

[REVIEW]

CHIRONOMID MIDGE ALLERGY

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***Chironomidae* larvae and midges cause allergic reactions in approximately 20 percent of exposed people; predominantly aquarists using insect larvae as fish food and environmentally exposed subjects living in areas abounding in water are affected. Our studies include 642 subjects of whom 205 are aquarists; 85 are occupationally and 352 environmentally exposed people. 123 of them were shown to be sensitized to these insects. Using highly purified allergens, we could demonstrate that *Chironomidae* hemoglobins (*Chi t I*) represent the major allergenic components causing rhinitis, conjunctivitis and bronchial asthma. Considerable immunological cross-reactivity exists between hemoglobins of the same and closely related *Chironomidae* species. Genetic factors seem to be important for the susceptibility of this type of allergy. In studies with peptides of *Chi t I* component III, we identified several epitopes recognized by human IgE antibodies and/or T-cells.**

Key words: allergy — B-cell-/T-cell epitopes — *Chi t I*-IgE antibodies — *Chironomidae* — genetic factors

Our interest as clinicians in the insect family *Chironomidae* began when in 1978 several aquarists consulted our outpatient department because of severe allergic reactions. Contact with freeze-dried "red mosquito" larvae (species *Chironomus* (*C.*) *thummi*), a common fish food, induced immediate-type hypersensitivity such as conjunctivitis, urticaria, sneezing, rhinorrhea and bronchial asthma. In addition, several studies performed in areas abounding in water proved *Chironomidae* midge swarms also to be responsible for asthma and rhinitis outbreaks in Japan⁹⁾¹¹⁾¹⁶⁾, Wisconsin/USA¹⁰⁾, the Nile region in the Sudan⁸⁾ and in Egypt⁷⁾.

Identifications and characterization of the major allergen of the genus Chironomus

In order to identify the causative components in *C. thummi* larvae we used different chromatographic methods. Two fractions, red in color, were obtained from Sephadex G-75 gel electrophoresis, containing all of the allergenic material as shown both in skin tests and in RAST studies¹⁾. As we know now, these two fractions represent the monomeric and dimeric hemoglobins. Studies with the immunoblot method and with the HPLC-

separated twelve individual *C. thummi* hemoglobins demonstrate that all of them strongly cross-react and bind IgE antibodies of allergic patients (Fig. 1). The same is true for hemoglobins of at least some other species of the genus *Chironomus*. Corresponding results were obtained with rabbit hyperimmune serum against the total hemoglobin fraction of *C. thummi*, *Chi t I*⁴⁾.

Interestingly, hemoglobins or cross-reactive components of adult *Chironomidae* midges also bind IgE antibodies and produce allergic reactions in the skin test⁴⁾. Sequence analyses of the individual *C. thummi* hemoglobins (*Chi t I*) components (I-XII) were performed by Braunitzer and coworkers of the Max-Planck-Institute for Biochemistry in Martinsried. It could be shown that each of these twelve hemoglobins consists of 136-151 amino acid residues (see example in Fig. 2); homology varies from 30 to 85%⁸⁾. It should be mentioned that their primary structures are quite different from vertebrate hemoglobins. The molecular weights are approximately 16 and 32 kD for the monomeric and dimeric forms respectively; each *Chironomus* hemoglobin chain is complexed with one heme group which is covalently bound to the histidine residue in position F8¹⁷⁾.

