

23751

Insect hemoglobins (*ChitI*) of the Diptera family Chironomidae are relevant environmental, occupational, and hobby-related allergens

X. Baur and V. Liebers

Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin (BGFA), Institut an der Ruhr-Universität-Bochum, Gilsingstraße 14, W-4630 Bochum 1, Federal Republic of Germany

Received March 8, Accepted May 27, 1992

Summary. Six hundred and forty-two persons with hobby-related ($n = 205$), occupational ($n = 85$), or environmental contact ($n = 352$) to the midge and larval allergen *ChitI* were studied. Frequencies of IgE-mediated sensitization in these selected populations were 36.1%, 24.7%, and 9.6% respectively. Occupationally sensitized subjects who had been heavily exposed showed higher levels of antibodies, were more frequently diagnosed as having bronchial asthma and less frequently as having conjunctivitis, and had a significantly shorter latency period when compared to environmentally exposed people or aquarists. Our results are evidence for a higher risk of sensitization and of bronchial asthma in highly exposed subjects.

Key words: Allergy – Chironomidae – *ChitI* – IgE antibodies

Introduction

ChitI comprises the 12 homologous hemoglobins of the Diptera species *Chironomus thummi*, a widely distributed insect species [5]. Our recent studies revealed *ChitI* to be a potent inhalative allergen [2–4]. Many aquarists using *C. thummi* larvae as fish food have hitherto been examined. The present study compares for the first time three *ChitI* exposure groups: aquarists, workers predominantly engaged in fish food production, and inhabitants of certain areas abounding in water where larvae or adult midges of different species of the family Chironomidae occur in large numbers.

Material and methods

Skin prick test. *ChitI* was prepared from *C. thummi* larvae as previously described [5], and a skin prick test solution of 0.1 mg/ml was used. Wheal reactions $\geq 50\%$ of the induced by 0.1% of histamine were regarded as positive. Skin prick tests were performed in 124 exposed subjects.

Correspondence to: X. Baur

Antibody determination. We quantified *ChitI*-specific IgE antibodies by the RAST method (radio-allergo-sorbent test [1, 2]). *ChitI* was bound to cyanogen bromide-activated paper disks in our laboratory. Total IgE serum levels were measured by PRIST (Pharmacia).

Subjects. In total, 642 Chironomidae-exposed persons from different areas were examined. Of the 290 from Germany, 205 used *C. thummi* larvae as fish food and 85 were employed in fish food factories or in scientific laboratories dealing with these insects. All these German subjects had a physical examination (inspection, auscultation, percussion of the chest, etc.) and 150 of them underwent lung function testing (airway resistance measured by oscillometry or by a body plethysmograph). Ninety-four atopic railway workers from Venice (Italy), where the species *C. salinarius* is predominant, were screened. Previously published data [12] on 26 symptomatic Sudanese subjects heavily exposed to “green nimitti midges” (*Cladotanytarsus lewisi*) were included in the study. Sera as well as clinical data of 229 consecutively taken Japanese atopics in contact with midges of the species *C. plumosus* and *Tokunagayusurika akamusi* were obtained from the HLA & Allergy Workshop organized by D. Marsh, Baltimore. Data concerning three patients exposed to large numbers of *C. plumosus* midges near lakes in Wisconsin, USA, were delivered by S. Kagen. The total number of environmentally exposed persons examined was thus 352.

Each case history was evaluated by a trained physician of the respective area using a standardized questionnaire.

Statistical analysis. Data were analyzed by Student's *t*-test or the χ^2 method (a *P* value of 0.05 was taken as the limit of significance).

Results

All 642 Chironomidae-exposed subjects were examined for *ChitI*-specific antibodies by RAST ($n = 620$) and/or skin prick tests ($n = 124$) (Table 1). Of these subjects, 20% were sensitized to *ChitI*. Among aquarists, 36.1%, among the occupationally exposed group, 24.7%, and among environmentally exposed persons, 9.6%, had *ChitI*-specific IgE antibodies. There was a close correlation between results of skin tests and RAST, positive results of antibody measurements being associated with positive skin tests in 98.6% (73/74) of the cases. Four subjects showed weakly positive skin test reactions in spite of negative RAST results.

