

Humoral immune response to the insect allergen *Chi t I* in aquarists and fish-food factory workers

Liebers V, Hoernstein M, Baur X. Humoral immune response to the insect allergen *Chi t I* in aquarists and fish-food factory workers. Allergy 1993; 48: 236–239. © Munksgaard 1993.

Our examination of 225 subjects who had been exposed to the insect allergen *Chi t I* involved the degree of allergen exposure, the exposure-associated symptoms, and their relationship to the presence of specific IgE and IgG antibodies as well as sensitization to ubiquitous allergens. It could be shown that specific IgE antibodies found in 34% of these subjects were closely associated with symptoms ($P < 0.01$), whereas no relationship between IgG antibodies and complaints could be observed. Conjunctivitis (63%) and rhinitis (62%) were predominant, followed by asthma (45%) and urticaria (37%). Antibody levels of patients suffering from asthma were highest. In addition, symptoms were associated with the degree of exposure. While nearly all IgE-sensitized subjects of the medium-, high-, and very high-exposure group were symptomatic, only 57% of the sensitized individuals of the low-exposure group reported complaints. Furthermore, specific IgE antibodies were most frequently present in the groups with medium (46%) and high (54.5%) exposure, whereas IgG antibodies predominated in individuals with very high exposure (69.1%). In the low-exposure group, most subjects (73.6%) had neither IgE nor IgG antibodies.

In addition, within *Chi t I* sensitized subjects, sensitization to common allergens and elevated total IgE levels were more frequently present than within non-*Chi t I* sensitized individuals, indicating a predisposition to allergy.

Inhalative uptake of organic materials elicits specific immune responses in a proportion of exposed persons. The initiation of the immune response and its type depend, among other factors, on the nature and amount of the airborne substance(s). In addition, immune responses are determined by genetic factors, since restriction takes place as a result of specific binding of immunogenic peptides by corresponding structures of MHC class II molecules encoded within the HLA-D loci (7, 8).

Aquarists often use larvae of the Diptera species *Chironomus thummi thummi* in freeze-dried form or as a component of mixed flake food. Hemoglobins (*Chi t I*) of the larvae of this insect species are well-characterized allergens (2, 3, 9, 13).

In this retrospective study, we evaluated the data of 225 subjects who had been in contact with *Chi t I*. The intention was to identify the dependence of serum levels of specific IgE and IgG antibodies on the degree and duration of exposure as well as their

correlation with symptoms, total IgE, and sensitization to common allergens.

Material and methods

Study group

The case histories of 225 subjects (68 females, 157 males) who had responded to our advertisement in aquarists' journals were recorded, and blood samples were taken. A trained physician, using a standardized questionnaire, obtained a careful case history and made the anamnestic diagnoses used in the following investigation. All subjects of the study were volunteers who had contact with fish food because of their hobby or occupation and were interested in testing their allergic state.

The group was subdivided according to duration and intensity of exposure. Before skin testing and antibody determination, the degree of exposure was calculated according to the following information:

V. Liebers¹, M. Hoernstein², X. Baur¹

Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin (BGFA)¹, Bochum Klinikum Großhadern, Medizinische Klinik I², Pneumologische Abteilung, Universität München

Key words: *Chi t I*-sensitization; specific IgE.

Dipl.-Biol. V. Liebers
Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin (BGFA)
Gilsingstr. 14
4630 Bochum 1
Germany

Accepted for publication 1 May 1992

- 1) the amount of *C. thummi thummi* larvae used per month;
- 2) the form of fish food (*C. thummi thummi* larvae are sold freeze-dried as frozen bars or live in water);
- 3) the frequency of contact per month (some subjects use *C. thummi thummi* only once or twice a week, whereas other subjects, especially in fish-food production, have daily contact with the larvae).

The form of fish food was important since the larvae in freeze-dried form are most allergenic owing to dust occurrence in handling. An estimated amount of less than 5 mg of *C. thummi thummi* larvae allergen per month was assumed to be low, 5–25 mg to be medium, and more than 25 mg to be high exposure. An extremely high degree of exposure (> 100 mg) was assumed in 15 workers engaged in industrial fish-food production. The following proportion of fish food was related to the hemoglobin allergen: 0.001% of the frozen larvae, 0.002% of the living larvae, 0.024% of mixed fish food, and 0.1% of the freeze-dried larvae.

