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Occupational Allergy to Adult Chironomid Midges among Environmental Researchers

Abstract

A case of occupational allergy to chironomid midges in research work is described. A researcher was exposed to adult chironomid midges during his research and developed allergic rhinitis after 10 years of such exposure. Using the midge extract of adult *Chironomus plumosus* (CP) (Linnaeus, 1758), both immediate skin test and the ophthalmic challenge test gave positive results. IgE antibody against adult CP was also demonstrated by the radioallergosorbent test. Four of the five serum samples of the environmental researcher examined showed a positive radioallergosorbent test to at least one of the adult midges breeding around eutrophic Japanese lakes. Enzyme-linked immunosorbent assay inhibition test and immunoblot experiments indicated that the remaining hemoglobin is one of the major allergens of adult CP. These results demonstrate that the exposure to adult chironomid midges is an important occupational hazard among environmental researchers.

Key Words

Adult chironomid midges

IgE antibody

Occupational allergy

Remaining hemoglobin

Introduction

In recent years, hypersensitivity to chironomids (Diptera, Chironomidae) has been described worldwide. In Sudan and Egypt, endemic allergy to the green nimitti midge (*Cladotanytarsus lewisi*) has been reported. A substantial part of the Sudanese population living along the Nile river are sensitized and suffer from asthma and allergic rhinitis as a consequence of the exposure to the environmental airborne allergens after mass hatchings of the midges [1–3]. Atopic patients living around a reservoir who were sensitized with the lake fly, *Chironomus plumosus* (CP), were reported in Wisconsin, USA [4]. In Germany, hemoglobins (Hbs; *Chi t I*) of the larvae of *Chironomus thummi thummi* (CTT) were shown to be the major allergens among aquarists and workers in fish food factories [5–7]. Railway workers allergic to *Chironomus salinarius* were also reported in Venice, Italy [8].

In Japan, the increasing occurrence of adult chironomids from waters polluted with organic wastes has become a nuisance. A marked increase of adult chironomids was observed around eutrophic lakes. The midges make swarming pillars, rest on house walls, rush to electric lights and die at house corners.

Important roles of chironomids in allergic reactions and bronchial asthma have been investigated in Japan since 1985. The species involved were *Polypedilum kyotoense* (PK) [9], *Tanytarsus oyamai* Sasa [10], *Chironomus yoshimatsui* [11], CP of a subfamily of Chironominae, *Tokunagayusurica akamusi* (TA) [12] and *Cricotopus sylvestris* (CrSy) [13] of another subfamily of Orthoclaadiinae. Positive skin test rates ranging between 7.6 and 23.5% were induced by different midge extracts among asthmatic children in Toyama [14]. High levels of specific IgE against midge allergens were also detected in serum samples of the pa-

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tients. However, there are no reports on sensitization to the adult stage of the midges under working conditions.

We describe a symptomatic research worker in whom some allergological investigations were performed. We also studied the IgE antibody to several species of chironomids in serum samples among research workers dealing with the adult midges. The role of hemoglobin (Hb) in allergies to adult midges will be discussed.

Materials and Methods

Case Report

A 43-year-old male research worker who was generally in good health, with the exception of a history of pollinosis to Japanese cedar (Sugi; *Cryptomeria japonica* D.Don) beginning at 19 years of age. Mild seasonal rhinitis occurred each year during early spring (March–April).

Between 28 and 30 years of age he investigated the breeding conditions of some insects such as silk worms. At the age of 31, he began to study the population ecology of chironomid midges around Japanese lakes. He collected the adult midges using light traps and/or a suction tube. During this period, he was almost always exposed to large amounts of live as well as dead midges. This kind of work continued almost every day for several months of the research period every year. Around the lakes, CP emerges abundantly from late August to September. TA also emerges abundantly from November to December [15].

Ten years after starting this work, he began to suffer from sneezing and rhinorrhea. These symptoms occurred when he collected the chironomids and ended soon after ceasing his work. Nasal symptoms, sometimes complicated by ocular symptoms, occur and continue every year from August through December when he collects CP or TA. He has not yet experienced an asthmatic attack. He never complained of food allergies. The patient has never smoked. During recent years, the symptoms reduced thanks to wearing a mask and other protective clothing.