Table 1. Results of allergological tests and exposure data of examined subjects

<i>ChitI</i> contact due to:	Country	Number of tested subjects	Exposure to insect stage	Skin tests performed <i>n</i> (%)	RAST performed <i>n</i> (%)	Sensitized subjects ^a <i>n</i> (%)
Hobby	Germany	205	Larvae	90 (43.9)	204 (100)	74 (36.1)
Occupation	Germany	85	Larvae	5 (5.8)	85 (100)	21 (24.7)
Environment	Italy ^b	94	Midges	0 (0)	94 (100)	9
Environment	Japan ^b	229	Midges	0 (0)	229 (100)	10
Environment	Sudan ^c	26	Midges	26 (100)	5 (19.2)	12 (9.6)
Environment	USA ^c (Wisconsin)	3	Midges	3 (100)	3 (100)	3
Total		642 (100)		124 (18.5)	620 (96.7)	129 (20)

^a Sensitization assessed on the basis of RAST results or, if unavailable, skin test results with *ChitI*

^b Atopic people only

^c Heavily exposed symptomatic people; clinical data of the Sudanese patients are from Tee et al. [12]

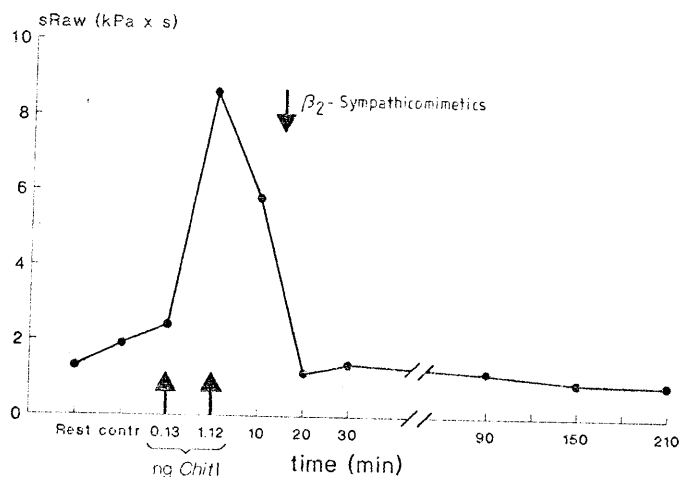


Fig. 1. Strong immediate asthmatic reaction after inhalative challenge by 1.25 ng of *ChitI*. This aquarist has suffered from bronchial asthma and conjunctivitis for 5 years owing to the handling of freeze-dried *C. thummi* larvae. Specific airway resistance (*sRaw*) was measured with a whole body plethysmograph (Jaeger, Würzburg, FRG). An increase in *sRaw* of > 100% is considered a significant response

Table 2. Diagnoses of *ChitI*-sensitized subjects^a

Diagnosis	<i>ChitI</i> contact: occupation (<i>n</i> = 21)	<i>ChitI</i> contact: hobby (<i>n</i> = 74)
Bronchial asthma*	76.2%	36.5%
Rhinitis	81.0%	74.3%
Conjunctivitis	57.1%	77.0%
Urticaria	52.3%	36.5%

* $P < 0.01$ (χ^2 test)

^a Hobby-related Chironomidae contact was due to the use of frozen, lyophilized, or living larvae as fish food from once per week up to twice daily. Occupationally exposed subjects were workers of a fish food factory or research institutes; the former were in intensive contact with the allergen in the production or packing area, the latter were exposed almost daily since they were engaged in the purification and characterization of *ChitI* components

1. *ChitI*-exposed, but healthy subjects (*n* = 108)
2. *ChitI*-sensitized subjects without further sensitization (*n* = 40)
3. Atopics sensitized to at least one out of 20 common allergens but without *ChitI* sensitization (*n* = 40)
4. *ChitI*-sensitized atopics also sensitized to common allergens (*n* = 51)

Total IgE level was shown to be lowest in nonatopic *ChitI*-negative subjects and highest in individuals sensitized to *ChitI* as well as to common allergens (publication in preparation).

Of all *ChitI*-sensitized people, 74.8% were atopics, i.e., showed a significant skin test response to at least one common allergen. Among the atopics, 9.6% of the Italian, 4.4% of the Japanese, and 50.5% of the German group had IgE antibodies to *ChitI* (Fig. 2).

