

Comparison of fiberglass-based histamine assay with a conventional automated fluorometric histamine assay, case history, skin prick test, and specific serum IgE in patients with milk and egg allergic reactions

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A microfiberglass-based histamine assay (HR_M) was compared with an automated fluorometric histamine assay (HR_A). Twenty-four subjects with and 24 without a case history (CH) of milk and/or egg allergy were tested by HR_M and HR_A , skin prick test (SPT), and specific serum IgE (RAST). Six different concentrations of milk, egg, and anti-IgE were used to stimulate washed leukocytes (250 μ l for HR_A) and whole blood samples (25 μ l for HR_M) in parallel. When we compared scores representing basophil sensitivity, correlation coefficients (r_s) were positive ($r_{(anti-IgE)} = 0.88$, $r_{(egg)} = 0.95$, $r_{(milk)} = 0.88$, $P < 0.001$), but no significant correlations were found after exclusion of the negatives in both tests. In some individual dose-response curves, the scores obtained by HR_M were shifted to higher allergen and anti-IgE concentrations. A high degree of concordance was found in positive and negative responses between the two tests: anti-IgE 91%, egg 92%, milk 86%. Finally, we found a good concordance between, on one side, HR_M and, on the other, CH, SPT, and RAST (HR_M vs. CH/SPT/RAST): egg 92/82/82%; milk 89/74/67%. We conclude that HR_M is in good qualitative, but poor quantitative, agreement with the autoanalyzer-based fluorometric histamine assay.

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Introduction

Immediate type allergy is one of the most common mechanisms involved in allergic reactions to foods (5). Thus, *in vitro* and *in vivo* tests for the evaluation of food hypersensitivity are based on the measurement of elevated specific IgE levels in response to food antigens, the measurement of the wheal and flare response after antigen challenge of the skin, or the calculation of histamine dose-response curves from stimulated blood basophils. A new *in vitro* method has recently been developed to determine basophil histamine release from whole blood (9, 16). Several studies with inhalant allergens have demonstrated that it might be an appropriate *in vitro* assay for the investigation of IgE-mediated allergic reactions (10, 12). However, the use of leukocyte histamine release to evaluate allergic reactions to foods

is still controversial. This study was designed to investigate the correlation between the new method (HR_M) and a conventional method (HR_A) (15).

Furthermore, the concordance between HR_M and HR_A , case history of milk/egg allergic reactions, SPT, and RAST was determined.

Material and methods

Patients

Twenty-four patients (aged 2 to 47 years, mean 19 years) with a case history of an immediate (less than 2 h after oral intake) allergic reaction to egg and/or milk were included in the study. Food-induced symptoms included diarrhea, rhinorrhea, dyspnea, vomiting, or urticaria. Informed consent was obtained from all patients or their parents, respectively. Twenty-four nonallergic subjects (aged 3 to 45 years,

mean 18 years) served as controls. Two patients with spontaneous histamine release after incubation with a buffer had been excluded from the calculation (6).

Reagents

The following reagents were purchased: Dextran (Pharmacia, FRG), PIPES (Sigma, FRG), and human serum albumin (HSA) (Merieux, France).

Sedimentation of red blood cells was carried out in a solution containing 2.5 ml 6% Dextran, 0.4 ml 50% glucose (Braun, FRG), and 1 ml 0.1 M EDTA per 10 ml blood. We used a PAG buffer containing 25 mmol/l PIPES, 110 mmol/l NaCl, 5 mmol/l KCl, 0.1% glucose, and 0.003% HSA. Cell washes were done with PAG with or without 10 mmol/l EDTA. Histamine release experiments were carried out in PAG, supplemented with 1 mmol/l CaCl_2 and 1 mmol/l MgCl_2 (PAG-CM). To determine the total cellular histamine content, we used 0.4 mol/l HClO_4 to lyse the cells.

Allergens and anti-IgE

The stock solution of anti-IgE (410 000 IU/ml, Behringwerke, FRG) and allergens from the same lot (Soluprick egg 1:100 w/v and Soluprick milk 1:20 w/v, ALK, Denmark) were diluted in PAG-CM on the day of testing to ensure maximum response.

Anti-IgE was used at final concentrations of 0.41, 2.05, 9.02, 41, 205, 902, and 4200 IU/ml. For histamine release assays and titrated skin prick tests, the egg and milk extracts were used at final concentrations of 0.0026, 0.009, 0.03, 0.11, 0.38, 1.4, and 5% of the stock solution.