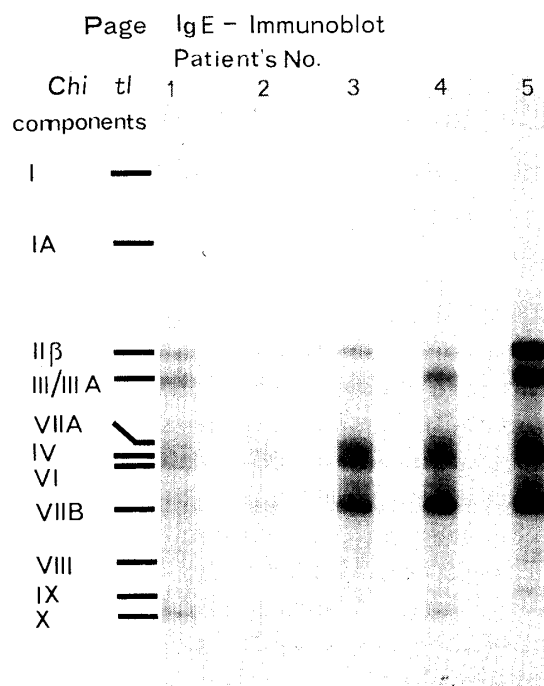


Fig. 1. Polyacrylamide gel electrophoresis of *Chi t I* under non-denaturing conditions with subsequent immunoblotting¹⁴⁾ using sera of five sensitized patients. All twelve *Chi t I* components (I-X) are shown to bind human IgE antibodies more or less.

As we know from studies of Steigemann and Weber¹⁷⁾ who performed X-ray analyses of component III at 1.4 Å resolution, the tertiary structures of *Chironomus* hemoglobins are very similar to those of myoglobin. They are coiled into eight helical sequences, named A to H, which are connected by six non-helical segments. The heme group is located within a hydrophobic pocket between helices E and F.

Demonstration of Chi t I as an important environmental allergen in areas abounding in water

Screening of atopic subjects from Venice and from four different Japanese regions showed 9.6% and 0–6.3% of these study groups to be sensitized to *Chi t I* (Tables 1, 2). Hypersensitivity to a distantly related *Chironomus* midge *Tokunagayusurika akamusi* was found four times more frequently in Japanese atopics (6.3–23.5%). 2% of the Japanese groups had IgE antibodies for *Chi t I* as well as for the *Tokunagayusurika akamusi* extracts. Among green nimitti (*Cladotanytarsus*

Table 1 *Chi t I*-specific IgE antibodies in environmentally exposed subjects

Area	Study group	No. of subjects	<i>Chi t I</i> +
Venice	Atopics	94	9.6%
Japan	Atopics	229	4.4%
Sudan	<i>C. lewisi</i> -sensitized	26	46.2%

Table 2 *Chi t I*-specific IgE antibodies in 229 Japanese atopics

Area	Atopics (no.)	<i>Chi t I</i> +	Tak a +	<i>Chi t I</i> and Tak a +
13 Kumamoto ¹	95	6.3%	12.6%	4.2%
20 Tokyo ²	32	3.1%	6.3%	0%
33 Kanagawa ³	31	0%	6.5%	0%
36 Fukuoka ⁴	71	4.2%	18.3%	1.4%
Total	229	4.4%	14.4%	2.2%

Sera and clinical data were kindly provided through the HLA and Allergy workshop by ¹Ishikawa, ²Okudaira, ³Yasueda, ⁴Nishimura.

Tak a = crude extract of *Tokunagayusurika akamusi* larvae.

lewisi) allergic Sudanese patients, 46.2% were shown to be sensitized to *Chi t I*.

Chi t I-related immunological cross-reactivity between 33 different Chironomidae species

We prepared crude extracts of *Chironomidae* larvae and/or adult midges (imagines) from 33 species, collected from different areas or reared in the laboratory. Pool serum from two allergic patient groups were used. Pool serum "Aquarianer" was obtained with equal amounts of samples taken from five German asthmatic aquarists who have been in contact with *C. thummi* larvae for 2–12 years. Pool serum "USA" consisted of serum samples (kindly provided by Steven Kagen) of three sensitized patients from Wisconsin, USA, who live near lakes and have suffered from conjunctivitis, rhinitis and/or bronchial asthma when in contact with *C. plumosus* midges. All these probands showed positive results to *C. thummi* hemoglobin *Chi t I* in skin test and/or RAST. Both patient groups' pool sera exhibit moderate to strong IgE reactivity to larval and midge extracts of all but one species (*C. salinarius*) of the genus *Chironomus* and *Glyptotendipes* (Fig.

