

Anaphylaxis induced by horsefly bites: Identification of a 69 kd IgE-binding salivary gland protein from *Chrysops* spp. (Diptera Tabanidae) by western blot analysis

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Anaphylaxis from insect venom is mostly caused by Hymenoptera stings but has been also reported after bites by various blood-feeding arthropods. The horseflies (Tabanidae, comprising some taxonomically poorly defined subgroups such as deerflies, yellowflies, or clegs) is a hematophagous family of Diptera, with several species that feed on human beings. Bites by tabanids are painful and may induce a local wheal and flare response. However, they have also been believed to cause severe systemic reactions.¹⁻⁴ The involvement of specific IgE antibodies in these reactions has been proposed in earlier studies.²⁻⁴ The insufficiency of currently available diagnostic tools in the analysis of hypersensitivity to biting insects, however, makes the stigma of anecdote adhere to many reports and may have caused general underreporting of anaphylaxis caused by non-Hymenoptera insects in the literature.

CASE REPORT

A 57-year-old man was bitten on his left foot by an unidentified horsefly species. Within 10 minutes he had generalized itch, urticaria, and angioedema, followed by pharyngeal constriction, shortness of breath, and a final 30-minute episode of unconsciousness. He recovered without specific therapy after an unknown period of

time. A second horsefly bite received 1 week later on the chin again provoked a systemic reaction. Despite immediate administration of astemizole, the patient had nausea, vomiting, shivers, profuse perspiration, and impairment of the sense of taste. There was no history of atopy or preceding insect hypersensitivity.

METHODS

The patient was referred to the allergy clinic 4 months later. He was intradermally tested with honeybee and yellow jacket venom (ALK) and skin prick tested with mosquito extract (Stallergenes) and common inhalant allergens. Because of a lack of an appropriate allergen extract, no horsefly species could be used for skin testing. Total IgE (Pharmacia) as well as specific IgE (as determined by RAST) against Hymenoptera (i.e., honeybee, yellow jacket, hornet, and fire ant), mosquito (*Aedes* spp.), Chironomids, and horsefly (*Tabanus* spp.) were assessed (all extracts obtained from Pharmacia).

Host-seeking female horseflies (*Chrysops* spp.) were collected in the field, immediately frozen in dry ice, and stored at -20° C. Salivary glands were removed from 10 specimens in phosphate-buffered saline under a stereo microscope, transferred to fresh buffer, homogenized under liquid nitrogen, and centrifuged for 30 minutes at 13,500 rpm. The supernatants were dialyzed for 3 days in distilled water and lyophilized, yielding about 1 µg of protein per salivary gland pair. Resuspended proteins were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis and either stained with silver nitrate or blotted onto nitrocellulose membranes. Nitrocellulose strips were incubated with 1:10 diluted sera from the patient and four nonatopic control subjects without a history of insect hypersensitivity. Specific IgE was detected by 1:10 diluted iodine 125-labeled rabbit anti-human IgE (Pharmacia). Specific IgG was detected by monoclonal rabbit anti-human IgG (Jackson Dianova) followed by ¹²⁵I-labeled donkey anti-rabbit IgG (Amersham).

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J Allergy Clin Immunol 1998;101:134-6.

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0091-6749/98 \$5.00 + 0 1/54/85987