Histamine release and automated fluorometric histamine assay (HR_A)

For the sedimentation of red blood cells, 20 ml of venous blood was gently mixed with 8 ml of the Dextran solution described above. After 60–90 min, the supernatant containing most of the basophils was supernatant to another tube and centrifuged for 8 min at $400 \times g$. After two washes in PAG-EDTA and PAG for 8 min at $400 \times g$, aliquots of the cells were resuspended in PAG-CM and challenged in plastic tubes with anti-IgE or allergen for 40 min at 37°C in a water bath. All concentrations were tested in duplicate at a final volume of 500 μl . To assess spontaneous release, we incubated the cells in PAG-CM alone. After pelleting the cells by centrifugation for 15 min at $800 \times g$, the histamine-containing supernatants were removed and either stored at 4°C for a maximum of 4 days, or immediately measured by an automated, continuous-flow

fluorometric system using Ophthalaldehyde (OPT) (15). The release of histamine was expressed as a percentage of the total histamine content in the sample. Release of more than 15% of the total histamine content was defined as a positive response (Table 1).

Fiberglass-based histamine assay (HR_M)

Heparinized blood (5 ml) was drawn from each patient and control subject, stored at room temperature, and used within 24 h. A 96-well microtiter plate, coated with a fiberglass matrix which selectively binds histamine, was used for the histamine release experiments (16). The plates were further precoated with anti-IgE, egg, and milk in the above mentioned concentrations. To each well, 25 μl of PAG-CM without glucose and HSA were added, and the plates and blood were prewarmed at 37°C for 30 min. After this preincubation, 25 μl of heparinized whole blood was added to each well, and the plates were incubated at 37°C for 60 min. During this time the released histamine bound with high affinity to the fiberglass matrix. After incubation, the plate was rinsed twice with deionized water with an SLT-immunowasher. Interfering substances from the blood were removed from the fiberglass matrix by adding a solution of Tris (Bie & Berntsen, Denmark) and Renafix (Bie & Berntsen, Denmark) to the wells for 30 min at 37°C , followed by another rinse. The histamine bound to the fiberglass and then reacted with 75 μl OPT reagent (10 mg OPT dissolved in 2 ml methanol alkalized by 60 μl 0.05 N NaOH). After 10 min, the histamine-OPT reaction was stopped by adding 75 μl of 0.6% HClO_4 . Histamine fluorescence values were collected and measured with a Gilson 122 fluorometer and a Gilson autosampler 221 capable of making 96 determinations in 5 min. Based on a histamine, 2 HCl standard curve, the absolute histamine values were computed, using software for MS-DOS-compatible microcomputers. A histamine release of greater than 15 ng/ml of whole blood was defined as a significant cell response (Table 1).

Scoring system for HR_A and HR_M

The individual results were divided into three score groups on the basis of the allergen concentration

Table 1. Cut-off values for the individual tests

	Negative	Positive
HR_A	<15%	$\geq 15\%$ histamine
HR_M	<15 ng/ml	≥ 15 ng/ml histamine/blood
SPT (skin index)	<0.5	≥ 0.5
RAST	<class 1	$\geq$ class 1

inducing greater than 15% histamine release (HR_A) and greater than 15 ng/ml histamine in whole blood (HR_M) (Table 2).

Skin prick test

Skin prick tests were carried out by the same person with standard needles on the volar surface of the patient's forearm with the allergen concentrations mentioned above. The wheal induced by histamine, 2 HCl (1 mg/ml) represented the positive control. After 10 min the allergen-induced wheals were marked with a pen and transferred to a strip of tape. Skin indices were calculated by the formula $(D_{A/Max} + d_{A/Min}) : 2 / (D_{H/Max} + d_{H/Min}) : 2$. $D_{A/Max}$ and $d_{A/Min}$ represent the wheal diameters of the allergen-induced wheal; $D_{H/Max}$ and $d_{H/Min}$ the wheal diameters of the histamine-induced wheal. The allergen reaction was considered positive when the skin index was greater than or equal to 0.5 (Table 1).

Specific serum IgE

Allergen-specific IgE was determined by the Phadebas-RAST and expressed in classes. A RAST class greater than or equal to 1 was considered positive (Table 1).

Statistical analysis

The relationship between the scores for both histamine assays was analyzed by the Spearman rank test. The correlation coefficient ρ (r_s) was corrected for ties. Concordance based on the positive or negative outcome of the tests was compared by 2×2 tables. The chi-square test was used to test significance.