L S A D Q I S T V Q A S F D K V K G D P V G I L Y A V F K A D P S I M A K F T Q
 1 40
 F A G K D L E S I K G T A P F E T H A N R I V G F F S K I I G E L P N I E A D V
 41 80
 N T F V A S H K P R G V T H D Q L N N F R A G F V S Y M K A H T D F A G A E A A
 81 120
 W G A T L D T F F G M I F S K M
 121 136

Fig. 2. Amino acid sequence of *Chi t I* component III as published by Buse et al.⁵⁾

*Single letter amino acid codes

Phe : F Leu : L Ile : I Met : M Val : V Ser : S Pro : P Thr : T Ala : A Tyr : Y
 His : H Gln : Q Asn : N Lys : K Asp : D Glu : E Cys : C Trp : W Arg : R Gly : G

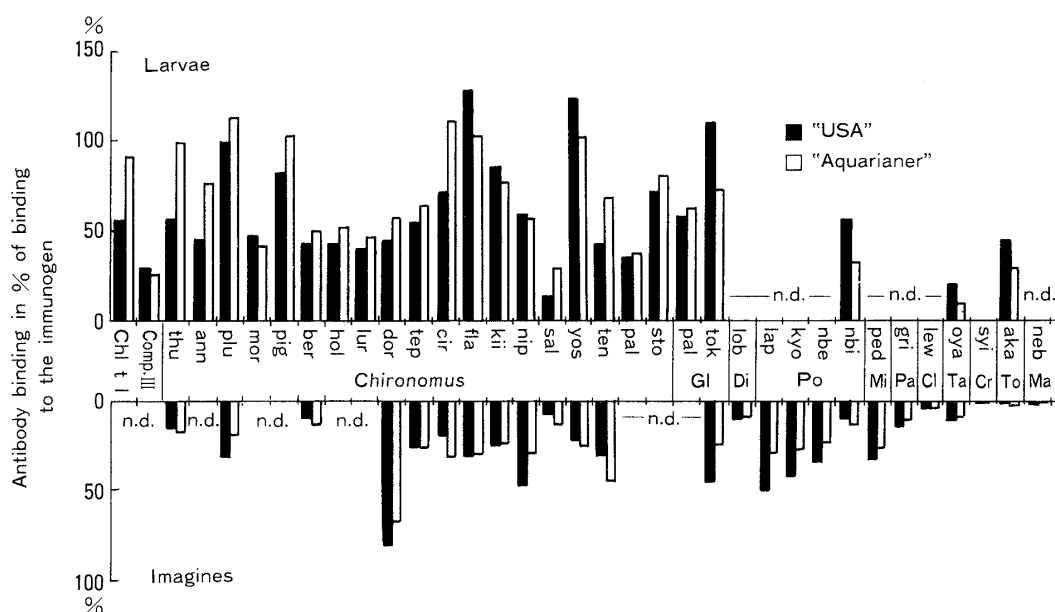


Fig. 3. Measurement of IgE antibody binding to *Chi t I*, to *Chi t I* component III and to crude larval and/or imagines extracts of 33 species of the Diptera family *Chironomidae*. Pool sera of five aquarists sensitized to *Chironomus thummi* larvae ("Aquarianer") and pool serum obtained of three patients sensitized to *Chironomus plumosus* imagines⁴⁾ was used for these investigations.

