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Histamine Release from Human Basophils by the Insect Allergen Chi t I¹

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Abstract. Hemoglobins of the Diptera species *Chironomus thummi thummi* (Chi t I) are potent inhalant allergens. Chi t I-specific histamine release was measured by a new radioimmunoassay in whole human blood taken from 20 sensitized patients, 11 exposed nonsensitized probands, and 11 nonexposed controls. The sensitized patients, who all had positive skin tests and radioallergosorbent test results with Chi t I, showed a significantly higher histamine release than the two other groups. However, within the patient group, the percentage of released histamine did not correlate with the intensity of the skin test response or the concentration of Chi t I-specific IgE antibodies. Our results demonstrate that this method is a sensitive and specific in vitro test for evaluation of IgE-mediated sensitization.

Introduction

Larvae and adult midges of the Diptera family Chironomidae are known to elicit IgE-mediated diseases in many exposed people [1-8]. In the larvae from the species *Chironomus thummi thummi* (synonym: *Chironomus riparius*), which are often used in freeze-dried form as domestic fish food, most or all relevant allergens are hemoglobins. The primary structures of 12 individual hemoglobin components have been described [9, 10], and the tertiary structure of one (component III) was elucidated by X-ray analysis [11, 12].

The midges are an important environmental cause of conjunctivitis, rhinitis, and bronchial asthma in water-rich areas in Japan [5-8], the United States of America [3], Egypt, and the Sudan [2, 4]. They contain in addition to the hemoglobin structures further allergenic components [6-8].

The aim of this study was to investigate histamine release (HR) in whole human blood from patients and controls, using the well-characterized hemoglobin allergen (Chi t I) from the species *C. thummi thummi* and a new radioimmunoassay for histamine [13]. This commercially available assay allows an easy and

quick determination of histamine for clinical laboratories. In contrast, other determinations with fluorometric, chromatographic, and radioenzymatic techniques [14-18] are expensive and/or need a well-equipped laboratory.

Patients and Materials and Methods

Patients and Controls

Venous blood samples were taken from 31 fish hobbyists who had used freeze-dried chironomid larvae of the species *C. thummi thummi* for at least 2 years. 17 of them were atopic, and 20 had noticed symptoms associated with contact and showed clear evidence of IgE-mediated sensitization. 11 unexposed persons (9 healthy persons and 2 atopics sensitized to other inhalant allergens) served as controls.

Allergen Preparation

Crude extracts of *C. thummi thummi* larvae were prepared as described [1]. The hemoglobins were precipitated with ammonium sulfate at a final concentration of 80% (w/w). After centrifugation (60 min at 15,000 g) the precipitated hemoglobins were dissolved, desalted on a Sephadex G-25 column (Pharmacia, Uppsala, Sweden) in 0.01 M ammonium hydrogen carbonate buffer, and freeze-dried.

Clinical Test Methods

For prick skin tests, a concentration of 0.01 mg/ml of Chi t I was used. With respect to the diameter of the wheal reaction observed after 20 min, grading was performed as following: < 2 mm = 0; 2-4 mm = 1; 4-7 mm = 2; 7-10 mm = 3; > 10 mm = 4.

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Table 1. Clinical and laboratory data of the study groups