He showed positive immediate (after 20 min) reactions on intradermal skin testing (extracts of adult CP at a dilution of 1/10,000 w/v, 0.01 mg/ml) according to the criteria of Ishizaki [16], but did not respond to saline. He also showed positive immediate reactions, consisting of itching and redness of the conjunctiva on ophthalmic challenge testing (adult CP extracts at a dilution of 1/100 w/v, 1 mg/ml). High IgE antibody titers against adult CP were demonstrated using the radioallergosorbent test (RAST score = 2) and against Japanese cedar pollen (RAST score = 2). Total IgE was 310 IU/ml.

Subjects

Five male environmental research workers, aged 23–54 years, who had been exposed to adult chironomids from which they inhaled allergen over periods of 5–20 years were included in this study. None of them complained of any other work-related allergic symptoms except for the allergic case mentioned above. All of them gave their informed consent before participating in the study. Blood was drawn by venipuncture and the serum was separated and immediately deep-frozen until analysis.

Table 1. Chironomid species used to detect specific IgE antibody

Species	Antigen source	Major habitats	Distribution in Japan
CrSy	culture	eutrophic lakes	common
TA	capture in field	eutrophic lakes	abundant
PaG	culture	eutrophic rivers	common
PK	culture	eutrophic rice paddies	abundant
DL	culture	eutrophic lakes	common
GT	culture	eutrophic lakes	abundant
CaB	culture	eutrophic lakes	rare
CP	capture in field	eutrophic lakes	abundant
CY	culture	eutrophic rivers	abundant
CTT ¹	Pharmacia	eutrophic rivers	absent

¹ Larval CTT antigen produced by Pharmacia, Sweden.

Antigen Preparation

Seven species of chironomidae (CrSy, CY, PK, *Camptochironomus biwaprimus*; CaB, *Dicortendipes lobiger*; DL, *Glyptotendipes tokunagai*; GT, and *Paratanytarsus grimmii*; PaG) were cultured from eggs to adult midges by the method of Kawai and Konishi [17]. Two more species, TA and CP, were collected from major habitats around the lakes where the research work was being performed. The mature larvae of CP were bred from the egg masses oviposited by the female adults. The names of the chironomid species used to detect specific IgE are listed in table 1. A schematic representation of the evolutionary relationships of each chironomid species is given in figure 1, according to the system of Baur et al. [18].

Frozen adult midges were defatted with diethyl ether, homogenized and extracted using phosphate-buffered saline (PBS) (pH 7.2). The larvae of CP were also extracted with 0.025 M phosphate buffer (pH 8.6). Supernatants were collected after centrifugation at 12,300 g for 20 min. The filtrate of supernatant was used as the crude extract. All procedures were performed at 4°C. The partial purification of the larval allergens of CP was performed as described by Kawai et al. [19]. Briefly, a Sephadex G-75 column (3.1×61.5 cm; Pharmacia, Uppsala, Sweden) was equilibrated with a buffer consisting of 0.1 M sodium acetate (pH 6.4) and 0.035 M magnesium chloride according to the method of Braun et al. [20]. The crude extract (2.5 ml) was applied to the column and fractions (4.3 ml) were collected at a flow rate of 10 ml/h. Absorbances at 280 and 415 nm were monitored to estimate protein and hemoglobin concentrations, respectively. The fractions on each peak of B or C were gathered and saturated with 80% ammonium sulfate. After centrifugation, the precipitates were collected and dialyzed against 0.025 M phosphate buffer, pH 8.6, and then lyophilized. A large proportion of the proteins in each peak of B and C contained dimeric Hbs (dHb, mw 28,000–32,000) and monomeric Hbs (mHb, mw 15,000–16,000), respectively, as described for Hbs of CTT. Purity of mHb was higher than that of dHb, judged from the ratio of the absorbances of 415–280 nm; 2.6 for mHb and 1.7 for dHb as shown in figure 2. Japanese cedar pollen was extracted using Coca's solution [21] and dialyzed against PBS, pH 7.0.

