

Patterns of immunoglobulin E sensitization to chironomids in exposed and unexposed subjects

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SUMMARY

We present our study of four patients with occupational type I allergy to red midge larvae (group 1) who we compared with seven individuals also sensitized to red midge larvae but who had not apparently been exposed to them (group 2). All patients showed elevated specific IgE against *Chironomus thummi*, and positive skin prick tests (SPT) and provocation tests with red midge larvae. We carried out SPT with *Dermatophagoides pteronyssinus*, *Acarus siro*, shrimp, cockroach and common mosquito (*Culex pipiens*) and specific serum IgE was also determined. The red midge larvae wheal size was significantly larger in group 1 ($x = 149.2 \text{ mm}^2$) than in group 2 ($x = 18 \text{ mm}^2$). Significant differences between levels of IgE anti-*C. thummi* were also found in group 1 ($x = 52.12 \text{ kU/l}$) and in group 2 ($x = 1.5 \text{ kU/l}$). The patients in group 2 had high levels of specific IgE against other allergens (*D. pteronyssinus*, shrimp and/or common mosquito) and had positive SPTs with these allergens, while the patients in group 1 were only sensitized to chironomids. The IgE-immunoblotting profile was quite different in both groups. The patients in group 1 seemed to have IgE against Chi t 1, the main *Chironomus* allergen, while the patients in group 2 had IgE against several protein bands in *Chironomus*, mosquito, shrimp and *D. pteronyssinus* extracts, some of them with equivalent molecular weights. We believe that the patients in group 2 were sensitized to *Chironomus* as a result of cross-reactivity with other insect or crustacean species.

Key words: Chironomids - Occupational allergy - Insects - Mites - Red midge larvae cross-reactivity

INTRODUCTION

Aquarists often use larvae of the *Chironomus* species (red midge larvae), a nonbiting midge (order: Dip-

tera, family: *Chironomidae*) (1) as fish food. Hemoglobins (Chi t 1) of red midge larvae are well-characterized allergens and they may cause IgE-mediated diseases in humans. Patients predominantly suffer from rhinitis, conjunctivitis, urticaria and bronchial asthma (2). In Japan and Sudan, nonbiting midge allergy is a common cause of bronchial asthma from environmental exposure to adult chironomids and their larvae (3, 4). In Europe, allergy to chironomids has been reported in occupationally exposed individuals (2, 5) as well as in unexposed individuals (6-8) and there is no explanation for it.

Here we present our study of four patients with occupational type I allergy to red midge larvae who we compared with seven individuals also sensitized to red midge larvae but who had not apparently been exposed to them, with the aim of establishing the differences between the two groups of patients.

MATERIALS AND METHODS

Patients

Group 1 was made up of four patients with occupational red midge larvae allergy who are described according to sex and age in Table 1 (patients 1-4). All four patients suffered from rhinoconjunctivitis, asthma or contact urticaria when they handled lyophilized or frozen red midge larvae (*Chironomus* spp.) which they used as fish food for their aquarium. The case of patient no. 2 has been reported extensively (9).

Group 2 was made up of seven individuals suffering from asthma and/or rhinoconjunctivitis whose sex and age are detailed in Table 1 (patients 5-11). They were sensitized to red midge larvae and had positive skin

Table 1

Allergological study results (I). Patients number 1-4 correspond to the group with occupational allergy from chironomids (group 1) and patients 5-11 to the patients apparently not exposed (group 2).