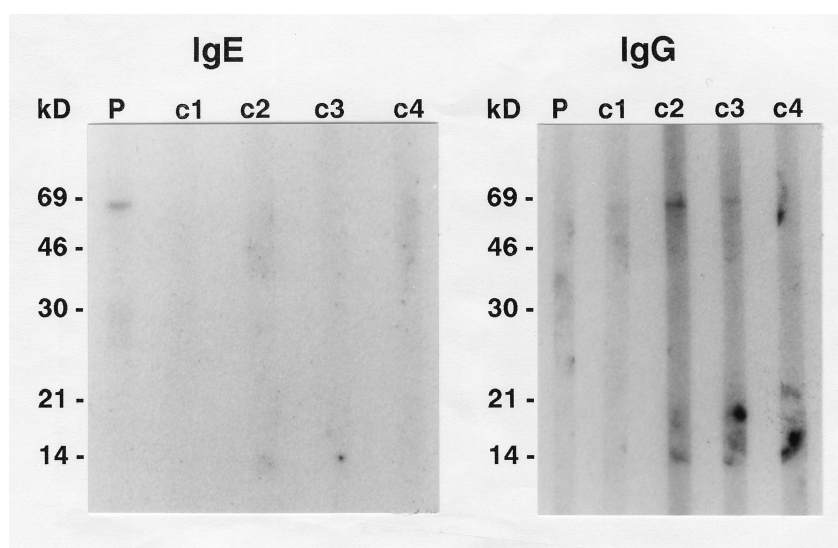


FIG. 1. Serum IgE and IgG reactivity to salivary gland extract from the horsefly (*Chrysops spp.*) showing antibody binding to a 69 kd protein in patient (lane P) and control subjects (lanes c1-c4).

RESULTS

Skin test responses were completely negative to inhalant allergens and mosquito extract, as well as to the vespid venoms (apart from a weakly positive reaction with 0.1 μ g/ml intradermal honeybee venom that was not considered relevant). RAST results were negative for all tested insects. Total IgE levels were elevated (234 kU/L).

Silver-stained gels showed about nine faint protein bands between 30 and 65 kd and two more prominent bands close to each other near the 69 kd protein marker. Results of IgE binding were moderately positive in the patient to a protein around 69 kd but completely negative in all control subjects (Fig. 1). IgG binding was restricted to the putatively same protein. IgG bands were faint and overlaid by interfering unspecific binding of the radiolabeled antibody, which impeded our analysis. IgG binding that was considered specific occurred in three control subjects. No corresponding IgG band was present in the patient (Fig. 1).

DISCUSSION

A major problem with horsefly hypersensitivity is the lack of well-characterized allergen extracts that can guarantee high specificity and adequate sensitivity of allergy testing. There is some evidence from skin tests,^{1,2} RASTs,^{3,5} and basophil histamine release tests² that adverse reactions to horsefly bites are mediated by specific IgE. The reliability of these findings, however, may be inadequate because they were obtained with whole-body extracts of questionable specificity.⁴ By using extracts from isolated salivary glands we were able to demonstrate IgE binding to a 69 kd protein in a patient with two anaphylactic reactions to horsefly bites, provid-

ing further evidence that IgE-mediated anaphylaxis to horseflies does exist. The 69 kd *Chrysops* salivary protein is the first allergen identified from tabanids. Because salivary-gland extracts may not easily become commercially available, it seems important to critically reevaluate the usefulness of whole-body extracts in comparative studies.

More than 30 cases of horsefly allergy have been recorded so far, involving generalized urticaria, angioedema, bronchial constriction, and shock.¹⁻⁵ Allergy to tabanids may be less exceptional than assumed considering possible underreporting caused by poor diagnostic facilities and a lack of medical alertness. Furthermore, some horsefly species mimic vespid pigmentation patterns and may have been mistaken for Hymenoptera.

The comparatively weak IgE binding in the immunoblot may reflect a rapid fall in specific IgE titers within the 4-month period after the last bite. Otherwise, primary sensitization may have occurred not to *Chrysops spp.* but to a different horsefly species, with the weak IgE binding seen here reflecting cross-reactivity within this insect family. Not all tabanid species are relevant pests. *Haematopota spp.* is the most important genus for human beings in the northern hemisphere. *Chrysops spp.*, like *Tabanus spp.*, is of secondary importance but was used in this study because specimens could be successfully collected in the field. All three genera, by vicarious species, are represented in the northern hemisphere. Accordingly, allergy testing should be performed with *Haematopota spp.* rather than with *Chrysops spp.* or *Tabanus spp.*, which to our knowledge are currently used in commercial extracts.

Concomitant sensitization to Hymenoptera venoms in subjects allergic to horseflies seems to be frequent,³⁻⁵ but

the 69 kd *Chrysops* allergen is unlikely to be a cross-reacting homologue of major Hymenoptera allergens, all of which lie between 20 and 50 kd. Rather, cross-reactivity may be expected with saliva from other blood-feeding insects. Interestingly, several salivary proteins around 65 kd have been identified in mosquitoes,⁶⁻⁷ one of which was recently shown to be an allergen.⁸

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