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Clinical cases

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Mosquito bite hypersensitivity

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SUMMARY

We report a patient who experienced systemic anaphylaxis after several mosquito bites. The skin tests were positive, by prick and intradermal tests, with two species of common mosquito (*Aedes communis* and *Culex pipiens*) and also with red midge larvae (*Chironomus*). Specific IgE against *Aedes communis* was demonstrated. We carried out SDS-PAGE and immunoblotting with whole body extracts of *Aedes communis* and *Culex pipiens* and with red midge larvae. The immunoblotting results showed specific IgE against two proteins of approximately 30 kDa with the extract of *Culex pipiens*. We also found IgE against one protein of a slightly lower molecular weight with *Aedes communis*, and against several proteins (the most important with a molecular weight between 30 and 70 kDa) with the *Chironomus* extract. Cross-reactivity between these insect species was suspected.

Key words: *Aedes communis*. Anaphylaxis. Cross-reactivity. *Chironomus*. *Culex pipiens*. Immunoblotting. Mosquito allergy.

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INTRODUCTION

Blood-sucking mosquito bites (order: Diptera, family: Culicidae) frequently produce immediate wheal-and-flare reactions mediated by mosquito saliva-specific IgE antibodies (1, 2, 3). They can also produce delayed bite papules mediated by eosinophils and lymphocytes (2). Anaphylactic mosquito bite reactions are, however, very rare (4, 5).

Here we report a patient who suffered an anaphylactic reaction possibly due to several mosquito bites, and the results of immunoblotting analysis.

CASES REPORT

The patient was a 26-year-old man who in June 1996 experienced generalized pruritus with facial angioedema, nasal congestion, rhinorrhea,

sneezing, conjunctivitis, the sensation of a foreign body in the throat, and dyspnea. The symptoms disappeared a few hours after the administration of subcutaneous epinephrine and intravenous dexchlorpheniramine and methylprednisolone. The clinical symptoms occurred at about 1 a.m. in the open air. No relationship could be established between the consumption of medicines or food capable of producing allergy, but the patient recalled that he was bitten by several mosquitoes. On other occasions the patient had experienced immediate local reactions (wheal 2-3 cm in diameter) to mosquito bites. The ingestion of fish or raw or undercooked prawns had resulted in oral allergy syndrome, which also occurred with the consumption of sunflower seeds and peanuts.

Three months later physical examination was normal. A complete blood cell count, erythrocyte sedimentation rate and blood biochemistry were normal. Total serum IgE (CAP-System, Pharmacia, Uppsala, Sweden) was 830 kU/L. Specific IgE (CAP-System, Pharmacia) to *D. pteronyssinus* (0.76 kU/L), *Aedes communis* (1.21 kU/L), shrimp (0.43 kU/L) and *Chironomus thummi* (0.68 kU/L) was elevated, but was negative for echinococcus, bee and wasp.

Skin prick tests (SPT) for routine inhalant allergens, latex and *Anisakis simplex* (ALK-Abelló, Spain) were positive (wheal > 3 mm of diameter) with *D. farinae*, cockroach and grass pollen. SPT with commercial foods (Leti laboratories, Spain) were positive with peanut, chick-pea, bean, lentil, sunflower seed, shrimp and sole. SPT with red midge larvae (*Chironomus* spp.) (5% w/v, ALK-Abelló) was positive, as were SPT with two commercially available whole body extracts from the mosquitoes most frequently found in our area: *Aedes communis* (0.1% w/v, Leti, Spain) and *Culex pipiens* (10% w/v, Stallergenes-Pasteur, France). Intradermal tests were carried out with commercially available whole body extracts of *C. pipiens* (0.01% w/v, ALK-Abelló) with consecutive dilutions between 0.00001 and 0.01% w/v. These were positive at 15 min but negative at 4 h and 24 h (table I). SPT and intradermal tests with the extracts mentioned above were all negative in six controls (one reported immediate wheal reactions after mosquito bites).

