

Allergenicity of Chironomidae in asthmatic patients

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In order to clarify the relation between asthma and Chironomidae, we examined the cross-reactivity between Chironomidae and other common allergens.

We noted significant correlations between positive skin tests with Chironomidae and with other allergens. The radioallergosorbent inhibition test, however, suggested that there may be no cross-reactivity or, if any, only very low cross-reactivity between midge allergens and mite, house dust (HD), silk, shrimp, or mosquito allergens.

INTRODUCTION

Asthma and rhinitis caused by Chironomidae were first reported in Northern Sudan,¹ and afterwards were studied extensively by the use of RAST.² Hypersensitivity to Chironomidae is no longer recognized as a problem restricted to the Sudan. Hypersensitivity to Chironomidae has been also reported in Germany,³ United States of America,⁴ Japan,⁵ and Sweden.⁶ In Germany, hypersensitivity is found among persons who deal with fish food, using the larvae of Chironomidae.⁷

In our previous studies it was found that specific IgE and IgG antibodies to Chironomidae in asthmatic patients in Tokyo were significantly greater than those in normal subjects. It was also found that there was a high correlation between positive skin test to midge and to mite.⁸ Recently, it has been reported

that there may be cross-reactivity of allergens between Chironomidae and crustacea in Sweden,⁹ suggesting that skin test to Chironomidae may become positive if the allergen of Chironomidae cross-reacts to other allergens. In the case of IgG antibodies, we demonstrated that there may be no cross-reactivity of midge allergens to those of mite, silk, and mosquito;⁷ however, cross-reactivity of IgE antibodies was not yet fully examined. In the present study we investigated the correlation of Chironomidae skin tests to those of other allergens, and the cross-reactivity of specific IgE using RAST inhibition test.

MATERIALS AND METHODS

Extract of Allergens for RAST

Extracts of adult midges (*Tokunagayusurika akamusi* and *Chironomus yoshimatsui*) and larval midges (*T. akamusi*) were prepared as reported previously.⁵ *T. akamusi* is the species commonly found in lakes in Japan. The adult midges emerge in early November to the beginning of early December.⁸ *C. yoshimatsui* is the species most commonly and exclusively found in bottom sediments of sewage ditches in Japan and the adult midges emerge in all seasons except winter.⁸ An extract from a cultured strain of mosquito (*Culex tritaeniorhynchus*), which is one of the most common

mosquito species in Japan, is a gift from Dr. K. Sato (Institute of Tropical Medicine, Kanazawa Medical University, Japan). Lyophilized extracts from house dust (HD), mite (*Dermatophagoides farinae*), shrimp (*Panacus japonicus* 36%, *Panacus semisulcatus* 36%, *Metapenaeopsis barbata* 28%) and silk were kindly provided by Torii Pharmaceutical Co Ltd, Japan.

Extract of Allergen for Skin Test

1:20 (wt/vol) extracts of adult and larval midge (*T. akamusi*) were used for skin prick tests. Thirty other common allergens for prick test including mite (*Dermatophagoides farinae*), HD, ragweed, Japanese cedar, other pollens, dog hair, shrimp, other foods, and fungi were supplied from Torii Pharmaceutical Co Ltd, Japan. The dilution of extract used for prick testing was 1:100 for mite and fungi, 1:20 for pollens, and 1:10 for other allergens including HD. The extracts of adult and larval midge were the same as those for RAST. The other extracts were prepared from similar materials and by the same procedures as the extracts for RAST. The extract of mosquito was not used in the prick test (see "Discussion").

Skin Test

Prick tests using the extracts of adult and larval midge (*T. akamusi*) were done in 303 randomly selected adult (165 females and 138 males) asth-

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Received for publication January 26, 1988.

Accepted for publication February 28, 1989.

matic patients in the Allergy Clinic, Tokyo University Hospital and in the Allergy Clinic, Teikyo University Hospital, both of which are located in Tokyo. The age range of the patients was 17 to 76 years, and 92% of the patients were 20 to 55 years old. Four percent were over 55, and 4% were less than 20 years old. In the same patients, 30 other common allergens described above were examined by prick test. Evaluation of the skin test reaction was done according to the criteria described by Vanselow.⁹

RAST

Paper discs of adult and larval midges were prepared as described previously.⁵ Paper discs of mite, HD, silk, shrimp, and mosquito were prepared by the method of Ceska.¹⁰ Solutions of 10 mg protein/mL were used at the conjugation to discs. The protein concentrations were determined by Lowry's method.¹¹