No.	Sex/age	Clinical picture	Positive SPT to common inhalant allergens	Positive SPT (in mm ²)					
				RML	Dpt	As	Sh	Bg	Cp
1	F/27	R-C, A, CU	—	88	—	—	—	—	—
2	M/37	CU	Ol	234	—	—	—	—	16
3	F/39	R-C	Ol, Gr, Ch, Cl, Al, Ca	65	—	—	—	—	12
4	M/38	C, A, CU	Gr	210	—	—	—	—	—
5	M/9	A, OSA	Dpt, Pl, Ld	9	16	40	54	9	20
6	M/26	R, Ana, U, OAS	Df, Ol, Gr, Ar, Ch, Pl	9	—	—	—	25	90
7	M/15	R-C	Gr, Ch	9	—	—	—	—	12
8	F/17	R-C, CU	Dpt, Df, Gr, Ol, Ar, Ch, Pl, Ca, Cl	16	12	—	—	—	—
9	M/10	R-C, OAS	Ol, Gr, Ch, Pl, Ca	28	—	—	—	—	66
10	M/24	R-C, A	Dpt, Df, Ld, Ol, Gr, Ar	30	42	—	—	—	25
11	M/14	R-C	Gr, Ol, Ch	25	—	—	—	9	54

SPT, skin prick tests; RML, red midge larvae extract; Dpt: *D. pteronyssinus*; As: *A. siro*; Sh: shrimp; Bg: *Blattella germanica*; Cp: *Culex pipiens* (mosquito); Ht: histamine at 10 mg/ml, F: female, M: male; R-C: rhinoconjunctivitis; A: bronchial asthma; CU: contact urticaria; C: conjunctivitis; OAS: oral allergy syndrome; Ana: anaphylaxis; R: rhinitis; U: urticaria; Ol: *Olea*; Gr: grass; Ch: *Chenopodium*; Cl: *Cladosporium*; Al: *Alternaria*; Ca: cat; Df: *D. farinae*; Ld: *L. destructor*; Ar: *Artemisia*; Pl: *Plantago*.

prick tests (SPT), high serum specific IgE levels and positive red midge larvae provocation tests. None of them had been exposed to chironomus because they had not handled fish food containing this allergen. Moreover, they were not exposed to fish food at home or work. Nevertheless, we cannot exclude environmental exposure due to the fragmentation of chironomus bodies and subsequent dispersion in the air.

Preparation of extract

The following raw materials were used: lyophilized red midge larvae of *Chironomus* spp. without additives (Vita Kraft, Biozoo SA, Spain), *Aedes communis* and *Chironomus thummi* mosquito bodies from Allergon (Engelholm, Sweden), *Dermatophagoides pteronyssinus* mite bodies from ALK-Abelló (Madrid, Spain) and fresh shrimps bought in the local market.

The raw materials were extracted at 5% (wt/vol) ratio for 1 h at room temperature and centrifuged. The supernatant was dialyzed, filtered through 0.45 µm Millipore and stored in aliquots at -20 °C until use. Protein content was determined using the Lowry method.

Skin prick tests

SPT were carried out with 50% glycerinated red midge larvae extract and the following commercially available extracts: *D. pteronyssinus* (100 BU/ml, ALK-Abelló, Madrid, Spain), *Acarus siro* (5,000 E/ml, Ifidesa-Aristegui, Spain), shrimp (1 mg/ml, Leti, Spain), cockroach (*Blattella germanica*) and mosquito (*Culex pipiens*) (both 1:10 wt/vol Stallergenes S.A., France). Histamine dihydrochloride (10 mg/ml) and saline solution were used as positive and negative controls respectively. SPT

were performed on the anterior forearm with a standardized lancet (Dome Hollister, Germany). Wheals were read at 15 min and expressed in mm² as the multiplication of the longest diameter by its perpendicular at its midpoint. A wheal >9 mm² than that produced by the negative control was considered positive.

Total and specific IgE

Both total and specific serum IgE were determined by an enzymatic immunoassay (CAP-FEIA), which was performed according to the manufacturer's instruction (Pharmacia Diagnostics, Uppsala, Sweden). Results of specific IgE were expressed in kU/l. Values higher than 0.35 kU/l were considered elevated. Anti-*C. thummi*, anti-*D. pteronyssinus*, anti-shrimp, and anti-mosquito (*A. communis*) IgE were determined.