Allergen preparation and skin prick test

Chi t I was prepared from *C. thummi thummi* larvae as previously described (6). Skin prick tests were performed in 94 volunteers (conc. 0.1 mg/ml). Wheal and flare reactions greater or equal to 25% of those induced by 0.1% histamine were regarded as positive. The intensity of skin test reactions was subdivided into four groups:

- 1) 25% compared with the histamine reaction;
- 2) 50% compared with the histamine reaction;
- 3) 100% compared with the histamine reaction;
- 4) 200% compared with the histamine reaction.

Antibody determination

Chi t I specific IgE and IgG antibodies were quantitated by the RAST method (radioallergosorbent test) and a modified RAST technique using protein A instead of anti-IgE.

Each subject underwent RAST screening with disks coupled with extracts from mixed molds, mixed animal dander, wheat flour, *Dermatophagoides pteronyssinus* (Pharmacia), mixed grass pollen, mixed tree pollen, mixed flower pollen (Bencard), and *Chi t I* (bound to cyanogen-bromide-activated paper disks in our laboratory). Further RAST reagents, including the reference system, were bought from Pharmacia. Results greater than 0.35 U/ml were regarded as positive. Total IgE in the serum was determined by Phadebas IgE PRIST (Pharmacia).

Statistical analysis

Data were analyzed with paired *t*-test or Chi-square test; 0.05 was taken as the limit of significance. Correlation is given as Spearman's correlation coefficient.

Results

Most subjects had been exposed to *C. thummi thummi* larvae for 5–25 years (63.3%) and were assigned to the medium-exposure group (49.3%). The study group consisted of 190 aquarists, 32 workers in a fish-food factory, and 3 employees of a research institute.

IgE antibodies specific to *Chi t I* were detectable in 34% ($n = 76$) of the whole study group, ranging from 0.41 to 635 U/ml. Specific IgG antibodies were found in 20% ($n = 45$) of all cases studied; 27 of them also had *Chi t I* specific IgE antibodies, while 131 showed neither IgE nor IgG antibodies (Fig. 2).

In the skin prick test, *Chi t I* produced results in 90 subjects corresponding to those of RAST; thus, skin test reactions are associated with positive RAST results. In four cases, weakly positive skin test reactions were observed in spite of a negative RAST result. With regard to skin test groups, 5 patients were assigned to group 1, 19 to group 2, 17 to group 3, and 10 to group 4. Forty-three patients showed no skin test reaction. Correlation between skin test reactions and specific IgE was significant ($P < 0.001$; $r_s = 0.7918$).

Respiratory, conjunctival, and/or cutaneous complaints induced by fish food were reported by 34.2% ($n = 77$) of the total population and by 86% ($n = 65$) of IgE-sensitized subjects (Table 1). Only 8%

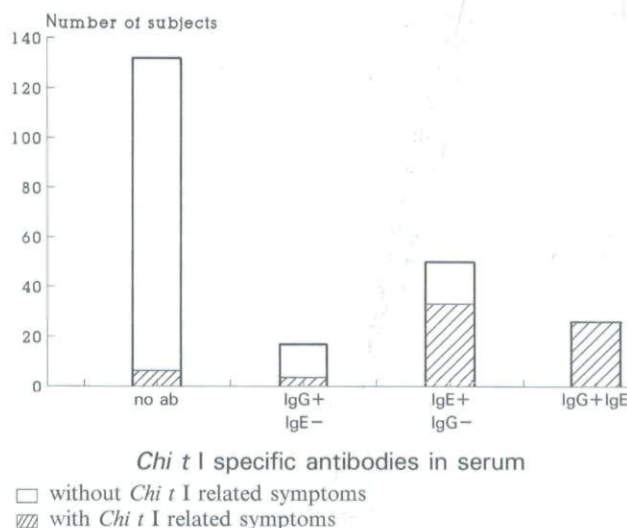


Fig. 1. Association of allergic complaints caused by fish food with specific immune response to *Chi t I*. The presence of IgE antibodies is strongly associated with complaints caused by fish food.