Sub- ject No.	Age years	Total IgE U/ml ^a	IgE antibodies to common allergens ^b	Chironomid- induced symptoms ^c	Chi t I skin test 0.001 mg/ml	Anti-Chi t I IgE PRU/ml ^b	Anti-Chi t I IgG U/ml ^d	Histamine in whole blood, nM ^e	HR with anti-IgE, µg		
									0.02	0.2	2.0
Chi t I-sensitized patients											
1	60	279	-	B	4	20.1	0.57	90	18	27	67
2	45	190	-	R, C	3	8.4	0.32	1,050	6	7	32
3	37	94	T, F	(A), (B)	3	12.0	0.78	525	4	9	34
4	21	39	-	R, U	2	5.0	0.53	630	19	29	53
5	45	657	G, T, F	A, R, C	4	24.3	1.04	117	54	48	48
6	54	222	-	B, R, C	3	12.0	0.79	210	71	86	71
7	56	150	G, C	A, R, C	3	22.0	1.43	504	71	50	14
8	43	777	G, T, F, H	R, C, U	4	150.0	0.96	160	100	100	53
9	43	732	-	A, B, R, C, U	3	55.0	1.11	740	33	30	11
10	58	166	-	R, C	2	52.0	0.53	252	79	71	64
11	30	24	-	R, C	1	6.0	0.54	1,300	20	24	34
12	56	199	G	(A), (B), (R), (C)	3	21.6	0.38	580	14	16	14
13	38	39	T	C	2	0.4	0.08	109	94	63	38
14	30	160	-	A, B, R, C, U	3	11.8	0.75	400	55	50	17
15	40	110	T	R, C, U	4	23.5	0.18	175	13	19	10
16	46	241	G, T, C	(R), U	4	48.0	0.67	130	11	17	8
17	26	2,198	H	(A), (R)	n.d.	0.8	0.08	273	23	50	27
18	32	18	C	R, C	4	137.0	1.94	485	10	6	24
19	36	1,392	G, T, F, H, C	A, B, R, C, U	n.d.	635.0	0.38	268	59	48	15
20	45	1,180	G, T, H, M, C	C	3	535.0	1.35	282	21	5	32
Chi t I-exposed, nonsensitized persons											
1	62	33	G	0	0	0.06	0.29	180	32	38	27
2	63	45	0	0	0	0.06	0.60	80	55	71	33
3	56	414	0	0	0	0.04	0.27	160	6	15	8
4	53	3	0	0	0	0.04	0.29	483	12	24	8
5	56	52	0	0	0	0.06	0.75	231	22	27	17
6	54	7	0	0	0	0.04	0.27	118	54	64	39
7	48	19	T	0	0	0.06	0.15	588	4	7	4
8	55	22	0	0	0	0.03	0.06	360	24	27	10
9	51	390	T	0	0	0.14	0.05	190	5	10	3
10	25	198	G, M, C	0	0	0.06	0.48	248	3	13	4
11	29	15	G	0	0	0.09	0.50	340	7	5	9
Chi t I-nonexposed persons											
1	23	148	G, Fl, S	0	0	0.04	0.23	315	38	50	27
2	35	433	0	0	0	0.09	0.23	300	3	7	10
3	32	18	0	0	0	0.04	0.18	152	26	65	55
4	65	70	0	0	0	0.12	0.23	135	20	24	20
5	48	108	H	0	0	0.06	0.09	368	22	20	8
6	59	4	0	0	0	0.01	0.06	71	51	45	24
7	53	17	0	0	0	0.03	0.15	504	48	48	23
8	45	73	0	0	0	0.06	0.10	406	17	27	7
9	29	12	0	0	0	0.05	0.26	460	16	2	9
10	28	95	0	0	0	0.10	0.54	647	2	25	2
11	23	51	0	0	0	0.10	0.18	906	1	5	2

^a Upper limit of normal range: 120 U/ml. ^b Upper limit of normal range: 0.35 U/ml; C = cat dander, F = flower pollen, Fl = flour, G = grass pollen, H = house dust mite (*D. pteronyssinus*), M = moulds, S = soybean, T = tree pollen. ^c A = Asthma, B = bronchitis, C = conjunctivitis, R = rhinitis, U = urticaria; parentheses indicate doubtful relationship with exposure to chironomid larvae. ^d Upper limit of normal range: 0.64 U/ml. ^e Upper limit of normal range [13]: 500 nM.

Total serum IgE concentrations were determined using the Phadebas IgE PRIST (Pharmacia). The results are expressed in units per milliliter. Radioallergosorbent test screening with common allergens was performed with discs coupled to extracts from *Dermatophagoides pteronyssinus*, animal dander and wheat flour (Pharmacia), mixtures of grass pollen, tree pollen, flower pollen (Bencard, Neuss, FRG), and moulds (Pharmacia). In case of additional relevant exposure, the allergen spectrum was broadened. The specific IgE antibodies were determined with Pharmacia's radioallergosorbent test.

To determine anti-Chi t I IgE antibodies, Chi t I was coupled to cyanobromide-activated paper discs. The antibodies were measured as previously described by radioallergosorbent test [1]. The results were expressed according to the PRU reference system (Pharmacia). Sera which gave values > 17.5 PRU/ml were diluted and retested; values > 0.35 PRU/ml were regarded as positive.

For measurement of specific IgG antibodies, Chi t I was bound to glutaraldehyde-activated microtiter plates; in each well 0.5 µg Chi t I dissolved in 100 µl of 50 mM sodium carbonate buffer (pH 9.3) was pipetted.