Provocation tests

We carried out provocation tests with red midge larvae extract in all patients but patient no. 1, who did not want to partake in the test. The main clinical symptoms observed by the patients (rhinitis, conjunctivitis or contact urticaria) determined the target organ for provocation. Conjunctival provocation tests were performed in six patients. In brief, increasing concentrations (0.005, 0.05, 0.5 and 5 mg/ml) of red midge larvae extract were prepared, and two drops of the first allergen dilution were instilled into the conjunctival sac of one eye and control saline solution in the other. If no reaction was detected within 20 min, the following concentration was applied into the same eye. The criteria for a positive test were congestion of the conjunctival mucosa and itching in the eye.

Nasal provocation tests were performed in four patients. The same concentrations as for the conjunctival test were used. In patients without nasal symptoms and after anterior rhinoscopy, two drops of saline solution were instilled into one nostril, and if no reaction was detected within 20 min, two drops of the increased concentrations of allergen were instilled into the other nostril. We considered the test positive if the patient showed at least three of the following symptoms: sneezing, itching, increase of rhinorrhea, increase of blocking of the nose, increase of nasal edema or increase of nasal paleness. To avoid the depositing of allergen in the lower airways the patients were asked not to breathe in deeply.

A rub test was carried out in patient no. 2 by delicately rubbing the forearm for 30 sec with frozen red midge larvae. It was considered positive if contact urticaria was produced.

SDS-PAGE and IgE-immunoblotting

SDS-PAGE was carried out according to Laemmli (10) using non-reducing conditions and a 15% polyacrylamide running gel. The extract proteins (100 µg protein/lane) separated by SDS-PAGE were transferred onto PVDF membranes (0.4 x 5 cm) as described by Towbin *et al.* (11). After blocking with 5% bovine serum albumin (BSA) in PBS, immunodetection of IgE-binding proteins was achieved by incubating with a 1/10 dilution of patient serum in 1% BSA in PBS and then with a 1/3,000 dilution of mouse anti-human IgE monoclonal antibody (MAB) HE-2 ascitic fluid (12). Immunodetection of IgE-binding proteins was achieved by enhanced chemiluminescence following the manufacturer's instructions (ECL-Amersham, UK). As a control, blots with extract proteins were also incubated with a negative serum (NC, negative control). These assays were only performed in one patient from group 1 (patient no. 3) and five patients from group 2 (patients 5, 6, 8, 10 and 11).

The rest of the patients were not tested as sera were not available).

Statistical analysis

For the comparison of quantitative variables the Mann-Whitney test was used, and for nonquantitative variables the Fisher's exact test was used.

RESULTS

The characteristics and results of the allergy study can be seen in Table 1 and Table 2. In group 1, there were two males and two females. In group 2, there were six males and only one female. The average age in group 1 was 35.3 years (range 27-39), while that of group 2 was 16.4 years (range 9-26) ($p < 0.01$).

All the patients except for no. 1 were atopic as they showed positive SPT with several common inhalant allergens. Patient no. 1 was monosensitized to red midge larvae.

The SPT wheal size with red midge larvae was larger in group 1 ($x = 149.2 \text{ mm}^2$, $SD = 85.1$) than in group 2 ($x = 18 \text{ mm}^2$, $SD = 9.5$) ($p < 0.01$). Significant differences ($p < 0.01$) were also found in the levels of IgE against *C. thummi* found in group 1 ($x = 52.12 \text{ kU/l}$, $SD = 26.5$) and those found in group 2 ($x = 1.5 \text{ kU/l}$, $SD = 1.2$) ($p < 0.01$).

