate systemic reaction within 5 min of a honeybee sting, with urticaria, angioedema, severe hypotension, and fecal and urine incontinence, which required his admission to the intensive care unit. Previously, he had tolerated bee stings well. An allergologic study was then performed, and sensitization to honeybee venom was demonstrated by skin prick test with 1 µl/ml (wheal 10×12 mm) and by the CAP commercial method (class 3). He was treated with specific immunotherapy and experienced adverse effects that required a dose reduction on several occasions.

Specific allergologic tests were performed to study the present angioedema attacks.

In vivo methods. Skin prick tests with common inhalant allergens and foods were weakly positive for grass pollens. Saline solution and histamine (10 mg/ml) were used as negative and positive controls, respectively. Tests were considered positive when the wheal had a diameter greater than 3 mm.

The prick-to-prick test was positive for propolis (wheal 10×5 mm) and negative for beeswax and honey. Propolis was tested in six controls (two beekeepers, two nonatopic subjects, and two atopic subjects) and did not elicit any reactions. *In vitro methods.* Specific IgE against bee venom by the CAP commercial method was positive (3.12 kU/l, class 2).

For analysis of the antigen (bee propolis), a propolis extract was prepared in phosphate-buffered saline (PBS) at 10% w/v; after dialysis, the extract proteins were covalently bound to BrCN-activated paper disks by the method of Ceska et al. (2). The protein concentration of propolis extract was 73.5 µg/ml.

Measurement of propolis-specific IgE was carried out by RAST. The propolis disks were incubated with 50 µl of sera from patient/controls and afterward with ≈100 000 cpm of ¹²⁵I-anti-IgE mAb HE-2 (3). As reference, disks sensitized with *Lolium perenne* were used, as well as four dilutions of a pool of sera from grass-pollen- allergic patients, previously calibrated with the Pharmacia Phadebas[®] RAST system. Specific IgE against propolis was positive for our patient (class 3) and negative for the pool of sera from beekeepers used as control.

Measurement of propolis-specific IgG was performed by ELISA. The ELISA plates were coated with different concentrations of propolis extract from 35 to 0.5 µg/ml (50 µl/well, at 4°C for ~18 h).

After saturation with PBS-BSA, the wells were incubated with 50 µl of sera from patient/controls at different dilutions and then with an antihuman IgG antibody labeled with peroxidase (Dako cod. PO214), 1/10 000 dilution. Lastly, the wells were incubated with peroxidase-substrate buffer, and the absorbance of wells was read at 490 nm. Specific IgG to propolis was positive for our patient and for the pool of sera (beekeepers) used as control.

In conclusion, we report the case of a child who presented an adverse, IgE-mediated reaction to propolis (previously sensitized to bee venom) demonstrated by means of *in vivo* and *in vitro* tests. To our knowledge, there are no reported cases of immediate hypersensitivity to propolis. Therefore, the allergologist should be aware of this allergen in beekeepers as a cause not only of contact dermatitis but also of allergic reactions.

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