Protein concentrations of these crude extracts were determined by the method of Lowry et al. [22] and stored at –20°C until use.

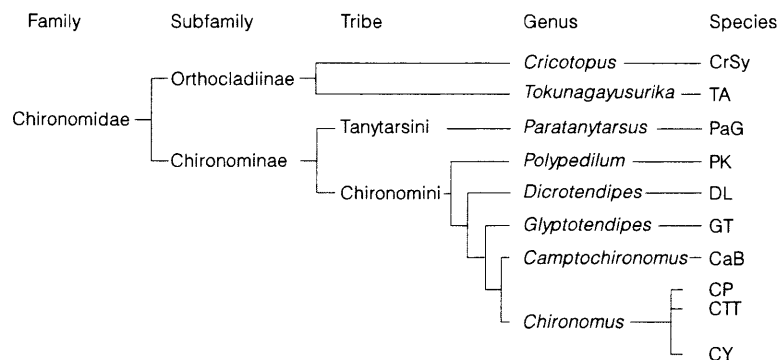


Fig. 1. Evolutional relationships of ten chironomid species studied.

Radioallergosorbent Test (RAST)

IgE antibodies were measured with RAST by the method of Ceska et al. [23] using paper discs. Briefly, 60 pieces of paper discs activated by cyanogen bromide were coupled with 3 mg of each crude extracts of chironomids. For detection for IgE, beta-D-galactosidase labeled antihuman IgE antibody was used (Phadezym-RAST kit, Pharmacia, Sweden) [24]. The paper discs of CTT larval antigen (i73) were also purchased from Pharmacia. Specific IgE was expressed as RAST score classified with the reference serum.

Enzyme-Linked Immunosorbent Assay (ELISA) Inhibition Test

ELISA inhibition was performed using a polystyrene microplate coupled with CP as described by Matsuoka et al. [25]. A microtitre plate with 96 flat wells (Nunc, Denmark) was coated with adult CP antigen of CP at a concentration of 10 µg/ml by overnight incubation and blocked with 1% bovine serum albumin (BSA) in Tris-HCl buffer saline at pH 7.5 (TBS). A 50-µl aliquot of serum containing high IgE antibody to CP adult was preincubated with 50 µl of the serial dilutions of antigens (0.1, 1.0, 10, 100 and 1,000 µg/ml) of CP adult, CP larva, mHb and dHb of CP larva, CrSy or Japanese cedar pollen for 3 h at room temperature vortexing slowly.

The ELISA was then performed using the preincubated sera. Peroxidase-conjugated goat antihuman IgE (Dako, Denmark; 1/100) was added as the secondary antibody. A mixture of 0.03% 2,2-azinobis-(3-ethylbenzthiazoline sulfonic acid) diammonium salt (Sigma, USA) and 0.003% H₂O₂ in 0.05 M citrate buffer (pH 5.0) was used as substrate.

Absorbance was measured at 410 nm after 1 h incubation at 37°C. The plastic plate was washed three times with TBS-Tween buffer before each step. Samples were measured in duplicate.

The percent inhibition was calculated using the following formula.

$$\text{Inhibition (\%)} = 100 - \frac{\text{ELISA with antigen}}{\text{ELISA without antigen}} \times 100.$$

PAGE Electrophoresis/Immunoblot Analysis

Polyacrylamide gel electrophoresis was performed under undena-tured conditions [26, 27]. We employed a vertical slab gel apparatus (Atto, Tokyo, Japan). About 100 µl of the crude extracts of adult CP, larval CP, mHb, and dHb were separated on 15% polyacrylamide (w/v) gel. Samples were applied in duplicate lanes of the same gel. After