No patients in group 1 were sensitized to *D. pteronyssinus*. However, in group 2, three out of seven had positive SPT with *D. pteronyssinus* and four of seven had elevated levels of IgE against *D. pteronyssinus*. Only patient no. 5 showed positive SPT with *A. siro*. Three patients in group 2 (nos. 5, 6 and 9) suffered from oral allergy syndrome with the consumption of seafood. All three had elevated IgE against shrimp but only no. 5 had positive SPT with shrimp. None of the patients in

Table 2
Allergological study results (II). Patients number 1-4 correspond to the group with occupational allergy from chironomids (group 1) and patients 5-11 to the patients apparently not exposed (group 2).

No.	Total IgE (kU/l)	Specific IgE in kU/l (class)				Provocation tests with RML (mg/ml)
		<i>C. thummi</i>	<i>D. pteronyssinus</i>	Shrimp	<i>A. communis</i>	
1	46	30 (4)	—	—	—	ND
2	86	56.1 (5)	—	—	—	RT+
3	263	88 (5)	—	—	—	NPT+ (0.5)
4	60	34.4 (4)	—	—	—	CPT+ (0.005)
5	1053	1.9 (2)	24.1 (4)	25.9 (4)	5.4 (3)	CPT+/NPT+ (0.5)
6	830	0.7 (1)	0.7 (2)	0.4 (1)	1.2 (2)	NPT+ (0.5)
7	518	0.7 (1)	—	—	1.3 (2)	CPT+ (5)
8	1154	0.5 (1)	—	—	3.2 (2)	CPT+ (0.5)
9	389	0.7 (1)	—	0.8 (2)	2.8 (2)	NPT+ (0.05)
10	944	3.1 (2)	36.5 (4)	0.4 (1)	9.8 (3)	CPT+ (0.05)
11	10	3.2 (2)	3.2 (2)	—	12.7 (3)	CPT+ (0.005)

RML: red midge larvae; ND: not done; RT: rub test; NPT: nasal provocation test; CPT: conjunctival provocation test.

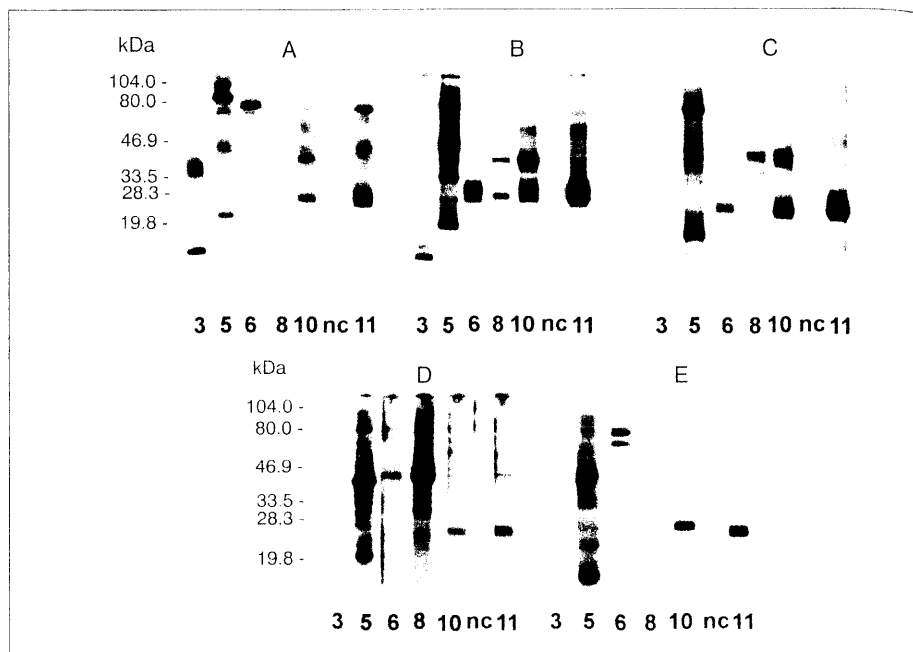


Fig. 1. IgE immunoblotting with serum of selected patients in groups 1 and 2. A) red midge larvae; B) *Chironomus thummi*; C) *Aedes communis*; D) shrimp; and E) *Dermatophagoides pteronyssinus* extracts. The figures at the bottom of each strip indicate the patient number (see Tables 1 and 2). NC: negative control. The molecular weight positions (kDa) were determined by prestained markers run in parallel (Biorad, Hercules CA, USA).

