

Histamine release from peripheral blood leucocytes in egg-allergic patients

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Summary

Twelve egg-allergic patients were selected on the basis of a clinical history of egg allergy and a positive skin-prick test (SPT) to whole egg. A study was then carried out on the ability of the patients' washed leucocytes to release histamine in the presence of whole egg, ovomucoid, ovalbumin and ovotransferrin. Histamine release (HR) from washed leucocytes was demonstrated in ten out of twelve patients, but only four out of ten released over 40% of their total histamine. Spontaneous HR ranged from 2.1–14.5% with a mean of 7.5%. There was good agreement between positive and negative HR, skin test and radioallergosorbent test (RAST) results. Concordance between the HR and skin test was found in 83%, HR and RAST in 71% and skin test and RAST in 78% of patients. However there was poor quantitative agreement between these three tests. When skin-prick tests and HR thresholds were compared, ovomucoid elicited the greatest skin test sensitivity in five out of six patients, whereas five of the same six were more sensitive to ovalbumin when judged by HR.

Introduction

Diagnosis of immediate-type hypersensitivity reactions to foods is generally made on the basis of clinical history, skin-prick tests (SPT) and by the presence of specific IgE antibodies. The determination of histamine release (HR) in response to foods from sensitized peripheral blood-leucocytes *in vitro* has not been widely applied in food allergy and there have been reports that washed leucocyte preparations from food allergic patients spontaneously release between 25–100% of their total histamine [1–3] although no such finding has been reported in other studies [4–7].

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With the above background in mind, we limited our study to a group of patients with a clinical history of egg allergy who exhibited immediate-type hypersensitivity reactions to egg as confirmed by provocation testing [8] and SPT. We wished to compare the response to three egg antigens by their ability to stimulate the release of histamine from basophil leucocytes *in vitro*, their ability to elicit skin-prick test reactions, and the specific serum IgE response to each antigen. These tests were also carried out in patients with a history of egg allergy but negative skin-prick and provocation tests, and also in control subjects with no history of egg allergy.

Materials and methods

Antigens and reagents

Purified egg antigens ovalbumin grade VI (OA), ovomucoid [trypsin inhibitor grade IV] (OM), ovotransferrin [conalbumin type I] (OT) were purchased from Sigma Ltd. The whole egg preparation was made by lyophilizing a batch of homogenised hens eggs.

Reagents for histamine assay

S-adenosyl-L-[methyl ^3H] methionine (^3H -SAME) was purchased from Amersham International PLC (England) at a concentration of 1 mCi/ml (specific activity 62 Ci/mmol). This was aliquoted and stored in liquid nitrogen. Histamine-N-methyl transferase (HMT) was isolated from rat kidneys as described elsewhere [9], aliquoted and stored at 10 mg/ml in 0.2 M phosphate buffer pH 7.9 at -70°C . Histamine standards were made up from histamine dihydro-chloride Sigma (Ltd) in Tris ACM buffer pH 7.4 (Tris A: 25 mM Tris(Hydroxymethyl)amino methane, 120 mM NaCl, 5 mM KCl, 0.03% Human Serum Albumin. Tris ACM: Tris A + 1 mM CaCl_2 and MgCl_2), aliquoted and stored at -20°C . The scintillation fluid used was 2:1 (v/v) toluene:triton X. The toluene contained 0.5% (w/v) diphenyloxazone (PPO) and 0.025% (w/v) dimethyl phenyl oxazolyl benzene (DM-POPOP).

Selection of patients

Twelve subjects were selected from eighty highly atopic patients who were screened for food allergy on the basis of clinical history and a positive skin-prick test to egg. Six of the patients were female and six were male. Their ages ranged from 16–46, mean 27-years-old.

The response of the majority of patients to egg was described by Amlot *et al.* [8] as oral allergy syndrome (OAS). The essential features of this syndrome consisted of one or more of the following symptoms: oral irritation, oral mucosal blebs, throat tightness and lip swelling within 5–15 min of food ingestion, sometimes followed by urticaria, asthma or anaphylaxis within 15–60 min and stomach ache, nausea, vomiting and diarrhoea (rarely) after 30–60 min.