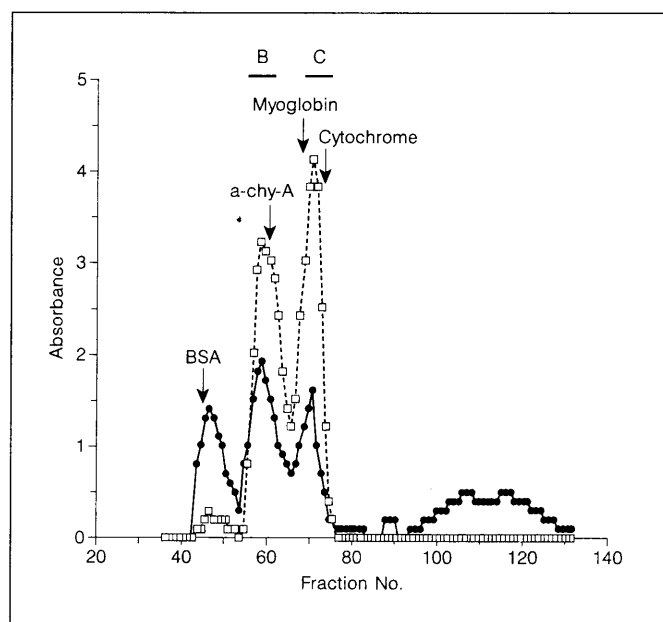


Fig. 2. Gel filtration of crude extract of CP larvae on Sephadex G-75 column. —●— = Absorbance at 280 nm, representing protein concentration. ----□---- = absorbance at 415 nm, representing Hb concentration. The column was calibrated with bovine serum albumin (BSA, mw 67 kD), α-chymotrypsinogen A (a-chy.A, mw 25 kD), myoglobin (mw 17 kD) and cytochrome c (mw 13 kD).

separation at 40 mA for 5 h, the gel was cut and one set of lanes were stained with Coomassie brilliant blue G-250 and another set of lanes were used for immunoblot analysis. The latter removed gel was placed on a poly(vinylidene fluoride) membrane (PVDF Clear Blot Membrane, Atto) in the transfer chamber and the proteins in the gel were transferred.

The transferred membrane was incubated overnight at 4°C with serum samples after blocking with 1% BSA in TBS. After washing with TBS, the strip was incubated with rabbit antihuman IgE antibody (DAKO; 1/1,000) as a secondary antibody. An alkaline-phosphatase-linked swine antirabbit IgG (DAKO; 1/1,000) was added as a tertiary antibody for detection of the IgE-anti-IgE complex. As substrate of alkaline phosphatase, 5-bromo-4-chloro-3-indolyl phosphate potassium salt and nitro blue monotetrazolium chloride (Vector Laboratories, USA) in Tris-HCl buffer, pH 9.5, was used.

Results

Specific IgE Levels

The protein concentrations of the allergen extracts were from 3.1 to 55.0 mg/ml.

Specific IgE antibodies to each species of chironomids in the serum samples of the five researchers are shown in table 2. High levels of IgE antibody against CrSy, TA, PK as well as CP were demonstrated in the allergic patient (case 1). A weaker positive result (RAST score 1) was also obtained from *Paratanytarsus grimmii* (PaG) in case 1. Weaker positive results (RAST score 1) were obtained from CrSy in cases 2, 3 and 4. No positive result was obtained from CTT larval antigen except in the serum sample of case 2.

ELISA Inhibition

The results of the ELISA inhibition tests are shown in figure 3. When the allergic sera were preincubated with increasing concentrations of the crude antigen of a CP adult, there was a dose-dependent inhibition in ELISA. When the serum samples were preincubated with the Japanese cedar or CrSy, there was no significant inhibition in ELISA. When the serum was preincubated with mHb, or dHb, clear dose-dependent inhibition patterns were obtained for both antigens. The inhibitory activity of mHb was slightly stronger than that of dHb.

PAGE Electrophoresis/Immunoblot Analysis

After staining the separation gel with Coomassie brilliant blue, 9 single bands were found in the adult CP lane. Six bands for larval CP, and three bands for mHb and dHb, respectively, were also distinguished (fig. 4a).