Individual *Chironomidae* species tested: *Chironomus* (*C.*) *thummi*, *C. annularius*, *C. plumosus*, *C. moratensis*, *C. piger*, *C. bernensis*, *C. holomelas*, *C. luridus*, *C. dorsalis*, *C. tepperi*, *C. circumdatus*, *C. flaviplumus*, *C. kiiensis*, *C. nipponensis*, *C. salinarius*, *C. yoshimatsui*, *C. tentans*, *C. pallidivittatus*, *C. storai*, *Glyptotendipes* (*Gl.*) *pallens*, *Gl. tokunagai*, *Dicrotendipes lobiger*, *Polypedilum* (*Po.*) *japonicum*, *Po. kyotoense*, *Po. nubeculosum*, *Po. nubifer*, *Microtendipes pedellus*, *Paratanytarsus grimmii*, *Cladotanytarsus lewisi*, *Tanytarsus oyamai*, *Cricotopus sylvestris*, *Tokunagayusurika akamusi*, *Makropelopia nebulosa*.

3). The other species give low or nonsignificant results. IgE antibody binding of both human pool sera to the larval extract of *C. thummi* is similar to the antibody binding to *Chi t I*. This demonstrates that *Chi t I* is the predominant or exclusive allergen of *C. thummi*. Interspecies cross-reactiv-

ity obtained with the "USA" pool serum is stronger than that of the "Aquarianer" pool serum. In all but one species (*C. dorsalis*), larval extracts produce higher IgE binding than midge extracts. This is also true for "USA" patients who are sensitized by midges instead of larvae. The

lower antibody binding in midges is probably due to their lower hemoglobin content. In the adult midge, hemoglobins are progressively degraded and their synthesis is stopped.

Stronger cross-reactivities, especially between distantly related species, were obtained with the rabbit antiserum against *Chi t I*; the only exceptions were *Cricotopus sylvestris* and *Macropelopia nebulosa*⁴⁾.

According to the pattern of reactivity of rabbit anti *Chi t I* component III, expression of the epitopes of this molecule varies considerably in the genus *Chironomus* and *Glyptotendipes*; they seem to be totally absent in all other species. Six monoclonal antibodies raised against *Chit t I* component III recognize epitopes in most closely related species or in only a few species of the genus *Chironomus*⁴⁾.

Identification of B-cell and T-cell epitopes of Chi t I component III

In order to localize antigenic determinants within the known 3 D-structure of *Chi t I* component III we analyzed a naturally derived set of relatively small peptides as well as several synthetic peptides.

First, we looked at the antibody binding sites, the so-called B-cell epitopes. The individual, purified peptides were coupled to CNBr-activated paper discs and tested for their binding to IgE antibodies of sensitized individuals. Four peptides bound IgE antibodies up to a value of five U/ml. These were the N-terminal region 1–15, sequence 91–101, which begins between helices F and G and extends into helix G and the C-terminal-sequence 110–135 (for the sequences see Fig. 1). The results of seven peptides were negative, that means they did not bind human IgE-antibodies to a significant extent³⁾¹³⁾¹⁴⁾.

To demonstrate the immunogenicity of individual peptides of *Chi t I* component III at the T cell level, we stimulated peripheral blood lymphocytes of hypersensitive patients in vitro. The stimulation indices were determined by ³H thymidine uptake. We examined 40 allergen-exposed sensitized patients and 17 non-exposed controls. The SI of patients ranged from 5 to 80, whereas nonexposed controls showed no reactivity (SI<5), when tested with *Chi t I*, containing each of the

twelve *C. thummi* hemoglobins. Using different component III peptides, it could be shown that nearly the whole sequence of the molecule stimulates T cells of some sensitized patients at least¹²⁾.

Genetic bases of Chi t I hypersensitivity in humans

The human immune response to foreign antigens is genetically restricted due to the different structures of MHC class II molecules, which are important for the presentation of immunogenic peptides. HLA D-subtyping of 61 *Chi t I* sensitized patients and 128 exposed healthy controls showed the immune response to *Chi t I* in non-atopics to be positively correlated with the HLA types DR1 and DQw 1.1. Furthermore, a negative association in this group with DRw52a and DQw3 exists¹⁸⁾. Preliminary results obtained in DQA typing indicate a reduction of type 0501 in *Chi t I* responders and strong reduction of type 03012 in non-atopic *Chi t I* non-responders²⁾.

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