

Chironomidae hemoglobin allergy in Japanese, Swedish, and German populations

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Hemoglobins of the Diptera (insect) family Chironomidae have been identified as causative allergens in asthmatic patients. In this study, 229 Japanese, 17 Taiwanese, and 92 Swedish sera from atopic patients were tested for antibodies against *Chi t I*, the hemoglobin from the European midge species *Chironomus thummi*, and against crude extracts from the Japanese midges *Tokunagayusurika akamusi* (*T. akamusi*) and *Cricotopus sylvestris* (*Cr. sylvestris*). Nearly 40% of patients showed a positive reaction to *Cr. sylvestris* extract, which contains no hemoglobin. This result is probably due to the presence of other partially cross-reacting allergens than hemoglobin. Nearly all tested Japanese serum samples showed cross-reactivity between *Chi t I* and *Cr. sylvestris*, a finding which is evidence for common epitopes in both midge species. Furthermore, an overall good correlation between the amounts of IgE antibodies against *Chi t I* and *Chi t I* component III was found in sera from Swedish, Japanese, and German patients.

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Chironomidae, popularly known as “nonbiting midges”, is one of the largest insect families. Approximately 10 000 species are distributed worldwide in areas rich in water (2, 3). Adult midges, as well as larvae, are reported to cause immediate-type allergic reactions. The non-cell-bound hemoglobins were identified as predominant inhalant allergens in many of the species (11, 12, 15). In Germany, Chironomidae species are not distributed in extraordinary numbers. Only a few people are exposed here; for example, fish enthusiasts who use larvae of the species *Chironomus thummi* (*C. thummi*) as fish food and workers engaged in fish-food factories (9, 10). However, nearly 20% of occupationally exposed persons were found to be sensitized (8). In countries or areas abounding in water, such as Sudan, Japan, Sweden, and southern Egypt, Chironomidae are important as sources of widespread environmental allergens (9).

The following study was performed to determine the relevance of Chironomidae sensitization in atopic populations of Japan and Sweden. We used IgE sera from atopic persons who had been exposed to airborne constituents of Chironomidae. The sera were tested for specific IgE antibodies against hemoglobins from *C. thummi* (*Chi t I*) and ingredients from the Japanese midges *Tokunagayusurika akamusi* (*T. akamusi*) and *Cricotopus sylvestris* (*Cr. sylvestris*). One further aim of this work was to determine

whether immunologic cross-reactivity among these different Chironomidae species exists. In addition, specific IgE antibodies against *Chi t I* component III were determined in all *Chi t I* sensitized Japanese and Swedish subjects, as well as in 41 German patients, to examine the correlation between the amount of IgE antibodies against *Chi t I* and *Chi t I* component III. Component III is a monomer and belongs to the 12 hemoglobin components which comprise *Chi t I*. It is well characterized by sequence analyses (6, 7, 14) and x-ray crystallography (4, 10).

Material and methods

Preparation of antigens

Crude extracts of *T. akamusi*, *Cr. sylvestris*, and *C. thummi* were prepared by homogenizing the larvae, as previously described (13). From the crude extract of *C. thummi*, *Chi t I* was then prepared by ammonium sulfate precipitation (2). Purification of *Chi t I* component III was performed on Sephadex G-75 and DEAE-cellulose exchanger (1, 5).

Patients

For screening the cross-reactivity of midges, Swedish and Japanese subjects were examined. Ninety-two sera from Swedish atopic patients were obtained

during an HLA workshop at the University Hospital, Linköping, Sweden. Furthermore, we received 246 serum samples from Japanese and Taiwanese atopic patients. The subjects live in eastern Japan (district of Kanto, $n = 63$), in southern Japan (district of Kyushu, $n = 166$), and in Taiwan (Republic of China, $n = 17$).