Radioallergosorbent Test (RAST) Inhibition

Three patients' sera that were positive by RAST against midge allergen, were pooled and used for RAST inhibition analysis. The pooled serum contained IgE antibodies to adult midges (*T. akamusi* and *C. yoshimatsui*, RAST score 4), mite (RAST score 4), HD (RAST score 4), silk (RAST score 3), shrimp (RAST score 2), and mosquito (RAST score 2). At first, 10-fold serial dilutions of allergens were prepared starting with 10 mg/mL, using RAST incubation buffer described by Ceska et al.¹⁰ Ninety microliters serum and 10 μ L allergen extract of several concentrations were mixed and incubated for one hour at 37°C. Then the serum was put into the tube in which there was a midge-coupled paper disc. The tube was incubated for three hours at room temperature. The paper disc was washed with RAST incubation buffer. Then 0.05 mL of anti-IgE (Pharmacia chemicals, Co Ltd, Uppsala, Sweden) labeled with io-

dine 125 was added and the mixture incubated for 24 hours at 4°C. These methods were essentially the same as described by Ceska et al.¹⁰ After the paper disc was washed, the radioactivity (Ra) was counted by an automatic gamma counter. A mixture of 90 μ L of patient serum and 10 μ L RAST incubation buffer was prepared, incubated with a midge-coupled disc and treated similar as above. This was used as an unabsorbed serum. The experiment was done in duplicate. Inhibition rate was expressed as % = [(Ra of unabsorbed serum - Ra of absorbed serum)/Ra of unabsorbed serum] $\times$ 100.

Statistical Analysis

Statistical analysis was done by using χ^2 test.

RESULTS

Skin Test

Out of 303 patients, 75 were skin test positive to both the adult and larval midge, 22 were positive to only adult midge, and 18 were positive to only larval midge. In total, 97 of 303 patients (32%) were positive to adult midges and 115 of 303 patients (38%) were positive to either or adult and larval midge.

The ten allergens that showed high rates of positive skin reactions in the 303 patients were mite, HD, midge, silk, Japanese cedar, *Candida albicans*, cat dander, ragweed, shrimp, and dog dander (Table 1). There were significant correlations between positive skin tests to midge and those to mite, HD, silk, shrimp, and dog dander (mite, house dust, shrimp, $P < .001$, silk, dog dander, $P < .005$). No correlation was noted, however, between positive skin test reactions to midge and those to Japanese cedar, *Candida albicans*, cat dander, and ragweed (Table 1).

RAST Inhibition Test

We studied RAST inhibition tests with extract from mite, house dust, silk, shrimp, and mosquito. The results are shown in Figure 1. In spite

of the high correlation in skin test, RAST of midges (*T. akamusi* and *C. yoshimatsui*) was not inhibited by mite, silk, and shrimp. In the case of mosquito and house dust, slight inhibition less than 10% was observed at the highest concentration (1 mg/mL in the mixture of allergen and serum). RASTs of *T. akamusi* and *C. yoshimatsui* were mutually inhibited in a dose-dependent manner and maximum inhibition was about 40% at the concentration of 1 mg/mL.

DISCUSSION

As shown in the results, 38% of 303 patients were skin test positive to either adult or larval midge. The rate was next to that of mite and HD. Since it has been reported that the major allergens of HD are derived from mite,^{12,13} midge appeared to be the second important allergen next to mite.

The results in the present experiment appear to suggest that cross-reactivity between IgE antibody to midge allergens and to several other common allergens might not be significant as judged by RAST inhibition.

We selected mite, HD, silk, and shrimp as inhibitory agents because the positive skin reactions to these allergens showed a good correlation with positive prick test responses to midge allergens (Table 1). The extract of mosquito was also selected as an inhibitory agent because the appearance of mosquitoes resembles midges very well, and both are Diptera. Our results further support our previous findings that specific IgG might not cross-react with those of mite, silk, and mosquito. We did not examine skin reaction to mosquito in the 303 asthmatic patients because its extract was not available before the prick test study was accomplished. A comparison between skin reaction to midge and to mosquito should be done in the future, although RAST inhibition tests suggested that the cross-reactivity between midge and mosquito might

Table 1. Correlation of Skin Test Between Midge (*Tokunagayusurika akamusi*) and Other Allergens

