

Detection of IgE antibodies to larvae and adults of chironomids by enzyme-linked immunosorbent assay

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Using the ELISA method, IgE antibodies to chironomid midges; *Tokunagayusurika akamusi* larva (TAL), *T. akamusi* adult (TAA), *Chironomus yoshimatsui* larva (CYL), *C. yoshimatsui* adult (CYA) and *Chironomus plumosus* adult (CPA), were measured in the sera of 36 children with bronchial asthma who visited Tamano City Hospital near Lake Kojiima in the Okayama prefecture. IgE antibodies to adult midges (TAA, CYA and CPA) were widely detected, but only few IgE antibodies to larvae (TAL and CYL). The amount of IgE antibody to CYA and to CPA correlated ($P < 0.001$), but the amount of IgE antibody to TAA and to CYA or CPA did not. This suggests that the allergenicity of CYA and CPA is similar, but the allergenicity of TAA is independent of that of CYA or CPA. On the other hand, the ELISA inhibition test showed that TAA allergenicity was independent of that of TAL, CYL, CYA, CPA and the house-dust mite, *Dermatophagoides farinae* (Df). The inhibition test also showed that CYA and CPA had similar allergenicity, but differed from TAL, TAA, CYL and Df. This indicates that the allergenicities of chironomid midges change during the metamorphosis from larva to adult, and are not common among all species.

Key words: allergen; bronchial asthma; chironomid midge; ELISA; IgE.

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Hypersensitivity to chironomid midges was first reported in northern Sudan and southern Egypt in the 1950s. The numbers of chironomids increased following the construction of dams in the Blue and White Niles. The increase in chironomids had been associated with a high incidence of allergic disorders, particularly bronchial asthma and rhinitis. *Cladotanytarsus lewisi* was the main species in those areas, and hypersensitivity to the species has been investigated by skin test, RAST and other immunological technics (4-6, 10, 11, 17-19).

Hypersensitivity to chironomid larva has been studied in the Federal Republic of Germany where they are used for fish bait or fish food. Many people who are occupationally exposed to chironomid larvae in fish-food

factories, as well as sports fishermen suffer from immediate-type hypersensitivity reactions (2, 3).

In Japan, the first bronchial asthma cases due to chironomid midge (*Polypedium kyotoense*) allergen were reported in the Toyama prefecture. Subsequently, studies using skin tests and RAST demonstrated that many of the bronchial asthma patients had anti-chironomid IgE antibody in their blood (8, 13).

Specific IgE antibodies to allergens have generally been measured by RAST. Abe et al. (1) measured house-dust mite-specific IgE antibody by enzyme-linked immunosorbent assay (ELISA). Using this assay, we detected IgE antibodies to chironomids in the sera of asthmatic children, and examined similarities and differences in the aller-

genicity of some of the relevant species of chironomids in Japan.

MATERIAL AND METHODS

Adult worms of chironomid midges, *Tokunagayusurika akamusi* (TAA), *Chironomus yoshimatsui* (CYA) and *Chironomus plumosus* (CPA) were collected at Lake Kojiima in the Okayama prefecture. *Tokunagayusurika akamusi* larvae (TAL) and *Chironomus yoshimatsui* larvae (CYL) were collected from the lake mud. The adults and larvae were stored at -20°C until use.

House-dust mite, *Dermatophagoides farinae* (Df) was cultured and isolated by the method of Sasa et al. (14) and Ishii et al. (7).

For the preparation of antigens the mites and worms of each species were homogenized in 0.1 M Tris-HCl buffer (pH 7.6) using a teflon-glass homogenizer. The homogenates were stirred with a magnetic stirrer for 20 h at 4°C and centrifuged at $100,000 \times g$ for 60 min. The supernatants were used as crude antigens. The protein concentration was determined by the method of Lowry et al. (12).

Human sera were collected from 36 bronchial asthmatic children in Tamano City Hospital. The age distribution was 3 to 14 years (mean 8.5). The sera were stored at -20°C until testing.

IgE antibody to each antigen in human sera was measured by ELISA using a polystylen microplate with 96 flat wells (Nunc, Denmark). The protein concentration of each crude extract used as antigen was adjusted to 10 μg protein/ml with 0.05 M carbonate buffer (pH 9.6) and 100 μl was applied to each well. After overnight incubation at 4°C , the plate was washed three times with phosphate buffered saline containing 0.05% Tween 20 (PBS/Tw) by shaking with a microplate shaker. Each well was blocked with 200 μl of phosphate buffered saline containing 1% BSA (BSP/BSA) for 1 h at 37°C . After three washings with PBS/Tw, 50 μl of human serum (the 1st Ab) diluted 1:1 in PBS containing 1% BSA and 0.05% Tween 20 (PBS/BSA/Tw), was applied to each well and the plate was incubated for 2 h at 37°C . After five washings with PBS/Tw, 50 μl of perox-