Antibody-binding assay

Measurement of antigen-specific IgE antibodies was performed with the EAST method (Phadezym RAST, Pharmacia), which uses BrCN activated paper disks coated by incubation in 0.1 M NaHCO₃ containing crude extract (1 mg/ml) of larvae from *T. akamusi* and *Cr. sylvestris* and isolated fractions of *C. thummi* (*Chi t I*, 1 mg/ml; *Chi t I* component III, 0.1 mg/ml). All 338 Swedish and Japanese serum samples were tested for specific IgE antibodies against *T. akamusi*, *Cr. sylvestris*, and *Chi t I*. Furthermore, patients who had positive results with *Chi t I* were tested with *Chi t I* component III. Correlation of these two allergens was determined in 11 *Chi t I* sensitized Swedish and 9 Japanese patients, as well as in 41 *Chi t I* positive German subjects.

For EAST inhibition studies, sera from eight *Chi t I* and *Cr. sylvestris* sensitized persons (40 µl each) were preincubated overnight with 0.1 mg *Chi t I* dissolved in 10 µl phosphate-buffered saline (PBS). In the opposing experiment with the same sera, we inhibited solid-phase *Chi t I* with *Cr. sylvestris* larval crude extract (10 µl; conc. 10 mg/ml). Another control for the specificity of inhibition was the preincubation of sera that were positive only for *Cr. sylvestris* with dissolved *Chi t I*.

Results

Ninety-two Swedish, 229 Japanese, and 17 Taiwanese serum samples were tested for specific IgE antibodies against the hemoglobin of *C. thummi* (*Chi t I*) and against crude extracts of *T. akamusi* and *Cr. sylvestris*. As shown in Figs. 1 and 2, 27 (29.3%) Swedish and 113 (45.9%) Japanese subjects showed IgE reactivity with at least one of the tested chironomid allergens, mainly *Cr. sylvestris*. IgE antibodies against *T. akamusi* were measured in 13% of the Japanese serum samples, whereas only 4.1% of these sera contained specific IgE against *Chi t I*. No Taiwanese subject had IgE antibodies against *Chi t I*. In contrast, more Swedish patients reacted with *Chi t I* (10.9%) than with *T. akamusi* (5.4%). However, both collectives included a number of persons who reacted with more than one of the midge allergens. Fig. 3a shows cross-reactivity between *Chi t I* and *Cr. sylvestris* tested by EAST inhibition with sera from eight Japanese *Cr. sylvestris* and *Chi t I* sensi-

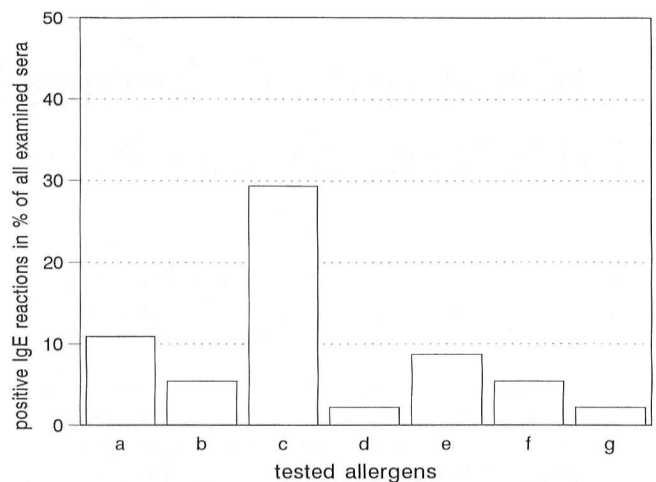


Fig. 1. EAST measurement of specific IgE antibodies against different midge allergens in 92 Swedish atopic patients (= 100%). Sensitization against: *Chi t I* (a); *T. akamusi* (b); *Cr. sylvestris* (c); *Chi t I* and *T. akamusi* (d); *Chi t I* and *Cr. sylvestris* (e); *T. akamusi* and *Cr. sylvestris* (f); and *Chi t I*, *T. akamusi*, and *Cr. sylvestris* (g).