Midge	Positive Rate							
	Mite	H.D.	Silk	Japanese Cedar	<i>Candida albicans</i>	Cat Dander	Ragweed	Shrimp
Positive to midge	115	91/115	74/115	55/115	21/115	18/115	9/115	20/115
Negative to midge	188	93/188	55/188	11/188	41/188	26/188	24/188	3/188
Total	303	184/303	129/303	66/303	62/303	44/303	33/303	23/303
P value (by χ^2 test)		<.001	<.001	<.005	NS	NS	NS	<.001

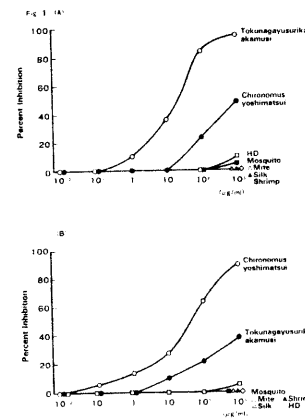


Figure 1. RAST inhibition test. (A) *Tokunagayusurika akamusi* and (B) *Chironomus yoshimatsui*. The ordinate indicates percentage inhibition and the abscissa, final concentration of allergens.

be, if any, low grade. We did not examine cross-reactivity between midges and dog dander because the extract of dog dander for RAST was not available. Existence of high cross-reactivity between the two allergens was not likely because the rate of positive skin test to dog dander was much lower than that to midges (Table 1). Eriksson et al⁶ reported that there was significant positive correlation between prick test to Chironomidae and to shrimp. They concluded that cross-reactivity may exist between Chironomidae and crustacea. Although positive skin tests to midges correlated to those of shrimp in our experiments, RAST inhibition analysis suggests the absence or, if any, only low degree of cross-reactivity between these two antigens. From these re-

sults, we concluded that the positive skin test to the midge might not be ascribed to the cross-reaction with other allergens but that the midge allergen might be discrete. RASTs of *T. akamusi* and *C. yoshimatsui* were mutually inhibited in a dose-dependent manner, indicating the presence of cross-reactivity among these midges. Baur et al¹⁴ reported the cross-reactivity among chironomid species. Tee et al¹⁵ also reported the presence of cross-reactivity between *Chironomus riparius* and *Chironomus lewisi* using RAST inhibition studies. Their reports have demonstrated hemoglobins as a common allergenic determinant. RAST of larval midges of *T. akamusi* is inhibited about 24% with those of *C. riparius* (Baur, personal communication). Thus, *T. akamusi* appears to be distantly related to the species in the genus of *Chironomus* in evolutionary terms, which may account for the lack of complete cross-reactivity in adult midges of *T. akamusi* and *C. yoshimatsui*.

As the pooled serum was obtained from only three patients, it could not be denied completely that there was some cross-reactivity between midge allergens and other common allergens. Further, it is not clear that the amount of allergens used for RAST inhibition test was enough to absorb cross-reacting antibodies, although relatively large amounts of allergens were used. A crucial conclusion must await further experiments using other methods such as immunoabsorption study described by Gleich et al.¹⁶ It was suggested from our results (Fig 1) that midges have allergenicities distinct from other common allergens. If it is true, a question arises, why skin reaction

to mite, HD, silk, shrimp, and dog dander showed good correlation with positive reactions to midge allergens. It was reported in our previous study that the correlation between midge-RAST scores and mite-RAST score was not significant, although there was a significant correlation between positive skin tests to both allergens.⁵ It is therefore suggested from this study together with the present study that patients allergic to one allergen might have a tendency to be sensitized to other allergens, even if the cross-reactivity between these allergens is not detected or, if any, it is of very low grade. Similar studies of RAST using allergens other than mite are in progress.

As reported in another paper, the midge allergens were able to release histamine from leukocytes from asthmatic patients who were positive to midge RAST.¹⁷ Kino et al demonstrated that there were particles containing Chironomidae allergen with a diameter smaller than 10 μ m in the air in Kyoto, Japan.¹⁸ The amount was comparable to that of ragweed allergen. Ito et al reported that 29.5% of randomly selected asthmatic patients were positive to midge RAST in Tokyo, Japan.⁵ Igarashi et al reported that a high rate of asthmatic responses was observed in midge RAST-positive asthmatic patients when they were challenged with midge antigen.¹⁹ These reports together with our present study suggest that chironomid midges may be very important in clinical allergy in Japan and other countries with similar situations concerning outbreak of chironomid midges. These also suggest that prick test with midge allergens might be useful in allergy clinic.