Table I
Results of skin testing with mosquito extracts, in mm wheal diameter read at 15 min.
Concentration in w/v

	<i>A. communis</i> 0.1% (Leti)	<i>C. pipiens</i> 10% (Stallergenes)	<i>C. pipiens</i> 0.01% (Abelló)	<i>Chironomus</i> 5% (Abelló)	histamine 10 mg/ ml	0.1 mg/ml
Prick	8 x 6	12 x 6	NP	6 x 4	6 x 6	NP
Intradermal					NP	17 x 13
0.00001%	NP	NP	13 x 11	NP		
0.0001%	NP	NP	12 x 10	NP		
0.001%	NP	NP	16 x 14	NP		
0.01	NP	NP	15 x 13	NP		

NP = Not performed. Prick and intradermal tests with saline solution were negative. The readings at 4 and 24 h of all tests were negative.

An open challenge test with peanut showed oropharyngeal itching. We did no challenge tests with other foods because the patient tolerated them if they were well cooked.

SDS-PAGE and immunoblotting were carried out with the following mosquito extracts: 1) *Chironomus*. A commercially available fish-food product was used as the raw material, consisting of lyophilized larvae of chironomid species without additives. The larvae were suspended in a 20 mM ammonium bicarbonate solution at 5% w/v, stirred with a magnetic stirrer for 2 h at room temperature, and centrifuged at 12,000 x g for 20 min. The supernatant was dialysed against water at 6° C, filtered and stored at -20° C. The protein content measured by the Lowry method was 19 mg/ml. 2) *A. communis* and *C. pipiens*. Whole bodies of adult mosquitoes supplied by Allergon were used. The raw material was suspended in NaCl at 10% (1.8% w/v) with a Potter homogenizer (1,000 x g) for 10 min, then centrifuged, dialysed against water at 6° C, filtered and stored at -20° C. The protein content measured by the Lowry method was *Aedes* 2.73 mg/ml and *Culex* 2.1 mg/ml.

SDS-PAGE was performed in 15% acrylamide minigels by the discontinuous system describe by Laemmli (6). Specific IgE detection by immunoblotting was performed after transfer by a diffusion method (7).

The immunoblotting results are shown in figure 1. The serum of the patients showed specific IgE against two proteins of approximately 30 kDa with the extract of *C. pipiens*. We also detected IgE against one protein of molecular weight (MW) slightly lower than *A. communis*, and against several proteins (the most important with a MW between 30 and 70 kDa) with the *Chironomus* extract.

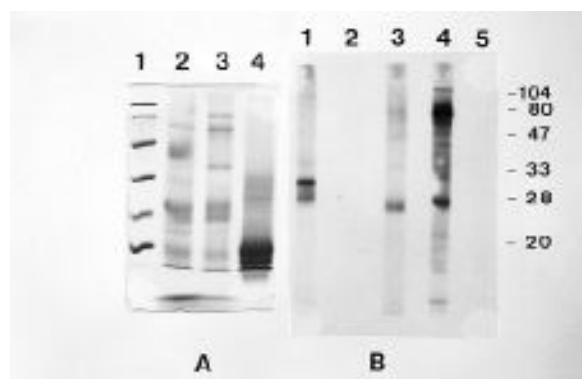


Figure 1.--A) SDS-PAGE of mosquito extracts. Molecular weight markers (lane 1): phosphorylase B (97.4 kDa), BSA (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa). Whole body extract of *Culex pipiens* (lane 2) and *Aedes communis* (lane 3). *Chironomus* spp. larvae (lane 4). B) Immunoblot analysis of IgE binding antigens in mosquito extracts: whole body of *Culex pipiens* (lane 1) and *Aedes communis* (lane 3). *Chironomus* spp. larvae (lane 4). Solvent was used as the negative control (lanes 2 and 5). Numbers on the right are the molecular weights of prestained standards run in parallel.

DISCUSSION

In contrast to bee and wasp stings, anaphylactic mosquito bite reactions are very rare (4, 5). Here we report a patient who suffered an anaphylactic reactions after several mosquito bites. We think that the anaphylactic reaction was due to mosquito bites, because of the temporal relation between the bites and the appearance of symptoms. Furthermore, the patient had apparently not been exposed to any other etiological agent (drug intake, suspect foods, physical exercise, etc.) and sensitization to hymenopterans, latex of *Anisakis* (other causes of anaphylaxis) was not demonstrated. However, the patient showed positive SPT and specific IgE againts two species of mosquito. This supports but does not confirm that mosquito bites were the cause of the anaphylaxis, as there are individuals who show specific IgE against mosquito in their serum but do not develop anaphylactic reactions (1-3). The only conclusive test would be to expose the patients to new mosquito bites, but this test is not standardized and we do not know how many bites would be necessary for an allergic subject to develop a systemic reaction, and the patient says he hasn't been bitten again by mosquitoes since then. The patient has oral allergy syndrome with certain foods, but he hadn't eaten them on this occasion and when he had done so on previous occasions the symptoms had been mild and very localized.