A positive challenge test to purified egg proteins was found in nine out of ten subjects (two not determined) by nasal provocation, in six out of twelve by buccal challenge and in four out of twelve by gastric challenge [8]. Three patients were also sensitive to milk by skin-prick test. All patients had positive skin tests and one or more of the following symptoms: hay fever, asthma and eczema (subjects 5–12). Controls included four atopic patients who had a clinical history of food allergy but did not give a positive skin-prick test to egg or milk. Three of the patients (subjects 13–15) suffered

from atopic eczema, exacerbated by egg in two cases. Controls also included five laboratory personnel, four atopic but none with any history of food allergy and all giving negative skin-prick tests to egg and milk.

Skin-prick tests

Patients were tested against whole egg at a concentration of 10 mg/ml. The threshold skin-prick test response was defined as the lowest concentration of egg antigen that elicited a weal 3 mm or greater than the negative control. Where possible, the purified protein to which the patient was most sensitive was used. Increments were in steps of 1, 3, 10, 30 $\mu\text{g/ml}$ etc. [8].

Histamine release from washed leucocytes

The washed leucocyte preparation was carried out as described previously by Siraganian & Brodsky [10]. Fifteen millilitres of blood were mixed with 0.01 M EDTA and sedimented at 37° C for 1 hr with 3.75 ml of 6% dextran in saline. The supernatant, containing leucocytes, platelets and plasma was transferred to a plastic universal tube (Sterilin Ltd, U.K.) using a soft nylon pipette and centrifuged at $300 \times g$ for 8–10 min at 4° C. The pelleted cells were resuspended in Tris A containing 4 mM EDTA (pH 7.4) and centrifuged at $250 \times g$ for 8–10 min at 4° C. After a second wash in Tris A and EDTA the cells were resuspended in 7 ml Tris ACM (pH 7.4) giving a range of cell counts of $3\text{--}10 \times 10^6$ cells/ml (mean = 7.0×10^6). The cell viability was consistently over 95% as measured by the trypan blue exclusion dye test. Antigen in Tris ACM (100 μl) was added to each Luckham LP3 tube followed by 100 μl of cells. The tubes were capped and placed in a 37° C incubator for 1 hr. The purified antigens were tested over a range of concentrations from 0.05 to 5 $\mu\text{g/ml}$ (final concentration) in ten-fold increments. For the whole egg samples an additional concentration of 50 $\mu\text{g/ml}$ was tested. The above determinations were carried out in duplicate. The spontaneous histamine release values were determined in triplicate (100 μl Tris ACM + 100 μl cells) as were the total histamine release values (100 μl cells + 6% perchloric acid). After 60 min the reaction was stopped by transferring the cells to an ice bath followed by centrifugation at ($800 \times g$) for 25 min at 4° C. Aliquots of 50 μl were taken from each supernatant and stored at -20° C for subsequent histamine assay.

Histamine assay

The single isotopic radioenzymatic assay was used, based upon the method of Guilloux *et al.* [11] as modified by Church *et al.* [12]. A 20 μl aliquot of each sample was taken (the total histamine samples previously diluted by 1/3 or 1/2 and adjusted to pH 7.5 with NaOH) and transferred to a 1 ml polypropylene tube (Sarstedt Ltd).

The enzyme and label were added together in 0.2 M phosphate buffer pH 7.9 to give a final concentration of 7.14 $\mu\text{Ci/ml}$ of ^3H SAME and 0.1 mg/ml HMT. Aliquots of this admixture (50 μl) were added to the sample and reference tubes at 4° C, vortex mixed briefly at room temperature and allowed to react for 4 hr at 4° C until stopped by the addition of 50 μl 0.5 M perchloric acid. The samples were spun at $1300 \times g$ for 15 min at 4° C, and 80 μl of the supernatant transferred to Luckham LP3 polypropylene tubes. NaCl-saturated 2.2 M NaOH (100 μl) was added to each tube, followed by 500 μl of chloroform. The tubes were mixed for 1 min on a multi-tube vortex mixer (SMI, Eastleigh, Hampshire, U.K.) and the upper, aqueous layer aspirated. NaCl-saturated 1