By immunoblot analysis using the allergic serum, the locations of IgE binding sites were colored by immunochem-

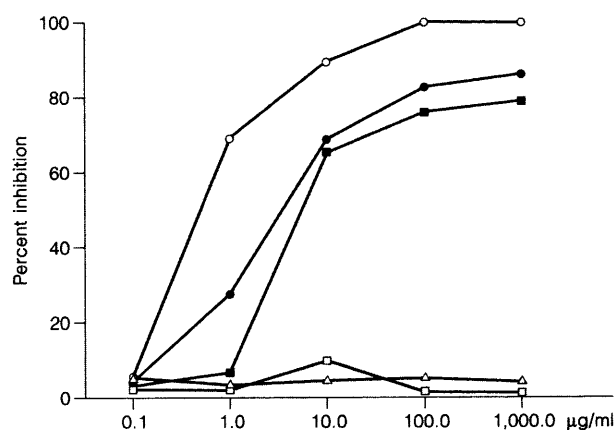


Fig. 3. ELISA inhibition was performed using microplate coating CP adult antigen. As inhibitors, adult CP (—○—), mHb (—●—), dHb (—■—), Japanese cedar pollen (—□—) and CrSy (—△—) antigens were used. Percent inhibition was calculated after the measurement of ELISA of mixtures of allergic sera and the inhibitors (see text).

Table 2. Specific IgE antibodies to each species of chironomid midge in serum samples of 5 research workers

	Case No.				
	1	2	3	4	5
Age, years	43	45	23	54	29
Symptom	+	—	—	—	—
Species					
CrSy	2	1	1	1	0
TA	2	0	0	0	0
PaG	1	0	0	0	0
PK	2	0	0	0	0
DL	0	0	0	0	0
GT	0	0	0	0	0
CaB	0	0	0	0	0
CP	2	0	0	0	0
CY	0	0	0	0	0
CTT ¹	0	2	0	0	0

Specific IgE is expressed with RAST score compared with commercially available reference serum. Adult midges were used to detect IgE antibody except CTT.

¹ A commercially available CTT disc (i73) was used to detect larval CTT antigen.

ical methods at these same mobility sites of Hbs in the larval CP, dHb and mHb lanes. Diffuse but clear locations of IgE binding are demonstrated in the adult CP lane at the same mobility sites of the major bands of Hbs as well as the low mobility sites above Hb bands (fig. 4b).

An immunoblot experiment using four nonallergic sera as well as nonallergic control serum did not show any clear IgE-binding sites.

Discussion

We describe here for the first time a case of occupational allergy to adult-stage chironomid. The researcher has developed allergic symptoms to the adult chironomid midges after 10 years of intensive exposure. Allergic symptoms are obviously induced while collecting and counting the adult insects. Skin as well as ophthalmic tests confirmed the sensitization. Moreover, specific IgE antibody to the CP adult was clearly demonstrated by RAST. Though he had a history of pollinosis and showed high IgE antibody level to Japanese cedar pollen, the independence of the chironomid allergenicity was clearly demonstrated by the ELISA inhibition test. A predisposition to allergy to common allergens may play an important role in chironomid allergy [28].

Baur et al. [8] described 642 cases allergic to larvae of chironomid. Eighty-five cases of them were occupationally sensitized subjects. They were exposed to the larval body fluid of chironomid when they deal with the larvae of CTT in fish food factories. In this case, the workers were sensitized by Hb-rich components from reddish 'bloodworms' larvae. The major allergens were identified as insect Hbs, *Chi t I* in the larvae. A similar occupational allergy to the larvae was reported from Sweden [29].

However, there are no reports describing occupational allergy to adult-stage chironomid midges. As mentioned above, some of the environmental researchers in Japan are exposed to adult chironomid midges exclusively. This kind of research work became important for the establishment for protective measures against the increasing midge nuisance and their impact on the environment.