Immunoblotting studies show IgE antibodies bound to multiple antigens in several mosquito species (8, 9). Antisaliva IgE antibodies bind to 22-, 20-, 36-, and 64-kDa antigens in *A. communis* saliva, the largest antigen being 36 kDa (1, 10, 11).

In our case we found specific IgE against one protein of MW slightly less than 30 kDa with the *A. communis* extract, which may correspond to the 30-kDa proteins discovered by Brummer et al. (10) or the 25.5-kDa protein discovered by Saínza et al. with whole body extract of *A. communis* in a patient with mosquito allergy (12).

The serum of our patient showed specific IgE against two proteins of approximately 30 kDa with the extract of *C. pipiens*. Saínza et al. (13) carried out immunoblotting with whole body extract of *C. pipiens* in the sera of 3 patients with *A. communis* allergy, and found only one IgE-binding protein of 80-90 kDa.

The patient's sera showed IgE against several proteins (the most important with a MW between 30 and 70 kDa) with the *Chironomus* extract. The major allergen of *Chironomus* is Chi t 1, correspondint to hemoglobins and representing approximately 80% of all proteins in the larvae. The MW of the monomeric and homodimeric forms is 16 and 32 kDa respectively (14). Our patient did not show IgE against the 16 kDa monomeric forms of Chi t 1, but we cannot rule out that he might have IgE against the 32 kDa homodimeric form.

Although there are few studies of the cross-reactivity (CR) between mosquitoes, the existence of species-shared antigens among different mosquito species has been demonstrated (8, 11). In other cases the immunoblot patterns varied individually, and it has been suggested that both

genus- and species-specific mosquito allergens exist (9). It should be taken into account that mosquito whole-body and salivary-gland extracts differ from pure saliva in total protein and antigen content (3).

Cross-reactivity has also been reported between chironomids, cockroach and *Anisakis* (15). Baldo et al. (16) found IgE-binding bands in the region of approximately 30 kDa in cockroach, common clothes moth, warehouse moth, and silverfish extract. These findings are similar to our results for IgE immunoblotting with the three insect extracts. Tropomyosin (MW 36 kDa) is claimed to be the connecting panallergen (15), and may correspond to the approximately 30-kDa protein found by us and by Baldo et al. (16). Eriksson et al. (17) observed concomitant sensitization to chironomids and *A. communis*, which may indicate a true CR between them. In our case, we also believe that CR exists between the three species of insect studied here, as the serum of the patient did not recognize Chi t 1 (16 kDa major allergen of *Chironomus*), but did recognize proteins with a MW of approximately 30 kDa which were common to the three insect species. Moreover, our patient had no contact with midge larvae.

In conclusions, mosquito bites can produce anaphylactic reactions. Cross-reactivity is possible between different mosquito species and even between a wide range of arthropods.

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RESUMEN

Presentamos el caso de un paciente que presentó un episodio de anafilaxia tras ser picado por varios mosquitos. Los test cutáneos fueron positivos, en prick e intradermoreacción, con dos especies de mosquitos común (*Aedes communis* y *Culex pipiens*) y con larvas de mosquito rojo (*chironomus*). Se encontraron niveles elevados de IgE específica frente a *Aedes communis*. Se realizaron SDS-PAGE e inmunoblottings con extractos de cuerpo entero de *Aedes communis* y de *Culex pipiens* y con larvas de mosquito rojo. Encontramos IgE frente a dos proteínas de aproximadamente 30 kDa con el extracto de *Culex pipiens*. Con el extracto de *Aedes communis* se demostró IgE frente a una proteína de peso molecular ligeramente inferior a 30 kDa. Se encontró IgE frente a diversas proteínas (las más importantes con pesos moleculares entre 30 y 70 kDa) en el inmunoblotting de *Chironomus*. Sospechamos que pueda existir reactividad cruzada entre las tres especies de insectos estudiadas.

Palabras clave: *Aedes communis*, alergia a mosquito. Anafilaxia. *Culex pipiens*. *Chironomus*. Inmunoblotting. Reactividad cruzada.

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