Results

Correlation between HR_A and HR_M

The comparison of both histamine assays was based on the score results obtained from 48 subjects. The histamine measurements were ranked (Table 2) on the basis of the anti-IgE/egg/milk concentration inducing more than 15% histamine release (HR_A) or

more than 15 ng/ml histamine in whole blood (HR_M) (Table 1). Comparing the quantitative histamine response to anti-IgE (Table 3), we obtained a high overall correlation between the two assays ($r_s = 0.65$, $P < 0.001$, $n = 48$); a low correlation was found after exclusion of the negative results detected by HR_A and HR_M ($r_s = 0.35$, $P < 0.05$, $n = 40$). However, the concordance between negative and positive test results obtained by the two methods was high (91%). In these eight excluded subjects (17%), both tests recorded no histamine release response after anti-IgE challenge. Four other subjects (8%) responded only in the HR_A or in the HR_M . Interestingly, in 14 cases (29%) there was a difference of one score interval between the two methods. Thus, 5–10-fold more anti-IgE was required for histamine release in the HR_M assay than for the HR_A test. In Table 4 the scores of the egg-induced histamine responses are shown. Only 37 individuals were included in the calculations since 11 patients were not tested with HR_A . For egg, we found a high overall score correlation between the two methods ($r_s = 0.84$, $P < 0.001$, $n = 37$), but not after exclusion of the negative results from the correlation ($r_s = 0.37$, $P = NS$, $n = 14$). However, the concordance between the two tests was high (92%). In three cases the score was positive in HR_A and negative in HR_M .

In Table 5 the scores for milk are shown. The overall correlation $r_s = 0.88$, $P < 0.001$, $n = 42$ and concordance (86%) were high, but no correlation

Table 3. Anti-IgE-induced histamine release

		HR _A score		
		0	1	2
HR _M score	0	8	2	0
	1	2	21	6
	2	0	4	5

48 patients were included in the correlation.

Concordance = 91%

$r_s = 0.65$, $P < 0.01$ χ^2 : $P < 0.001$

* $r_s = 0.35$, $P < 0.05$ * χ^2 : $P < 0.05$

* After exclusion of the negative results ($n = 8$).

Table 4. Egg-induced histamine release

		HR _A score		
		0	1	2
HR _M score	0	23	2	1
	1	0	3	3
	2	0	1	4

37 patients were included in the correlation since 11 patients were not tested with HR_A .

Concordance = 92%

$r_s = 0.84$, $P < 0.01$ χ^2 : $P < 0.001$

* $r_s = 0.37$, $P = NS$ * χ^2 : NS

* After exclusion of the negative results ($n = 23$).

Table 2. Individual results divided into three score groups based on the allergen/anti-IgE concentration inducing histamine release greater than 15% of the total histamine content (HR_A) or greater than 15 ng/ml blood (HR_M)

	Allergen	Anti-IgE
	[% of stock solution]	[U/L/ml]
Score 0 conc.	—	—
Score 1 conc.	0.11, 0.38, 1.4, 5	41, 205, 902, 4200
Score 2 conc.	0.0026, 0.009, 0.03	0.41, 2.05, 9.02

Table 5. Milk-induced histamine release

		HR _A score		
		0	1	2
HR _M score	0	24	3	3
	1	0	5	7
	2	0	0	0

42 patients were included in the correlation since 6 patients were not tested with HR_A.

Concordance = 86%

$r_s = 0.72$, $P < 0.001$ χ^2 : $P < 0.001$

* $r_s = 0.08$, $P = NS$ * χ^2 : NS

* After exclusion of the negative results ($n = 24$).

Table 6. Concordance between case history and histamine release in immediate hypersensitivity to egg

A:		Case history (egg)	
		Pos.	Neg.
HR _A	Pos.	11	1
	Neg.	2	22

Concordance = 92%

χ^2 : $P < 0.001$

B:

Concordance = 89%

χ^2 : $P < 0.001$

36 patients were included since 12 patients were not tested with HR_A.

was found after exclusion of the negative results ($r_s = 0.08$, $n = 18$, NS).

Correlation between the two histamine assays and CH

Concordance between a positive and negative CH versus positive and negative test results obtained by the two histamine assays is given in Tables 6 and 7. Almost similar concordances were found for egg (HR_A, HR_M: 92%/89%) and for milk (67%/64%), with a lower association for milk than for egg.