After incubation for 16 h the plates were washed three times with phosphate-buffered saline containing 0.1% Tween 20 and 0.1% bovine serum albumin (PBST), and the remaining binding sites were saturated by incubating with PBST (250 µl/well) for 1 h. 100-µl samples of diluted serum (1:500) were incubated overnight, and, after three washes, anti-Chi t I-specific IgG was detected by incubating with 100 µl ¹²⁵I-labeled protein A (1×10^5 cpm; New England Nuclear, Dreieich, FRG) for 3 h, washing, and measuring the bound radioactivity with a gamma counter. The results are expressed in arbitrary units (U/ml) on the basis of a standard curve obtained with a serially diluted serum with a very high concentration of specific IgG.

Values > 0.64 U/ml were regarded as positive, since preliminary studies of 30 unexposed control persons had shown a mean value of 0.30 U/ml and a SD of 0.17 U/ml.

HR in Whole Blood

To measure allergen-induced HR, 50 µl heparinized venous blood was diluted with 150 µl HR buffer, mixed with 100 µl of Chi t I allergen solution (containing 0.1, 10, or 100 µg in HR buffer), and incubated for 30 min at 37°C in closed plastic tubes. All further steps were done at 4°C. After centrifugation (5 min at 600 g), 100 µl of the supernatant was removed and stored at -20°C or analyzed immediately.

Total histamine content was quantified by analyzing supernatants after cell lysis. 50 µl of whole blood was mixed with 1 ml distilled water, frozen, thawed, and centrifuged for 20 min at 1,000 g.

As a positive control for HR with anti-IgE, we used different dilutions of goat antihuman IgE, ε-chain specific (Tago, Medac, Hamburg, FRG). 200-µl samples of diluted blood were incubated with 0.02, 0.2, or 2 µg antihuman IgE in 100 µl HR buffer as described above. Spontaneous HR was determined by using 100-µl samples of HR buffer instead of allergen solutions.

Monoclonal Antibody-Based Histamine Radioimmunoassay

Histamine was determined in the supernatants using the radioimmunoassay kit from Immunotech (supplied by H. Biermann GmbH, Bad Nauheim, FRG). This radioimmunoassay, which is precisely described by Morel and Delaage [13], uses ¹²⁵I-acylated histamine and a monoclonal antibody specific for acylated his-

tamine fixed to tubes. After acylation of 100-µl samples with 50 µl acylation reagent, the probes were mixed with 1 ml ¹²⁵I-labelled acetylated histamine; 0.5-ml samples were incubated for 18 h in antibody-coated tubes. The tubes were aspirated and counted in a gamma counter. The results were compared with those of the standard curve and multiplied by 6 to take into account the dilution of the blood. HR is expressed as percentage of total cellular histamine. All tests were done in duplicate; mean values are given. Variability was always < 15%.

Results

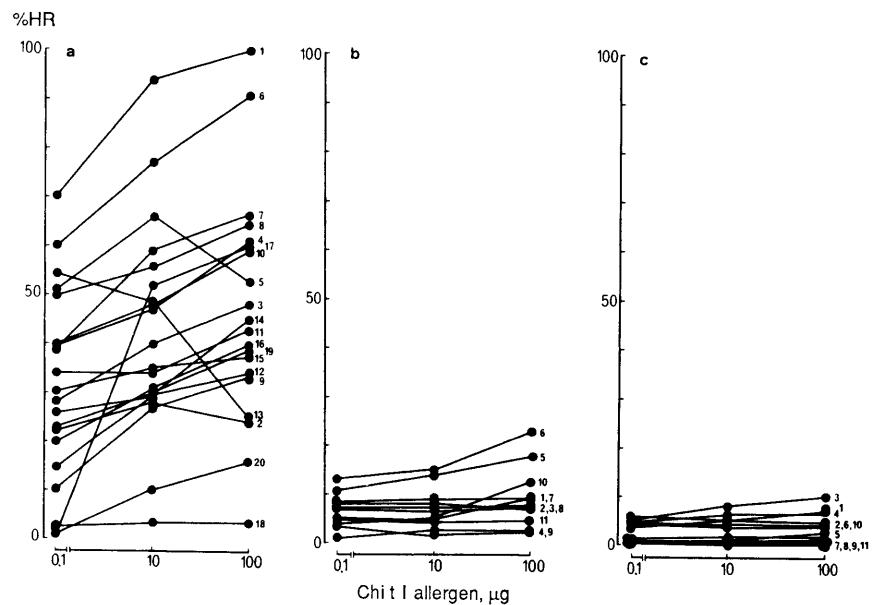
As shown in table 1, the 20 sensitized patients reporting chironomid-induced respiratory, conjunctival, and/or cutaneous complaints showed immediate skin test responses as well as positive radioallergosorbent test results to Chi t I. Twelve of this group also had IgE antibodies against common allergens. In comparison, all 11 fish hobbyists without symptoms when in contact with chironomid larvae and all 11 nonexposed controls exhibited negative results in skin testing and in radioallergosorbent test with Chi t I (table 1); 5 and 2, respectively, of these control persons were sensitized to other allergens. Total IgE levels range broadly in all groups; the highest values were found in some Chi t I-sensitized patients. Measurements of Chi t I-specific IgG antibodies gave weak positive results in 10 patients belonging to the sensitized group and in 1 exposed nonsensitized person and were negative in all other subjects.