Table 1. Frequency of symptoms related to fish food. Subjects are subdivided into those with (*Chi t I*+) and those without (*Chi t I*-) specific IgE antibodies

	Total population		<i>Chi t I</i> (+) subjects		<i>Chi t I</i> (-) subjects	
	n=225	100%	n=76	100%	n=149	100%
No symptoms	148	66%	11	14%	137	92%
Total symptoms	77	34%	65	86%	12	8%
Bronchial asthma	37	16%	34	45%	3	2%
Rhinitis	54	24%	47	62%	7	5%
Conjunctivitis	52	23%	48	63%	4	3%
Urticaria	33	15%	28	37%	5	3%

($n = 12$) of subjects without *Chi t I* specific IgE antibodies reported symptoms; 42% ($n = 5$) of them released *Chi t I* specific IgG antibodies. The close relationship of symptoms to specific IgE antibodies ($P < 0.01$) is shown in Figure 1. While 90% of subjects with IgE antibodies developed complaints, only 55% of the IgG positive group and only 5% of the group without antibodies reported symptoms. The incidence of allergic complaints within the different exposure groups is shown in Figure 2. The percentage of subjects reporting complaints increased with the intensity of exposure. With regard to IgE-sensitized persons, most subjects in the medium- (94%), high- (94%), and very high-exposure (100%) group reported symptoms, but only 57% of patients of the low-exposure group developed complaints. Among the different diagnoses, the *Chi t I* specific IgE antibody levels of persons suffering from asthma were the highest (the mean value of specific IgE in asthmatic people was 88.65 U/ml, and in nonasthmatic people it was 17.5 U/ml; $P < 0.01$).

When we compared subjects with specific IgE antibodies to *Chi t I* with non-*Chi t I* sensitized subjects, the first group showed more frequent sensi-

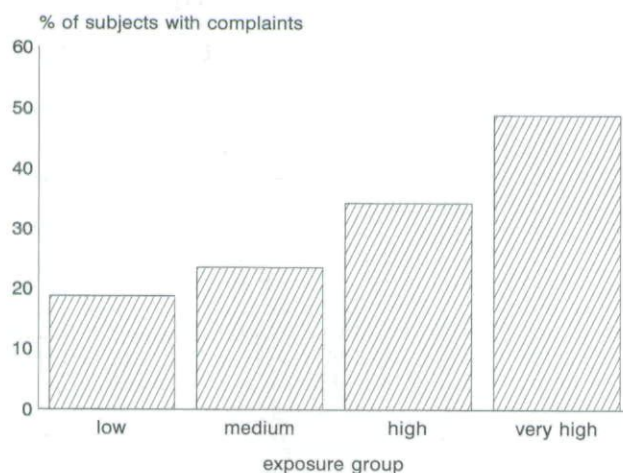


Fig. 2. Percentage of subjects of the whole study group with allergic complaints (rhinitis, urticaria, conjunctivitis, asthma) in the various groups of people exposed to *Chi t I*.

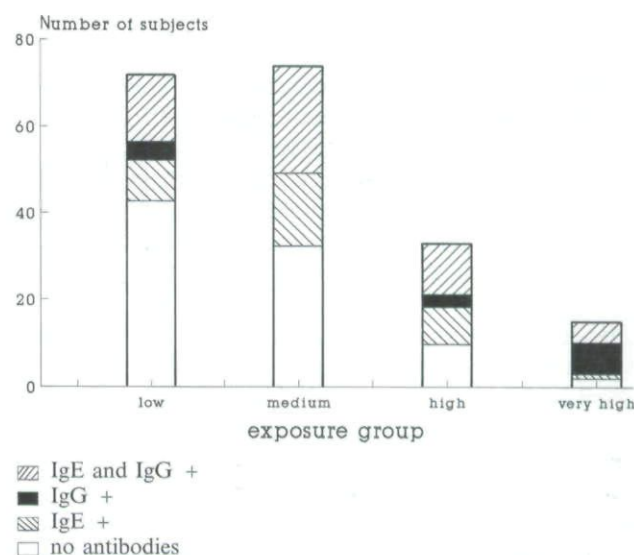


Fig. 3. Dependence of specific immune response to *Chi t I* on exposure degree, and numbers of subjects having the indicated antibodies in each exposure group.