Table 1. Specific IgE levels to food and inhalant antigens

Subject	Antigen					Total IgE (iu/ml)
	Whole Egg	Dust Mite	Grass Pollen	Cat	Dog	
1	4+	+/-	1+	-	+/-	740
2	4+	4+	4+	4+	4+	2000
3	+/-	-	-	1+	-	50
4	2+	-	1+	1+	1+	125
5	+/-	3+	3+	2+	1+	75 000
6	2+	4+	4+	3+	nd	9500
7	1+	2+	3+	2+	3+	12 000
8	1+	3+	4+	2+	nd	29 000
9	1+	4+	4+	4+	4+	16 000
10	2+	3+	3+	3+	+/-	16 000
11	1+	4+	-	2+	1+	75 000
12	-	-	3+	3+	nd	245
Negative controls						
13	-	4+	3+	2+	nd	1800
14	-	4+	2+	-	nd	2500
15	+/-	3+	4+	4+	4+	12 000
16	-	1+	2+	2+	1+	6200
17	-	4+	4+	2+	2+	500
18	-	-	-	-	nd	72
19	-	3+	2+	3+	2+	nd
20	-	3+	2+	-	nd	nd
21	-	4+	4+	3+	2+	80

Specific IgE levels to food and inhalant antigens in the sera of food allergic patients. The Pharmacia RAST scoring system is used; nd = not determined; - = negative.

m NaOH (200 μ l) was then added and the tubes remixed and upper aqueous layer aspirated again.

A 250- μ l portion of chloroform from each tube was transferred to 4 ml polypropylene scintillation vial inserts where they were allowed to evaporate to dryness. HCl (0.01 M, 200 μ l) was added followed by 3 ml of scintillation fluid. The samples were vortex mixed once more and counted in a Wallac B counter (LKB Ltd).

Radio-allergo-sorbent test

IgE antibodies to egg albumin, ovomucoid and ovotransferrin were measured by the radio-allergo-sorbent test (RAST) as described previously [13,14]. The specificity of these assays was confirmed by RAST inhibition [14]. Specific IgE antibodies were also measured to the common inhalant allergens: house dust mite, grass pollen, and cat and dog dander. One gram of CNBr-activated discs was coated with 1 mg of purified egg protein. Serum samples were assayed at 1/10 dilution in horse serum, and the results (adjusted for dilution) expressed as a score (1+ to 4+) using the Pharmacia Phadebas reference system. A score of +/- was recorded when the level of binding of I^{125} anti-IgE was greater than two times that obtained with a normal human serum but below 1+.

Table 2. Comparison of RAST, skin-prick test and basophil histamine release for whole egg

Subject and clinical history*	Histamine release		Antigen threshold (ng/ml)	Skin test (mm)	RAST
	Maximal†	Spontaneous			
1 OAS	100	10	0.03	7	4+
2 OAS	>87‡	13	0.07	9	4+
3 OAS	>55‡	8	6.3	6	+/-
4 OAS	56	14	10	7	2+
5 Nil	34	8	7,900	7	+/-
6 OAS	24	2	0.09	7	2+
7 OAS	27	7	22.4	8	1+
8 OAS	30	10	12 800	6	1+
9 OAS	23	8	19 952	4	1+
10 OAS	8	2	0.5	4	2+
11 Vomiting	—	2	—	7	1+
12 Eczema	—	6	—	4	—
Negative controls					
13 Nausea	—	18	—	1	—
14 Eczema	—	1	—	2	—
15 Past OAS	—	90	—	—	+/-
16 Ecz./Urt.	—	3	—	—	—
17 Nil	—	4	—	—	—
18 Nil	—	4	—	—	—
19 Nil	—	4	—	—	—
20 Nil	—	2	—	—	—
21 Nil	—	4	—	—	—
	§		¶	**	††

Comparison of patient response to whole egg by clinical history, skin test, RAST and histamine release (HR). *Symptoms induced by egg; †maximum HR as percentage of amount release by cell lysis (including spontaneous HR value); ‡HR value above top concentration of histamine standard in assay; §negative HR, i.e., max. HR < double spontaneous HR; ¶no response at 50 µg/ml whole egg; **negative SPT, i.e., <3 mm weal size; †† negative RAST; OPAS = oral allergy syndrome [8]; Ecz/Urt = eczema/urticaria.