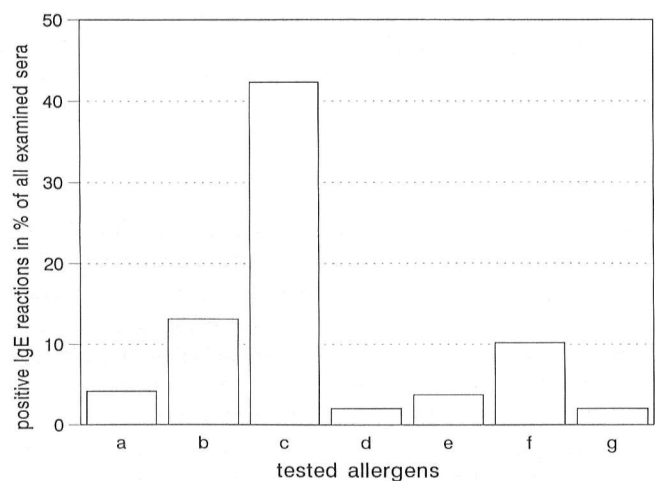


Fig. 2. EAST measurement of specific IgE antibodies against different midge allergens (a–g; see Fig. 1) in 246 Japanese and Taiwanese atopic patients (= 100%).

tized patients. In all but one case, the addition of *Chi t I* produced a decrease of IgE binding to *Cr. sylvestris*. In the reversed experiment, antibody binding decreased in all tested sera when we inhibited *Chi t I* on the disk with a solution of *Cr. sylvestris* (Fig. 3b). The addition of dissolved *Chi t I* to sera which were positive only for *Cr. sylvestris* had no influence on the IgE binding to *Cr. sylvestris*.

Sensitization against the highly purified *Chi t I* component III was studied as well. The correlation between reactions against *Chi t I* and *Chi t I* component III was examined with 11 Swedish, 9 Japanese, and 41 German *Chi t I* positive sera (Fig. 4). All patients with IgE antibodies against *Chi t I* component III were positive against *Chi t I*. On the other

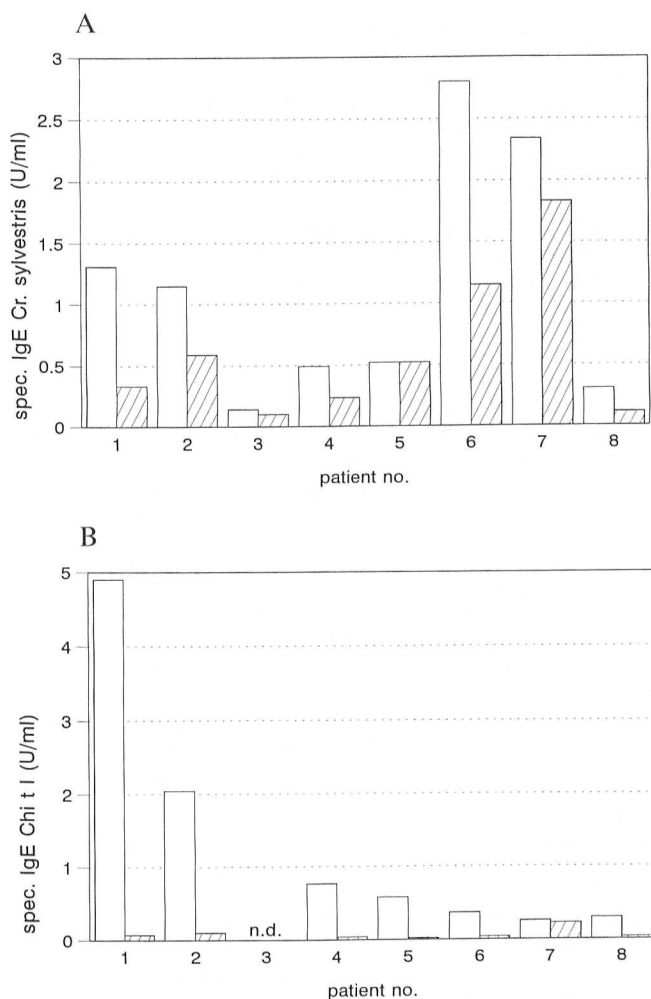


Fig. 3. Inhibition of IgE binding to solid-phase *Cr. sylvestris* crude extract by *Chi t I* (10 mg/ml) (A) and inhibition of IgE binding to solid-phase *Chi t I* by *Cr. sylvestris* crude extract (10 mg/ml) (B) in eight Japanese serum samples (hatched beams). Controls with PBS instead of dissolved *Chi t I* (A) and *Cr. sylvestris* (B) indicated by white beams; n.d. = not determined.

hand, 41.7% of *Chi t I* sensitized subjects did not react with component III. With the exception of two, these sera referred to EAST classes 1 and 2; 80% originated from Japanese and Swedish subjects. IgE binding to *Chi t I* and *Chi t I* component III was correlated with a correlation coefficient of $r = 0.58$. Patients with lower specific IgE (0.35–3.5 U/ml) showed a better correlation ($r = 0.7$) than those with higher amounts of IgE antibodies (3.5–17.5 and more units per milliliter).