idase-conjugated goat anti-human IgE (Miles Scientific, USA) (the 2nd Ab) diluted 1:50 in PBS/BSA/Tw was applied, and the mixture incubated for 2 h at 37°C . A mixture of 0.03% 2,2-azinobis-(3-ethylbenzthiazoline sulfonic acid) diammonium salt (Sigma Chemical Co., USA) and 0.003% H_2O_2 in 0.05 M citrate buffer (pH 5.0) was used as substrate. After the last five washings, 100 μl of the substrate was added to each well and the plate was incubated for 1 h at 37°C . Reaction was stopped by the addition of 50 μl of 1.25% sodium fluoride. Absorbance was measured at 410 nm with Sanko Myreader (Sanko Co., Japan). Antigen-free wells with only BSA/PBS were prepared for each serum. The specific absorbance of antigen-coated wells was calculated by subtracting the absorbance of the antigen-free wells. The test was carried out in duplicate wells for each serum.

ELISA inhibition test. The polystylen microplate was coated with TAA as antigen at a concentration of 10 μg protein/ml and was blocked by PBS/BSA. 100 μl of a pooled serum containing high IgE antibody to TAA (confirmed by ELISA as described above) was agitated with 100 μl of crude antigens of TAL, TAA, CYL, CYA, CPA and Df containing 1000, 100, 10, 1, 0.1, 0.01, 0.001 and 0 μg protein/ml, respectively, for 30 min at 37°C . The ELISA was then performed using the agitated sera as the 1st Ab. The same test was carried out with CYA for coated antigen; a pool serum containing high IgE antibody to CYA was agitated with each antigen and the ELISA performed.

RESULTS

IgE antibodies to each of the five antigens were measured by ELISA in the sera of the 36 asthmatic children. High IgE antibodies to TAA, CYA or CPA, but low IgE antibodies to TAL or CYL were detected. Mean absorbances of the sera to TAA, CYA and CPA were higher than those to TAL and CYL, the difference being statistically significant by the Welch test ($P < 0.001$) (Fig. 1).

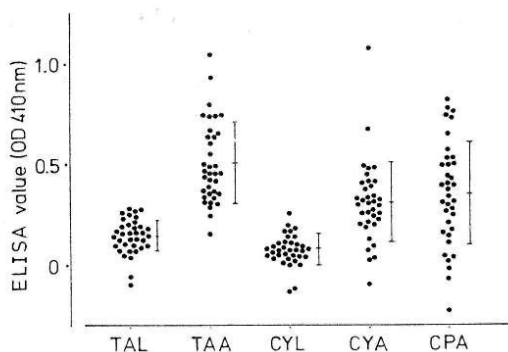


Fig. 1. Comparison of the amount of IgE antibody to various antigens in the sera of 36 asthmatic children (TAL: *Tokunagayusurika akamusi* larva; TAA: *T. akamusi* adult; CYL: *Chironomus yoshimatsui* larva; CYA: *C. yoshimatsui* adult; CPA: *C. plumosus* adult).

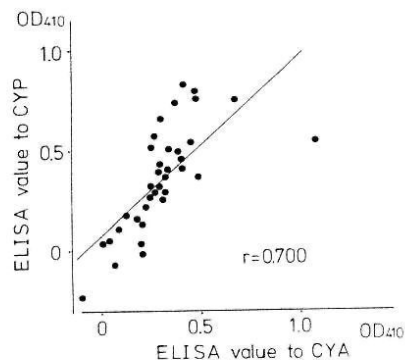


Fig. 2. Comparison of the amount of IgE antibody to CYA with that to CPA in the sera of 36 asthmatic children.

tween CYA and CPA in the sera of 36 asthmatic children was high ($Y = 0.903X - 0.075$, $r = 0.700$, $P < 0.001$) (Fig. 2). However, the correlation coefficient between TAA and CYA was low ($Y = 0.292X + 0.161$, $r = 0.294$). There was also no correlation between TAA and CPA ($Y = 0.197X + 0.255$, $r = 0.153$).

When the pooled sera containing high IgE antibody to TAA were preincubated with increasing concentrations of the crude antigen of TAA, there was a dose-dependent inhibition of ELISA. When the same sera were preincubated with the TAL, CYL, CYA, CPA and Df, there

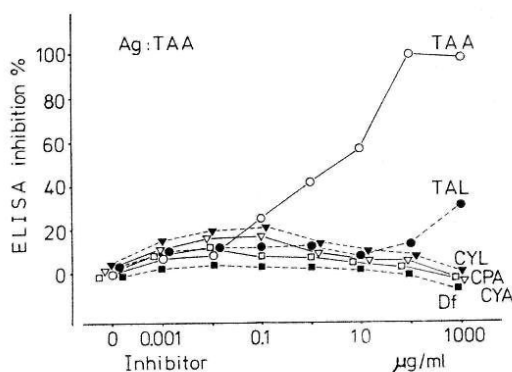


Fig. 3. ELISA inhibition test of IgE antibody to *Tokunagayusurika akamusi* adult (TAA) with *T. akamusi* larva (TAL: ●), adult (TAA: ○), *Chironomus yoshimatsui* larva (CYL: ▼), adult (CYA: ▽), *C. plumosus* adult (CPA: □) and *Dermatophagoides farinae* (Df: ■) antigens.