Correlation between HR_M and SPT/RAST

In Table 8, the positive and negative test results for HR_M versus SPT and RAST are shown. As can be seen from this table, similar concordances were found for HR_M versus SPT and RAST (82% and 81%) when results for egg responses were evaluated. The corresponding milk concordances were 74% for HR_M versus SPT, and 67% for HR_M versus RAST. Again the association was lower for the milk results, as compared with those of egg.

Table 7. Concordance between case history and histamine release in immediate hypersensitivity to milk

A:		Case history (milk)	
		Pos.	Neg.
HR _A	Pos.	9	9
	Neg.	5	19

Concordance = 67%

χ^2 : $P = NS$

B:		Pos.	Neg.
HR _M	Pos.	6	7
	Neg.	8	21

Concordance = 64%

χ^2 : $P = NS$

42 patients were included since 6 patients were not tested with HR_A.

Discussion

The value of basophil histamine release tests in the diagnosis of food allergy has been investigated to a limited extent (4, 11, 13). One difference between the histamine assays compared in this study is the use of washed leukocytes instead of whole blood. Also, the results of HR_A are expressed as a percentage of histamine related to the whole histamine content of the sample (15). Since the release of the total cellular histamine, either by detergents or by sonification of whole blood basophils, would result in interfering fluorescent products originating from the erythrocytes (16), the results of the HR_M can be expressed only as absolute amounts of histamine. In an earlier study (16) a strong correlation was found between test scores of washed leukocytes and washed blood cells when 10 inhalant allergens were tested in patients with asthma. In a similar study of inhalant allergens, the same dose relationship was found when whole blood instead of washed leukocytes was used to detect histamine release induced by the allergens (14). One might speculate that naturally occurring IgG antibodies to the tested foods are responsible for the shift of some dose-response curves to higher allergen concentrations as evaluated with HR_M of whole blood.

However, no explanation is readily apparent for the shift in one-third of the histamine release curves to higher concentrations of anti-IgE when the HR_M assay was used. Furthermore, commercially available food allergens are not characterized and standardized as well as inhalant allergens. Thus, the food extracts probably induce unspecific release in the HR_A test (4, 5), a fact which might explain the high number of questionable positive HR_A results by the highest concentrations of milk extract leading to a weak correlation in the milk-allergic group. When the concordance between CH, SPT, RAST, HR_A,

Table 8. Concordance between HR_{M} , skin prick test (SPT), and specific serum IgE by testing against egg and milk. (n) indicates number of patients included. In several patients HR_{M} , SPT, or RAST was not performed, and these patients were excluded from the calculations

	A. SPT (egg)		B. Spec. serum IgE (egg)		C. SPT (milk)		D. Spec. serum IgE (milk)	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
HR_{M}	Pos.	1	9	2	8	2	8	4
	Neg.	7	6	26	9	24	11	24
		Concordance = 82% Chi ² : P < 0.001 n = 46			Concordance = 81% Chi ² : P < 0.001 n = 43			Concordance = 74% Chi ² : P < 0.01 n = 43
								Concordance = 68% Chi ² : P = 0.052 n = 47

and HR_{M} was evaluated, a high correlation between the positive and negative test results was found (Table 8). Thus, basophil sensitivity to the egg extract evaluated by the test score in the two methods correlated well with SPT and RAST. Another study evaluating different diagnostic tests in egg-allergic patients concluded that, despite a good qualitative concordance (71–83%) between SPT and HR_{A} , a weak quantitative concordance was found between the tests (1).

Despite carrying IgE antibody on their surface, the basophils of some donors (8–20%) fail to release histamine after IgE-dependent stimulation (15–18). Therefore, it is necessary to include anti-IgE challenge as a positive control in all subjects; otherwise, the test results could be misinterpreted and lead to false negative results in the evaluation of immediate type allergies. In these subjects the test is of no diagnostic value, and other diagnostic procedures are required. Moreover, using the case history merely for the comparative investigation of diagnostic tests involves problems, including the subjective quality of the diagnosis and the verification of the last allergen contact. Thus, determination of test sensitivity and specificity in the diagnosis of food allergies ideally requires a double-blind, placebo-controlled oral challenge to establish the true diagnosis (4, 5).

In conclusion, this study indicates good qualitative agreement, but poor quantitative concordance, between HR_{A} and HR_{M} . As with any other tests for the presence of allergen-specific IgE antibodies, the diagnostic value of the histamine assays should be investigated in a prospective clinical study using double-blind, placebo-controlled food challenges and elimination diets.

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