In this study, high specific IgE were detected by the RAST method to CrSy, TA, PK as well as CP in the allergic case. These results suggest that detection of IgE antibody against several adult species of chironomid are useful tools to study chironomid allergy. Though CP was one of the dominant species around the lakes and suspected to be the main sensitizing allergen, the individual roles of CrSy, TA and PK should not be ignored. As TA, PK and CP have Hbs,

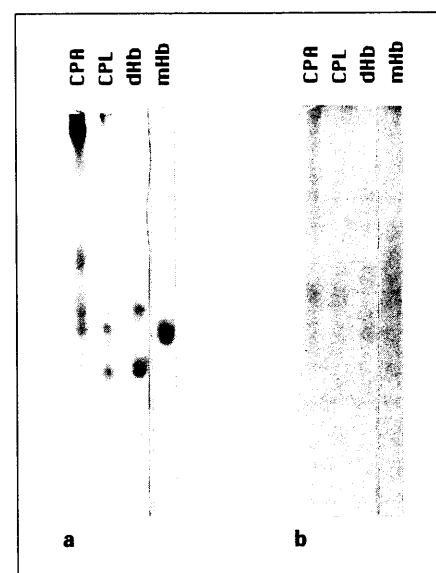


Fig. 4. Immunoblot analysis using allergic serum. **a** PAGE-electrophoresis of adult CP (CPA), larval CP (CPL), dHb, and mHb was performed and stained with Coomassie brilliant blue. **b** After electrophoresis, each protein in the gel was transferred to a poly(vinylidene fluoride) membrane and incubated with allergic serum. IgE binding sites were detected using alkaline-phosphatase-linked antirabbit IgG antibody after the reaction to IgE with rabbit antihuman IgE antibody. Origin of the electrophoresis is at the top.

more precise studies will be necessary on the cross-allergenicities of these species at the adult stage.

Positive RASTs to CrSy were detected in 4 of the 5 (80%) researchers examined. Recently, von Kampen et al. [30] reported that nearly 40% of atopic patients showed a positive reaction to the crude larval extract of CrSy and suggested that in crude larval allergens of CrSy there are allergens that partially cross-react with *Chi t I*. As CrSy has no Hbs and is evolutionally distantly related to other Chironominae species [31], the significance of positive RAST to CrSy, especially in cross-allergenicities with other allergens, should be clarified in further studies.

It is interesting to note that IgE against the larval allergen, CTT, was high in one case but not high in the allergic case. It might be explained by the fact that the CTT is completely absent from Japan, but some other indigenous species of chironomid may have some cross-allergenicities with CTT. In the allergic case, IgE antibody might react with more specific epitopes of CP than the common ones with CTT. Further study on adult chironomid midges will provide useful information on a wide range of cross-allergenicity and common allergens, which are found not only in insects but also in Arthropoda and Mollusca [32].

There are some controversies as to the major allergens in adult-stage chironomids. Larval Hbs were reported to be maximal in the fourth instar larva and decline through the pharate adult and were almost entirely absent in the mature adult [33]. However, the important roles of the remaining Hbs and Hb breakdowns in the adult stage of chironomid were described by Tee et al. [34] and Prelicz et al. [35].

In our experiment, ELISA inhibition test showed that Hbs in the larval stage have strong inhibitory activity against antigens of the adult CP, whereas Japanese cedar pollen or CrSy have none. Our results with the ELISA inhibition test strongly support the idea that larval Hb allergenicity can be retained through metamorphosis into the adult stage.

Furthermore, immunoblot experiments also suggest that retaining Hbs in adult CP are the responsible allergens, though the other components with slow mobility are suspected to be involved in other allergens. In this experiment, we employed enzyme-linked antibody instead of radioisotope-labeled antibody to detect the IgE complex for more convenient and safer processing. Thus, the sensitivity of this method was not so high and sites of IgE binding were a little obscure. More purification of the Hb fractions and the other components would be necessary for the characterization of such adult specific allergens.

Our results also suggest that some preventive measures are necessary for research workers to avoid allergen exposure from the point of view of industrial hygiene. A mask and glasses will be useful to reduce the amount of inhaled allergens. Further monitoring of environmental allergens will provide useful information for the avoidance of these allergens [36]. Early detection of IgE antibody reacting to adult allergens of chironomids may be useful for epidemiological studies and prevention of this kind of occupational allergy.

In this study, we demonstrated that occupational exposure to adult-stage chironomids elicits sensitization and allergy to these insects in environmental researchers. Caution should be advised to prevent this kind of occupational hazard.

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