group 1 were sensitized to shrimp. Three patients in group 2 and none in group 1 had positive SPT with *B. germanica*. Two patients (nos. 9 and 11) had an immediate wheal after mosquito bites with diameters of around 1-2 cm. Patient no. 6 suffered severe anaphylaxis after several mosquito bites. In group 1, two patients had positive SPT with *C. pipiens* with a small wheal size. In group 2, six patients showed positive SPT with *C. pipiens* and the wheal size was larger. None of the patients in group 1 had elevated IgE against *A. communis*, but all seven patients in group II showed elevated levels of IgE against this common mosquito.

Significant differences were observed when comparing the levels of IgE against *A. communis* in the two groups ($p < 0.005$). No differences were found as far as the provocation tests were concerned in the two groups.

Figure 1 shows the results of an IgE-immunoblotting experiment carried out with the serum of selected patients from both groups. In patient no. 3 (representative of group 1), two bands with molecular weight of about 17 and 34 kDa, which corresponds to the molecular weight described by the monomer and dimer of Chitin, were identified in red midge larvae extract (2). In *C. thum-*

mi extract a double band with molecular weight of about 16 kDa was recognized. In contrast, no IgE was detected against proteins of the other extracts studied. For patients no. 5, 6, 8, 10 and 11 (group 2) the situation was quite different. IgE was detected against several protein bands in all the extracts (Fig. 1), but no IgE was detected in red midge larvae and *C. thummi* extracts against protein band(s) in the range of the molecular weight of Chitin.

DISCUSSION

In our allergy clinic, we had the opportunity to study four patients with true occupational allergy to chironomid larvae. On another occasion, we had carried out SPT with red midge larvae in 465 patients suffering respiratory allergy and had found that they were positive in 19 patients (4.08%) (8). Moreover, seven of them had specific IgE against *C. thummi* and positive provocation tests with red midge larvae. The seven patients from this previous study, who were sensitized but had not apparently been exposed to red midge larvae,

med group 2 of our new study and were compared with the four occupationally exposed subjects (group 1).

The clinical pictures observed in the patients with occupational allergy from red midge larvae were the same as described in the literature (2) (Table 1). Two patients (nos. 1 and 4) suffered from asthma. Patients no. 1 and 3 had rhinoconjunctivitis. Patient no. 4 had conjunctivitis, and contact urticaria was observed in patients no. 2 and 4. The patients in group 1 were older than those in group 2 (35.3 vs. 16.4 years) ($p < 0.01$). The patients in group 1 may have become sensitized to contact with red midge larvae while carrying out their hobbies. However, it is possible that sensitization in group 2 occurred through cross-reactivity with other allergens, this allergy being more frequent in young people.

Patient no. 1 was monosensitized to red midge larvae, while none in group 2 were. This indicates the importance of testing red midge larvae in patients exposed to this fish food, as if it is not tested, we could label as inherent patients whose symptoms would disappear simply by avoiding exposure. This occupational allergy to chironomids is more frequent in atopic subjects.

In group 1, the SPT wheal size with red midge larvae was larger ($x = 149.2 \text{ mm}^2$, $SD = 85.1$) than in group 2 ($x = 18 \text{ mm}^2$, $SD = 9.5$) ($p < 0.01$). The levels of IgE against *C. thummi* in group 1 ($x = 52.12 \text{ kU/l}$, $SD = 26.5$) were also higher than those of group 2 ($x = 1.5 \text{ kU/l}$, $SD = 1.2$) ($p < 0.01$).