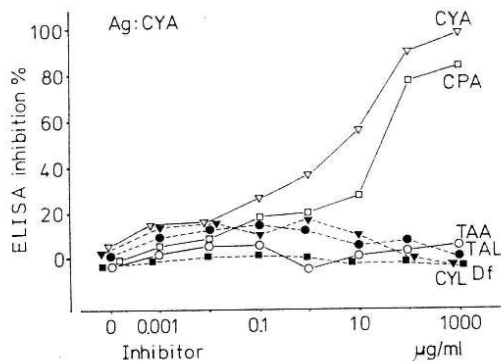


Fig. 4. ELISA inhibition test of IgE antibody to *Chironomus yoshimatsui* adult (CYA) with *T. akamusi* larva (TAL: ●), adult (TAA: ○), *Chironomus yoshimatsui* larva (CYL: ▼), adult (CYA: ▽), *C. plumosus* adult (CPA: □) and *Dermatophagoides farinae* (Df: ■) antigens.

was no appreciable inhibition of ELISA over the same range of concentrations as used in TAA (Fig. 3). When the pooled sera containing high IgE antibody to CYA were preincubated with increasing concentrations of the crude antigens of CYA or CPA, there were dose-dependent inhibitions of ELISA, and when the same sera were pre-incubated with TAL, TAA, CYL and Df, there was no inhibition (Fig. 4).

DISCUSSION

By 1977, 170 species of chironomids had been reported in Japan (15). Although many species were collected in Lake Kojima, we chose three major species: *Tokunagayusurika akamusi*, *Chironomus yoshimatsui* and *Chironomus plumosus*. These three species are abundant in Japan and their bodies are large, containing much protein. *T. akamusi* and *C. plumosus* are prevalent in eutrophic lakes such as Lakes Biwa, Suwa, Kasumigaura and Kojima. In these lakes, and also small rivers in urban areas, *C. yoshimatsui* is ubiquitous. The problem of *T. akamusi* midges is severe around Lake Kojima, breeding explosively in November. They invade houses, swarm around electric lights and frequently fly into eyes, noses and mouths. Dead midges form drifts on the roads and in house-corners, pollute the homes and clothes of the inhabitants, emitting an unpleasant smell of mud. Thus chironomid midge is a possible allergen source in the community, not only when alive but also as a dead component of house dust.

IgE antibodies to TAL, TAA, CYL, CYA and CPA were measured by ELISA in the sera of 36 bronchial asthmatic children who consulted the Tamano City Hospital near Lake Kojima. Large amounts of IgE antibodies to TAA, CYA or CPA, but small amounts to TAL or CYL were detected in the sera of these children. It is conceivable that the inhabitants come in frequent contact with adult chironomids, but less frequently with the larvae. The situation in Japan is similar to that of the Sudan in that hypersensitivity is due to the chironomid adults, whereas in the FRG it is due to the larvae. According to Schin and his colleagues (16), haemoglobins, which are considered to be potent allergens, undergo degradation during the metamorphosis of the midge. Our results suggest that the allergenicity of chironomids changes during metamorphosis from larva to adult.

In each serum, the amount of IgE antibody to CYA correlated well with that to CPA, while the amount of IgE antibody to TAA did not correlate with that to CYA or CPA. The same tendency was also observed in the ELISA inhi-

bition test. Our findings provide evidence that the allergens in CYA and CPA are similar and that the allergen in TAA differs from that in CYA or CPA. *C. yoshimatsui* and *C. plumosus* belong to the same genus and *T. akamusi* belongs to another. Thus, the difference in allergenicity is probably due to a difference in genus. Tee et al. (18), using skin test and RAST inhibition, evidenced that haemoglobins were the common allergenic determinants in two species of the same *Chironomus* genus. In contrast, Cranston et al. (4), using skin test, reported a limited degree of cross-reactivities among nine species of different genera. Furthermore, Kawai et al. (9) demonstrated cross-reactivities among 10 species of chironomid larvae as assayed by PCA reaction in rat skin. The TAL extract showed little reactivity with IgE antibodies to all of the other *Chironomus* genera. Those findings appear to support our results.

In the ELISA inhibition test, IgE antibodies to TAA and CYA were not inhibited by house-dust mite antigen which was a main allergen of house-dust, thus suggesting that the TAA and CYA allergens differed from house-dust mite allergen. Chironomids are large and probably have more allergenic protein than house-dust mites. Ito et al. (8) warn that midge antigens seem to be the second important allergen next to mite antigens in the metropolitan area of Tokyo, Japan. Further investigations are needed to prove the allergenic independency of chironomids and the existence of chironomid allergens in house-dust.

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