The histamine concentrations in whole blood after lysis are extremely high in 2 Chi t I-sensitized persons; however, there is no significant difference ($p < 0.05$) between the three groups (table 1).

HR was shown to be dose dependent using antihuman IgE. At 2 µg antihuman IgE, the HR was significantly higher ($p < 0.05$) in Chi t I-sensitized probands than in the other two groups. Taking together all subjects of the three groups with elevated total IgE (> 120 U/ml), there was a tendency towards higher HR compared to persons with normal IgE levels - mean values of persons with elevated IgE: 35.37 (0.02 µg anti-IgE), 36.92 (0.2 µg anti-IgE), and 27.64 (2 µg anti-IgE); persons with normal IgE: 24.95 (0.02 µg anti-IgE), 29.33 (0.2 µg anti-IgE), and 21.35 (2 µg anti-IgE); p values for all anti-IgE doses > 0.05.

HR by Chi t I was also dose dependent in the sensitized persons, with maximum values at about 95%, whereas only minimal HR at all doses was observed in probands of the two other groups, whether they are atopic or not (fig. 1). Two patients (Nos. 20 and 18)

Fig. 1. Chi t I allergen induced HR from whole blood. Dose-response curves of 20 Chi t I-sensitized persons (a) and of exposed nonsensitized (b) and nonexposed controls (c) are shown. Each curve represents the mean percent release from duplicate determinations. (The variability was always less than 15%.) Numbers refer to individual patients, as indicated in table 1. At all Chi t I concentrations the HR of sensitized patients is significantly higher than that of the other two groups ($p < 0.01$).



are exceptions insofar as they exhibit only borderline HR; interesting is that they belong to those with the highest levels of Chi t I-specific IgG. Within the sensitized group the degree of HR was not related to the intensities of the skin test responses and radioallergo-sorbent test results (all correlation coefficients < 0.11 ; table 1).

Discussion

In this study we measured the histamine release from peripheral blood leukocytes to investigate immediate hypersensitivity to the well-characterized insect allergen Chi t I. We used a new method with test reagents now commercially available.

It is shown that this method is very sensitive and differentiates between sensitized persons, exposed nonsensitized probands, and also nonexposed controls. There is no evidence for unspecific HR even in atopic exposed or nonexposed individuals with high total IgE levels and/or hypersensitivity to common allergens. Whether the borderline HR observed in 2 Chi t I-sensitized persons is due to the presence of specific IgG antibodies is unclear and requires further investigation. As demonstrated in several studies [19, 20] antibodies especially of the IgG subclass can influence allergen-induced HR mediated by IgE antibodies.

That within the sensitized group the degree of HR

does not relate to the levels of circulating specific IgE antibodies or the intensities of skin test reaction may be due to additional individual characteristics of the target cells. The percentage of histamine released after stimulation in whole blood by a certain amount of allergen may be influenced by the number of cell-bound IgE molecules and circulating IgE specific for the allergen investigated, interindividual differences in releasability of the basophils, and the presence of blocking antibodies – which cannot be totally excluded, although only low or no specific IgG was determined.

Our results indicate that this sensitive, commercially available in vitro assay is a useful test for detection of immediate-type sensitization. It also opens further interesting possibilities, e.g., immunotherapeutic studies of fragmented or modified allergens which are no longer allergenic but retain their immunogenic potencies.

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