Total IgE

Total IgE determinations were carried out by radioimmunoassay (Phadebas RIA, Pharmacia U.K. Ltd) in accordance with the manufacturer's instructions.

Results

All twelve patients were atopic as shown by at least one strongly positive RAST to common inhalant allergens (Table 1). Only two out of twelve subjects had higher RAST scores to egg than to inhalant antigens. The levels of total IgE were high in the majority of the patients studied (Table 1).

Of the twelve egg-allergic patients, ten gave a positive HR test to whole egg, defined as being greater than twice the spontaneous HR. None of the negative controls gave a positive HR test. Maximal HR values to whole egg are shown in Table 2. Whole egg

Table 3. Relationship between histamine release, skin-prick test and RAST values in egg-allergic patients

Histamine release <i>vs</i> skin-prick test ($n=47$)		
Concordance	+ / + : 25 }	39/47 = 83%
	- / - : 14 }	
Discordance	+ / - : 6 }	8/47 = 17%
H ₁ SPT	- / + : 2 }	
Histamine release <i>vs</i> RAST ($n=49$)		
Concordance	+ / + : 26 }	35/49 = 71.4%
	- / - : 9 }	
Discordance	+ / - : 2 }	14/49 = 28.6%
H/RAST	- / + : 12 }	
Skin-prick test <i>vs</i> RAST ($n=45$)		
Concordance	+ / + : 28 }	35/43 = 78%
	- / - : 7 }	
Discordance	+ / - : 3 }	10/45 = 22%
SPT/RAST	- / + : 7 }	

n =number of tests (approx. four tests/subject), values are for Subjects 1-12.

induced maximum HR in all subjects (apart from subject 8) and at lower concentrations than the purified egg proteins. Leucocyte HR was only greater than 50% of the total (minus spontaneous HR value) in three out of ten patients (Table 2). Those patients (1 and 2) who released most histamine in response to whole egg, reacted to the lowest doses of whole egg and had the highest levels of specific IgE. Three of the four patients who released more than 40% of total cell histamine had negative or low (1+) RAST scores with all the inhalant antigens tested and low levels of total IgE, whereas all eight subjects with low histamine release had low levels of egg-specific IgE but 3+ or 4+ RAST scores to at least one inhalant antigen and generally high levels of total IgE. There was good qualitative agreement between the three tests (Table 3) but the quantitative agreement was poor (HR *vs* SPT: $r=0.63$ ($P<0.001$); HR *vs* RAST: $r=0.2$ ($P>0.05$); SPT *vs* RAST: $r=-0.6$ ($P<0.001$)) (Table 3). The spontaneous histamine release values ranged from 2.1 to 14.5%, mean 7.5% for the egg-allergic patients.

The threshold response of SPT and leucocyte HR were compared to specific serum IgE levels for the individual egg antigens (Table 4). There was only partial agreement between SPT threshold, HR threshold (defined as the lowest concentration of antigen required to elicit a release of histamine twice the level of the spontaneous HR), and specific IgE levels for each of the different egg proteins. Of the six patients for whom HR and SPT data could be compared, subjects 1, 2 and 8 were most sensitive to ovomucoid by SPT titration but most sensitive to ovalbumin by HR. Subjects 3, 6 and 7 shared sensitivity to a single antigen (ovalbumin, ovotransferrin and ovomucoid respectively) by all three tests.

When the SPT and HR response to ovalbumin and ovomucoid only were compared, it was found that five out of six subjects were most sensitive to ovomucoid by SPT titration, whilst five out of six were more sensitive to ovalbumin by HR. These were found to be significantly different using the χ^2 test ($P<0.01$).