zation to common allergens (50% vs. 20%) and more elevated total IgE levels (55% vs. 28%) than the second group. With regard to the level of total IgE antibodies, the mean value of *Chi t I* sensitized subjects with further sensitizations to common allergens was significantly higher, as compared with *Chi t I* monosensitized subjects (498.5 U/ml vs. 108.4 U/ml; $P < 0.01$).

Chi t I-specific IgE is mostly elevated in persons with medium exposure, whereas *Chi t I*-specific IgG is mainly found in factory workers with extremely high exposure (Fig. 3). Exposure duration appears to influence neither specific IgE nor IgG (no significant correlation for IgE and IgG antibodies).

Discussion

Our study shows symptoms to be associated with the degree of *Chi t I* exposure. In addition, these symptoms are combined with *Chi t I*-specific IgE antibodies but not with IgG antibodies. Complaints observed in 8% of the subjects without *Chi t I*-specific IgE were probably due to sensitization to other allergens of the fish food or to common aeroallergens. Among symptoms, conjunctivitis and rhinitis were predominant. Antibody levels of patients suffering from asthma were highest. In addition, persons with elevated total IgE antibodies to common allergens were significantly more frequently RAST-positive for *Chi t I*.

A high degree of *Chi t I*-monosensitization (50%) exists, however. Whereas people with pollen allergy are commonly sensitized to different pollens, suggesting related allergenic components, the high percentage of *Chi t I* monosensitized subjects indicates

no crossreactivity with common allergenic components but, rather, a strong immunogenicity.

Chi t I-specific IgG antibodies show a relationship to the degree of exposure. Similar results have been reported for several occupational inhalative antigens (1, 16). In addition, the finding that people suffering from asthma develop the highest IgE antibody levels corresponds to previous results with indoor allergens (19). It has been shown that IgE antibodies can be an important risk factor for acute asthma (17) and that bronchial hyperreactivity can increase as a result of allergen exposure. Our results indicate that the IgE immune response to *Chi t I* is not necessarily associated with the presence of significant levels of IgG antibodies in the serum. Allergen-specific effects seem to influence the immunoglobulin class shifting. As reported, allergen-specific effects are also important in lymphocyte proliferation, skin tests, histamine release, and antibody determination. A positive correlation with skin test, histamine release, and total IgE level could be found for *Amb a I*, but not for *Amb a V* (5). Genetic factors responsible for recognizing defined antigenic structures may be important as well. As a matter of fact, an association between *Chi t I* immune response and certain genes of the HLA D-locus was found. Subjects without sensitization to common allergens expressing the HLA-type DR 1 or DQw 1.1 had a significantly increased risk of sensitization to *Chi t I*. On the other hand, DQw3-positive individuals are more commonly non-*Chi t I* responders (18). Associations between HLA D-types are also described for allergies to *Dermatophagoides farinae* (14, 15), ragweed pollen allergens (12), and bee venom (11).

In conclusion, our study shows *Chi t I* to be an important allergen affecting many aquarists. Furthermore, it is an important occupational (10) and environmental (4) allergen. Our data indicate that the intensity of exposure is relevant in eliciting the IgE antibodies finally inducing allergic symptoms. There was, however, no association between exposure duration and antibodies; we have no evidence whether this is due to a "healthy worker effect", since previously affected subjects were also included in our study. According to our results, lower exposure degrees significantly reduce the frequency of sensitization. Therefore, we strongly advise avoiding the allergen in the case of an established *Chi t I* allergy.