Of the 352 environmentally exposed people with specific IgE antibodies to *ChitI*, a similar number of subjects fell into each of the first three RAST classes but none were in the highest RAST class (37.5% RAST class 1; 33.3% RAST class 2; 29.2% RAST class 3). Among aquarists, 12.2% belonged to RAST class 1, 20.3% to RAST class 2, 23.0% to RAST class 3, and 44.6% to RAST class 4. Among the 21 occupationally *ChitI*-sensitized subjects, none could be allotted to RAST class 1;

ChitI-specific IgE antibodies were always associated with a case history compatible with or even clearly indicative of type I hypersensitivity. In ten doubtful cases, nasal or bronchial challenges were performed, resulting in immediate nasal and/or asthmatic reactions (see example in Fig. 1).

The prevalent diagnoses in occupationally exposed subjects were rhinitis (81.0%) and bronchial asthma (76.2%), whereas in aquarists conjunctivitis (77%) and rhinitis (74.3%) dominated (Table 2). In environmentally exposed subjects symptoms induced by *ChitI* are often ambiguous due to the coexposure to many other allergens.

The latency period, i.e., the interval between exposure onset and development of symptoms, was significantly longer in aquarists than in occupationally sensitized people (4.8 vs 1.6 years; $P < 0.05$; Student's *t*-test).

The total IgE of 239 German subjects was determined. We divided these people into four subgroups and compared their total IgE levels:

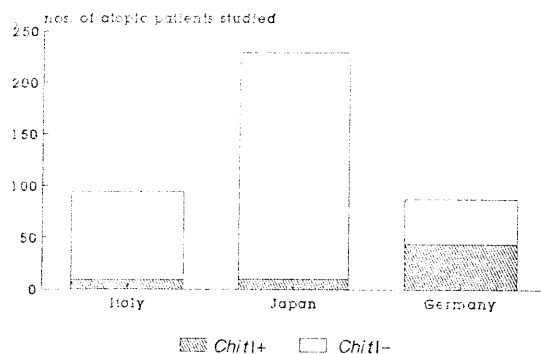


Fig. 2. Frequency of *ChitI* sensitization in 410 atopics from Italy, Japan (environmentally exposed), and Germany (hobby-related and occupational *ChitI* contact)

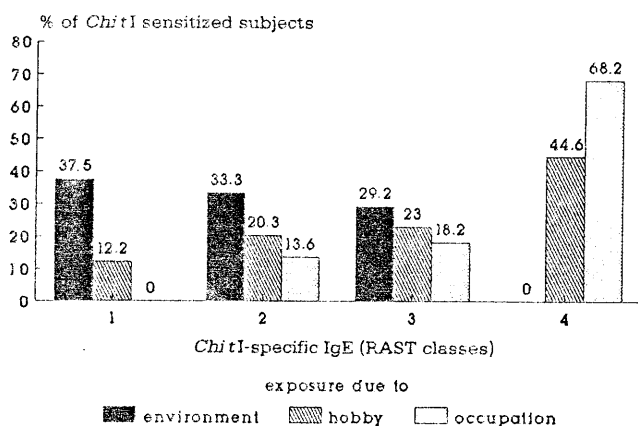


Fig. 3. *ChitI*-specific IgE in subjects with environmental, hobby-related, or occupational exposure

13.6% were RAST class 2 and 18.2% RAST class 3, but 68.2% had specific IgE levels above 17.5 U/ml (RAST class 4) (Fig. 3). These data are evidence for a relationship between intensity of exposure (usually low in the environment, moderate in aquarists, and very high in fish food factories) on the one hand and levels and frequency of specific IgE antibodies on the other hand.

Discussion

Our results clearly demonstrate *Chironomidae* hemoglobins to be potent allergens of occupational and worldwide environmental relevance. Until now, reliable data on the prevalence of this type of sensitization have not existed. We found 36.1% of partially selected aquarists and 24.7% of screened exposed workers to be sensitized to *ChitI*. The same was true for 4.4% of screened atopic Japanese and 9.6% of atopic people from the Venice area. The high prevalence of IgE-mediated sensitization in Sudanese people (46.1% [12]) and Americans (each of the three cases) seems to be due to the selection of heavily exposed and symptomatic people. It should be mentioned that several thousand Diptera species belonging to the family *Chironomidae* exist. Distribution and number vary greatly from area to area; only rough data

on the residential area of the tested people exist; thus precise information on allergen exposure is not available.