ACKNOWLEDGMENT

We thank Dr. Masahiro Nikami, Nippon Gene Company, Ltd for giving extracts of *T. akamusi*. We thank Dr. Sato, Kanazawa Medical University for giving the extract of mosquito. We also thank Torii Pharmaceutical Co Ltd for giving extracts of mite, HD, shrimp, and silk. This study was supported by Nissan Foundation for Science and Manabe Medical Foundation.

REFERENCES

1. Freeman P: A species of chironomid (Diptera) from Sudan suspected of causing asthma. *Proc Roy Entomol Soc London (B)* 1950; 19:58-59.
2. Gad El Rab MO, Kay AB: Widespread immunoglobulin E-mediated hypersensitivity in the Sudan to the 'green nimitti' midge *Cladotanytarsus lewisi* (Diptera: Chironomidae). I. Diagnosis by radioallergosorbent test (RAST). *J Allergy Clin Immunol* 1980;66:190-197.
3. Baur X: Hämoglobine von Chironomidae (Zuckmücken): bisher unbekannte, aggressive Inhalations-Antigene für den Menschen. *Kin Wochenschr* 1980;58:1163-1164.
4. Kagen SL, Yunginger JW, Johanson R: Lake fly allergy: incidence of chironomid sensitivity in an atopic population. (abstract) *J Allergy Clin Immunol* 1984;73:87.
5. Ito K, Miyamoto T, Shibuya T, et al: Skin test and radioallergosorbent test with extracts of larval and adult midges of *Tokunagayusurika akamusi* Tokunaga (Diptera: chironomidae) in asthmatic patients of the metropolitan area of Tokyo. *Ann Allergy* 1986;57:199-204.
6. Eriksson N, Johnsson P, Ryden B: Cross-sensitization between chironomids and crustacea. (Abstract) *Eur Congr Allergy* 1986.
7. Yamashita N, Ito K, Nakagawa T, et al: IgG and IgE antibodies to Chironomidae in asthmatic patients. *Clin Exp Immunol* 1987;68: 93-99.
8. Sasa M, Kawai K: Studies on chironomid midges of lake Biwa (Diptera, Chironomidae). *Lake Biwa Study Monographs, Lake Biwa Research Institute* 1987;3:1-119.
9. Vanselow NA: Skin testing and other diagnostic procedures. in Sheldon JM et al (eds): *A Manual of Clinical Allergy*. Philadelphia, WB Saunders Company, 1967; pp 55-77.
10. Ceska M, Eriksson R, Varga JM: Radioimmunosorbent assay of allergen. *J Allergy Clin Immunol* 1972;49:1-9.
11. Lowry OG, Rosebrough NJ, Farr L, et al: Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:263-275.
12. Voorhorst R, Spieksma FTHM, Varekamp H, et al: The house-dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house-dust allergen. *J Allergy* 1967;39:325-339.
13. Miyamoto T, Oshima S, Ishizaki T, et al: Allergenic identity between the common floor mite (*Dermatophagoides farinae*, Hughes, 1961) and house dust as a causative antigen in bronchial asthma. *J Allergy* 1968;42:14-28.
14. Baur X, Dewair M, Haegele K, et al: Common antigenic determinations of hemoglobin as basis of immunological cross-reactivity between chironomid species. (Diptera, Chironomidae): studies with human and animal sera. *Clin Exp Immunol* 1983;54:599-607.
15. Tee RD, Cromwell O, Cranston PS, et al: Evidence for haemoglobins as common allergenic determinations in IgE-mediated hypersensitivity to chironomids (non-biting midges). *Clin Allergy* 1985;14:117-127.
16. Gleich GT, Jacob GL: Immunoglobulin E antibodies to pollen allergens account for high percentages of total immunoglobulin E protein. *Science* 1985;190:1106-1108.
17. Yamashita N, Morita Y, Ito K, et al: Chironomidae as a cause of IgE-mediated histamine release in patients with asthma. *Ann Allergy In press*.
18. Kino T, Chihara F, Fukuda K, et al: Allergy to insects in Japan III. High frequency of IgE antibody responses to insects (moth, butterfly, caddisfly and chironomid) in patients with bronchial asthma and immunochemical quantitation of the insect-related air borne particles smaller than 10 μ m in diameter. *J Allergy Clin Immunol* 1987;79: 857-866.
19. Igarashi T, Murakami G, Adachi Y, et al: Common occurrence in Toyama of bronchial asthma induced by chironomid midges. *Japan J Exp Med* 1987;57:1-9.

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