The immunoblotting experiments carried out (Fig. 1) indicate that in contrast to the patients in group 2, those in group 1 were probably sensitized to Chi t 1, the main *Chironomus* allergen. In group 2, IgE against different protein bands in *Chironomus*, *Aedes*, shrimp and *D. pteronyssinus* extracts were detected, and notably two bands with a molecular weight of about 35 kDa and 25 kDa were observed in most of the cases. The 35 kDa band probably corresponds to tropomyosin, which has been described as a cross-reacting allergen in shrimp, mite and insects (13). The nature of the 25 kDa band is not clear. One possibility is the enzyme glutathione S-transferase, which has a molecular weight in the same range, has been described as an allergen in *D. pteronyssinus* (14) and cockroach (15) and may act as an allergen in other arthropod species.

Several authors have studied the correlation between chironomids and *D. pteronyssinus* allergy (4, 16-18), between chironomids and shrimp allergy (4, 6), between chironomids and mosquito allergy (4, 19) and between chironomids and cockroach allergy (20). Some have found cross-reactivity between them and others have not, but all of them have found that a relationship exists between them. Baldo *et al.* (21) reported that a "pan-allergy" exists between insect species and arthropods in general.

The subjects in group 2 could have become sensitized by inhaling particles of chironomids. The bodies

of dead adult midges break up and can be inhaled (22, 23). Another possibility is that they were sensitized to chironomus through cross-reactivity with other allergens such as mites, crustaceans or insects of other species (6, 19-21). In view of the results obtained in our study we are inclined to accept the latter, as the patients in group 2 did not show sensitization to Chi t 1 but rather to proteins which in some cases show equivalent molecular weight in the species studied.

Eriksson *et al.* (18) found that only 17% of chironomid-sensitized patients had positive SPT with Chi t 1 allergen, which could indicate that the sensitization of their patients is more often directed against other chironomus allergen.

CONCLUSIONS

There are patients who are sensitized to chironomids without apparently being exposed to these insects. These patients show levels of specific IgE and SPT wheal size inferior to those of patients who have a true occupational allergy to chironomids. The unexposed patients are sensitized to other species (crustaceans, mites, insects) which seem to share allergens with chironomids.

ACKNOWLEDGEMENTS

We thank the nurses of our allergy section, Adela Delicado and Rosalia Fernández-Pacheco, and the auxiliary nurses M^o Carmen Cid, M^o Cruz Rabadan and Rosa Valera, for their excellent collaboration in this study.

RESUMEN

Presentamos cuatro pacientes con alergia ocupacional tipo I frente a *Chironomus*, la larva del chironómido, y comparamos la respuesta inmunológica con la de un grupo de siete individuos sensibilizados a la misma larva pero no expuestos. Realizamos tests cutáneos con extractos de la larva, *Acarus siro*, cucaracha, mosquito y *Dermatophagoides pteronyssinus*, IgE específica, IgE total y test de provocación (nasal, conjuntival o de contacto). Finalmente, se realizó SDS-PAGE e inmunoblotting-IgE. En los resultados destacamos una diferencia estadísticamente significativa en la pápula en el test cutáneo a favor del grupo de pacientes expuestos a la larva, así como en las concentraciones de IgE específica. Por otra parte, los pacientes expuestos al alérgeno presentaban una monosensibilización, mientras que el grupo de pacientes únicamente sensibilizados mostraban polisensibilizaciones a los demás alérgenos testados. Finalmente el grupo de pacientes expuestos parecen tener IgE específica frente al alérgeno mayor Chi t 1, mientras que el grupo de pacientes sensibilizados muestra varias bandas (*Chironomus*, mosquito, gamba y extracto de *Dermatophagoides*) de pesos moleculares equivalentes. Concluimos que los pa-

cientes sensibilizados al *Chironomus* pueden serlo por una reacción cruzada a otros insectos o crustáceos.

Palabras clave: Quironómidos - Alergia ocupacional - Insectos - Ácaros - Reacción cruzada con la larva del quironómido

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