Our previous studies showed that hemoglobins are the only or predominant allergens at least of the *Chironomidae* species belonging to the *Chironomus* genus [1, 2]. In addition, strong immunological cross-reactivity between closely related species could be demonstrated with human, rabbit, and even some monoclonal antibodies [4].

Total IgE obviously roughly reflects number and degree of individual sensitizations. This may be due to the stimulation of polyclonal IgE production by specific sensitization.

Our data prove the prevalence of sensitization to depend on degree of exposure since people heavily exposed owing to the handling of freeze-dried larvae were more frequently and more intensely sensitized than persons having little contact with midges of the environment. In addition the former group suffered significantly more frequently from asthma. It should be mentioned that a relationship between the degree of exposure on the one hand and symptoms and specific IgE antibodies on the other hand was also found in a previous study comparing 187 aquarists and 37 workers of a fish food factory [7].

The findings indicate that a reduction in the allergen load might result in a decrease in symptoms in exposed workers. This is important since many allergologists would not have expected IgE-mediated sensitization to be associated with exposure intensity. A relationship between degree of exposure and risk of sensitization has also been observed for mite allergens in atopic children and young adults [6].

It should be mentioned that recent studies with highly purified and characterized allergens including *ChitI* demonstrate that genetic factors are also important for the development of IgE mediated hypersensitivity [9, 11].

References

1. Baur X, Dewair M, Fruhmman G, Aschauer H, Pflerschinger G, Braunitzer G (1982) Hypersensitivity to chironomids (non-biting midges): localization of the antigenic determinants within certain polypeptide sequences of hemoglobins (erythrocrucorins) of *Chironomus thummi thummi* (Diptera). *J Allergy Clin Immunol* 69:66
2. Baur X, Aschauer H, Mazur G, Dewair U, Prelicz H, Steigemann W (1986) Structure, antigenic determinants of some clinically important insect allergens: chironomid hemoglobins. *Science* 223:351
3. Baur X (1986) *Asthma, Alveolitis, Aspergillose-Charakterisierung ursächlicher Antigene*. Springer, Berlin Heidelberg New York
4. Baur X, Liebers V, Mazur G, Becker WM, Kagen S (1991) Immunological cross-reactivity of hemoglobins in the Diptera family *Chironomidae*. *Allergy* 46:445-451
5. Goodman M, Braunitzer G, Kleinschmidt T, Aschauer H (1983) The analyses of a protein polymorphism. Evolution of monomeric and homodimeric hemoglobins (erythrocrucorins) of *Chironomus thummi thummi* (insect Diptera). *Hoppe Seyler's Z Physiol Chem* 366:205

6. Lau S, Falkenhorst G, Weber A, Werthmann J, Lind P, Buettner-Goetz P, Wahn U (1989) High mite allergen exposure increases the risk of sensitization in atopic children and young adults. *J Allergy Appl Immunol* 84: 718-725
7. Liebers V, Hoernstein M, Baur X (submitted for publication) Humoral immune response to the insect allergen *Chit1* in aquarists and workers of a fish food factory. *Allergy*
8. Marsh DG, Zwollo P, Ansari AA (1989) Toward a total human immune response fingerprint: the allergy model. *Adv Biosci* 74: 65-82
9. Marsh DG, Zwollo P, Huang SK, Ansari AA (1989) Molecular genetics of human immune responsiveness to allergens. In: Chadwick D, Evered D, Whelan J (eds) *IgE, mast cells and the allergic response*. John Wiley, Chichester, p 171
10. Platts-Mills TAE, Chapman MD, Pollert S, Luszczynska GW, Ward J (1991) Specific allergens evoking immune reactions in the lung: relationship to asthma. *Eur Resp J* 4 [Suppl 13]: 68s-77s
11. Tautz C, Hoernstein M, Marsh DG, Baur X (1990) IgE response to chironomid allergen *Chit1* is associated with specific HLA-D-sequences. *J Allergy Clin Immunol* 85: 201
12. Tee RD, Cranston PS, Dewair M, Prelicz H, Baur X, Kay AB (1985) Evidence for hemoglobins as common allergenic determinants in IgE mediated hypersensitivity to chironomids (non-biting midges). *Clin Allergy* 15: 335