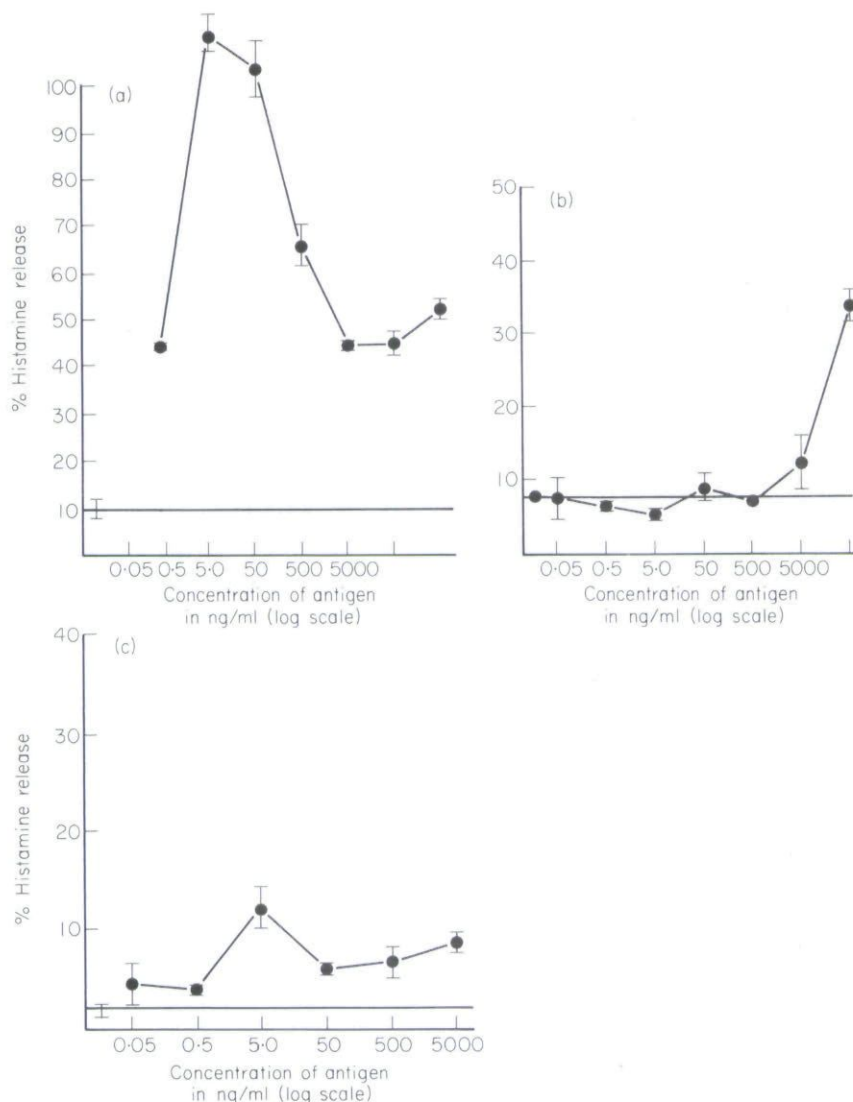


Fig. 1. (a) HR in response to whole egg (subject 1); (b) HR in response to whole egg (subject 5); (c) HR in response to ovotransferrin (subject 6). Spontaneous HR shown by line across each figure.

Discussion

Studies of HR from peripheral blood leucocytes of food allergic patients have produced conflicting results. Some studies show that the leucocytes of food allergic children spontaneously release high ($>25\%$) levels of histamine [1-3], whilst others have not confirmed this finding [4-7]. None of the egg-allergic patients described in this study gave a high spontaneous HR although one of the controls released 90% of total histamine. We were unable to test this individual again and therefore cannot be certain if this result was due to technical error. The discrepancy between our results and those described by May [1-3] may be partly explained by differences in the techniques used for histamine release but also because the earlier study [1] was of children with such

Table 4. Comparison of basophil sensitivity, skin-prick test threshold and specific IgE antibody levels to purified egg antigens