References

- BAUR X. Asthma Alveolitis Aspergillose – Charakterisierung ursächlicher Antigene. Berlin-Heidelberg-New York: Springer, 1986.
- BAUR X, DEWAIR M, FRUHAMNN G, ASCHAUER H, PFLETSCHINGER G, BRAUNITZER G. 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* 1982; 69: 66.
- BAUR X, ASCHAUER H, MAZUR G, DEWAIR U, PRELICZ H, STEIGEMANN W. Structure, antigenic determinants of some clinically important insect allergens: chironomid hemoglobins. *Science* 1986; 223: 351.
- BAUR X, LIEBERS V. Chironomid hemoglobins – relevant environmental and occupational allergens. *Arch Occup Environ Disease* (in press).
- BLACK P, MARSH D. Correlation between lymphocyte response and immediate type hypersensitivity to purified allergens. *J Allergy Clin Immunol* 1980; 66 (5): 394–401.
- BRAUN V, CRICHTON R, BRAUNITZER G. Über monomere und dimere Insektenhäoglobine (*Chironomus thummi*). *Hoppe-Seyler's Z Physiol Chem* 1968; 349: 197–210.
- FRENCH M, DAWKINS R, CHRISTIANSEN F, ZHANG W, DEGLI-ESPOSTI M, SAUERACKER G. Reply to the comment on: "Central MHC genes, IgA deficiency and autoimmune disease". *Immunol Today* 1991; 12 (4): 135.
- LAMB JR. Essential requirements for major histocompatibility complex recognition in T-cell tolerance induction. *Nature* 1984; 308: 72–4.
- LIEBERS V, SCHALBABA S, MAZUR G, BAUR X. Stimulation of human peripheral blood lymphocytes with chironomid hemoglobin allergen (*Chi t I*). *Int Arch Appl Immunol* 1991; 95: 163–8.
- LIEBERS V, BAUR X. Allergien am Arbeitsplatz – Gefahrstoffe für Fischzüchter und Beschäftigte in Forschungsinstituten und Fischfutterfabriken. Die BG, Fachzeitschrift für Arbeitssicherheit und Unfallversicherung 1992; 6: 364–6.
- LYMPANY P, KEMENY M, WELSH K, LEE T. An HLA-associated nonresponsiveness to melittin: a component of bee venom. *J Allergy Clin Immunol* 1990; 86 (2): 160–70.
- MARSH D, ZWOLLO P, HUANG S. Molecular and cellular studies of immune responsiveness to the short ragweed allergen *Amb a V*. *Eur Respir J* 1991; 4 (Suppl 13): 60–7.
- MAZUR G, BAUR X, LIEBERS V. Hypersensitivity to hemoglobins of the Diptera family Chironomidae; structural and functional studies of their immunogenic/allergenic sites. In: BALDO BA, ed. Molecular approaches to the study of allergens. Monographs in Allergy. Basel: Karger, 1990: 121.
- O'HEHIR R, ECKELS D, FREW A, KAY A, LAMB J. MHC class II restriction specificity of cloned human T lymphocytes reactive with *Dermatophagoides farinae* (house dust mite). *Immunol* 1988; 64: 627–31.
- O'HEHIR R, DOUGLAS Y, BARRY K, LAMB J. Clonal analysis of the cellular immune response to the house dust mite *Dermatophagoides farinae*. *Int Arch Allergy Appl Immunol* 1989; 88: 170–2.
- PIEN LC, ZEISS CR, CHESTER LL, et al. Antibody response to trimellityl hemoglobin in trimellitic anhydride-induced lung injury. *J Allergy Clin Immunol* 1988; 82: 1098–1103.
- PLATTS-MILLS T, CHAPMAN M, POLLART S, LUCZYNSKA C, WARD G. Specific allergens evoking immune reactions in the lung: relationship to asthma. *Eur Respir J* 1991; 4 (Suppl 13): 68–77.
- TAUTZ C, HOERNSTEIN M, MARSH DG, BAUR X. IgE response to chironomid allergen *Chi t I* is associated with specific HLA-D-sequences. *J Allergy Clin Immunol* 1990; 85: 201.
- WAHN U, LAU S, EHNERT B. Housing conditions for growth of mites and increased levels of dust mite allergens: the risk of sensitization. *Allergology* 1990; 11 (13): 439.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.