Discussion

In Germany, nearly 20% of occupationally exposed persons are sensitized against the hemoglobin of the European species *C. thummi* (*Chi t I*) (8). In this study, we examined 338 Swedish, Japanese, and Taiwanese serum samples for IgE antibodies against

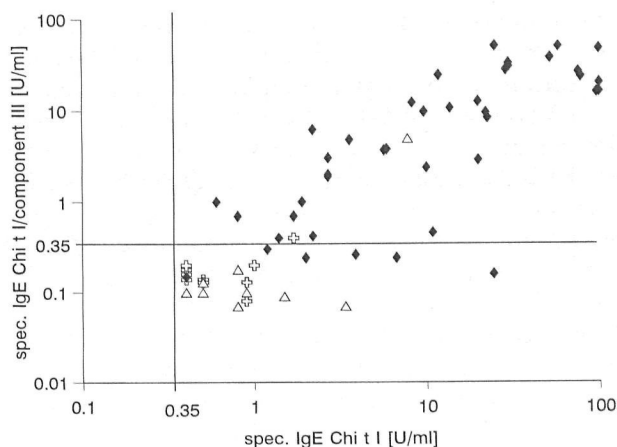


Fig. 4. Correlation between amounts of specific IgE antibodies from 11 Swedish (⊕), 9 Japanese (Δ), and 41 German (◆) subjects against *Chi t I* and *Chi t I* component III. IgE titers were measured by EAST.

Chi t I and for specific IgE against crude extracts of *T. akamusi* and *Cr. sylvestris*, species which are abundant in Japan (9). A large number of Japanese and Taiwanese patients was positive for *Cr. sylvestris* (42.3%) and *T. akamusi* (13.2%), and 4.1% had specific *Chi t I* antibodies. In this connection, it is interesting that more Swedish subjects reacted with Japanese *Cr. sylvestris* (29.4%) than with *Chi t I* (10.9%). The fact that so many EAST assays of *Cr. sylvestris*, which does not contain hemoglobin, showed positive results suggests that substances other than hemoglobin are relevant and act as partially cross-reacting antigens, too (15). *Chi t I* contains nearly 98% pure hemoglobin. There may be another allergenic substance in *C. thummi* midges, possibly explaining the lower number of persons reacting with purified *Chi t I* than with crude extracts of other midges. There are both distinct and common allergens in different Chironomidae species. The existence of common epitopes emerges from the fact that many patients were positive for more than one species, and the fact that cross-reactivity between *Cr. sylvestris* and *Chi t I* was detected. In previous studies with human and rabbit sera, and partially with monoclonal antibodies, a wide range of cross-reactivity among different Chironomidae species was found (3, 11, 15).

In our study, we further investigated the correlation between reactions against *Chi t I* and *Chi t I* component III. Patients with lower amounts of specific IgE antibodies showed a better correlation than those with higher amounts of IgE. However, in the case of high *Chi t I* specific IgE, an unequivocal correlation between *Chi t I* and *Chi t I* component III is not absolutely necessary, from the clinical point of view. As expected, all subjects reacting with component III were also positive for *Chi t I*, which con-

tains component III as well as 11 other hemoglobins. On the other hand, not all *Chi t I* positive subjects possessed specific IgE antibodies against *Chi t I* component III. This result indicates a possible sensitization against hemoglobin components other than component III. These components may share epitopes with other species living in the environment of the patients. Our examination of subjects and Chironomidae species from different countries showed that worldwide-sensitized subjects may develop allergic diseases caused by midge allergens, mainly hemoglobins. These antigens were shown to be identical as well as distinct in different species.

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