Subject	Antigen	Threshold antigen concentration for:		
		Basophil histamine release (ng/ml)	Skin-prick test (μ g/ml)	RAST
2	OA	0.08	300	120
	OM	0.1	100	15
	OT	0.5	300	33
1	OA	0.22	10	200
	OM	5.0	1.0	50
	OT	0.63	100	360
3	OA	141	300	<6
	OM	—	—	<6
	OT	—	—	<6
8	OA	1200	30	58
	OM	—	10	233
	OT	—	—	29
6	OA	10	—	41
	OM	25	100	60
	OT	0.7	100	126
7	OA	—	300	77
	OM	4.0	100	93
	OT	—	—	30
4	OA	630	—	<6
	OM	—	—	<6
	OT	—	—	<6
9	OA	—	—	25
	OM	—	100	33
	OT	—	—	35
11	OA	—	300	<6
	OM	—	—	<6
	OT	—	—	<6
5	OA	—	—	37
	OM	—	—	<6
	OT	—	—	35
10	OA	0.1	30	173
	OM	0.1	nd	55
	OT	1.25	nd	105

OM: Ovomucoid, OA: ovalbumin, OT: ovotransferrin, nd: not determined, —: no response at 5000 ng/ml, —: no response at 1000 μ g/ml. Antigens to which subjects are most sensitive in each test are in italic.

severe asthma that they had been admitted to hospital, suggesting that patient selection may have played a part.

The most striking finding was the relatively poor response of leucocytes from patients who were clearly allergic to egg by several criteria; a finding similar to reports published on other types of food allergy [4–7]. When Dockhorn [7] compared the amount of histamine released in response to inhalant and food antigens using

leucocytes taken from patients who had multiple allergies he found that HR to inhalant antigens was consistently higher (38–58%) than HR to foods (11–21%). Although we did not challenge the leucocyte of the food allergic patients described in this study with inhalant antigens, the majority (ten out of twelve) possessed higher serum IgE antibody levels to inhalant antigens than egg, suggesting that the poorer response to food antigens may be related to low specific IgE antibody levels. This suggestion is supported by the results of a study by Wahn *et al.* [15] who found a close correlation between the capacity of purified horse allergen extracts to induce HR from washed leucocytes and the level of specific serum IgE measured by crossed radio-immunoelectrophoresis. Likewise, Le Mao *et al.* [16] reported that the sensitivities of basophils to purified dust mite antigens reflected the proportion of specific IgE in serum and cell eluates.

Large amounts of total IgE in the serum may also explain why when comparing HR with RAST values, a positive RAST result was accompanied by a negative HR on twelve occasions (Table 3). In these tests, the subjects involved (nos 5, 7–9, 11) had low RAST results but high levels of total IgE (Table 1), suggesting that these high levels of IgE had given rise to non-specific binding, resulting in some false positive RAST scores as previously described [17], although the assay of samples at 1/10 dilution in horse serum substantially reduces this possibility. Overall, comparison of SPT, HR and RAST showed good agreement between a positive or negative result for the three tests (83–71%). However, the quantitative agreement was poor. Comparison of SPT and HR thresholds for ovalbumin and ovomucoid in six patients showed that the majority (five out of six) were more sensitive to ovomucoid than ovalbumin by SPT titration, but five out of six were more sensitive to ovalbumin by HR. Previous studies of egg allergy [18,19] showed patients to be most sensitive to ovomucoid by SPT, but when specific IgE was assayed by crossed radio-immunoelectrophoresis [20] or RAST [14], ovalbumin was shown to be the major allergen. In the study described here the levels of specific IgE were generally low and no major allergen was indicated by RAST.

The differences between SPT and HR with ovalbumin and ovomucoid could be due to the amount of specific IgE present on skin mast cells compared to peripheral blood basophils, but it seems probable that other factors such as IgG blocking antibody, present in the skin but not with washed cells, could be important. Indeed, preliminary results show higher levels of IgG antibody to ovalbumin than to ovomucoid (D. M. Kemeny, unpublished observations). Since IgG is present in both SPT and RAST this finding may explain why the correlation between HR and RAST ($r = -0.2$) was much poorer than that of SPT and RAST ($r = -0.6$).

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