

A Clinical and Immunological Study of Allergy to Hen's Egg White

IV. Specific IgE-Antibodies to Individual Allergens in Hen's Egg White Related to Clinical and Immunological Parameters in Egg-Allergic Patients

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Different subgroups of egg-allergic patients were established according to differences in allergic symptoms provoked by hen's egg white, total IgE-level, RAST (radioallergosorbent test) results to egg white, and age. In each group the pattern of specific IgE-antibodies to individual allergens in the egg white, determined by means of crossed radioimmunoelectrophoresis (CRIE), was compared with the pattern in a control group. The RAST value to egg white was found to exert the strongest influence on the CRIE results. A few statistically significant differences in CRIE results to certain allergens were also observed in other pairs of subgroups of egg-allergic patients. Possible explanations for this are discussed. It was concluded that CRIE studies of allergens in mixed protein solutions, using a series of sera from allergic patients, will most probably give the same results and the same classification of the allergens independent of the characteristics of the patients from which the sera were collected. However, it should be emphasized that the CRIE series must include a sufficient number of patients with high RAST values to the allergen in question. Otherwise some allergens may be missed and the classification of the allergens may be different.

Key words: allergens; crossed-radioimmunoelectrophoresis; hen's egg white.

Accepted for publication 3 May 1983

The introduction of crossed immunoelectrophoresis (CIE) and subsequently, crossed radioimmunoelectrophoresis (CRIE), has made it possible to study individual allergens in mixed protein solutions (11). The classification of the various allergens is usually based on CRIE studies, analysing series of sera from allergic patients, with respect to specific IgE-antibodies to each of the allergens in the protein solution. Information obtained in such CRIE studies, often presented in so-called allergograms (8), may serve as the basis for further work with this particular allergy as, for example, the production of improved allergen extracts for diagnostic and therapeutic use.

It is therefore crucial that a series of sera from allergic patients, used in order to establish an allergogram, are representative for the majority of patients suffering from this particular allergy. The possibility of variations in the pattern of specific IgE-antibodies in various groups of patients is also one of the factors to be considered when selecting patients for biological standardization of allergen extracts. Current knowledge about the significance of clinical and immunological parameters for the pattern of specific IgE-antibody to individual allergens is incomplete. Such information would be of interest particularly in the studies of food allergy, as patients suffering from

Patients	Symptoms					Age		IgE total	RAST %	Immunoprecipitates													
	a.d.	urt.	ast.	vom.	inh.	<2	>10			1	3	4	5	6	10	12	13	14	15	16	20	22	
35 H.C.	+	+	+					360	19														
36 V.V.	+							11800	19														
37 C.T.			+					370	19														
38 P.S.		+	+	+				400	18														
39 H.R.	+					+		40	18														
40 L.O.								710	17														
41 N.G.			+				+	230	16														
42 S.H.			+	+	+			550	16														
43 P.T.								80	16														
44 S.B.				+				380	16														
45 F.N.	+							760	16														
46 L.T.							+	1360	15														
47 R.J.	+	+	+					740	15														
48 G.T.						+		20	15														
49 S.P.			+				+	310	15														
50 H.A.	+							480	15														
51 O.T.	+					+	+	1640	13														
52 B.J.		+		+		+		80	12														
53 Ø.J.	+		+		+			620	12														
54 F.T.	+				+			400	12														
55 B.T.				+				6400	11														
56 W.Y.	+							1200	11														
57 B.A.		+					+	3100	11														
58 H.H.	+							360	10														
59 J.T.	+							11000	10														
60 L.A.	+					+		550	10														
61 B.K.								550	10														
62 H.L.	+							8200	9														
63 S.S.				+			+	530	9														
64 E.R.	+							2400	8														
65 H.A.								480	8														
66 R.K.	+	+					+	7080	7														
67 B.T.		+		+			+	940	6														
68 M.N.				+				360	6														

Fig. 1. Patients are arranged according to decreasing RAST values to egg white. Total IgE levels are expressed in kU/l. For each patient the presence of the various symptoms of egg allergy was recorded as + (a.d.: pruritus and exacerbation of atopic dermatitis, urt.: urticaria, ast.: asthma, vom.: vomiting, inh.: reaction from inhalation of egg or egg products). The CRIE results of each serum corresponding to various allergens (immunoprecipitates) is designated by varying the darkness of the respective square. CRIE reaction A (black square) is the strongest reaction.

MATERIAL AND METHODS

Radioallergosorbent test (RAST). Specific IgE-antibodies to egg white were determined using RAST-kits (Pharmacia, Sweden) and F_1 -discs (egg white discs, Pharmacia, Sweden) as described previously (8). The F_1 -allergosorbents have previously been shown to give results similar to those obtained with paper discs prepared with the hen's egg white reference antigen solu-

tion (9). Results were expressed in percent uptake of isotope applied to the discs. According to results obtained by RAST reference kit (Pharmacia, Sweden), the various RAST classes correspond to the following percentage uptake of isotope: RAST = 4: > 36%, RAST = 3: > 19%, RAST = 2: > 6% and RAST = 1: > 3%. The mean RAST value was calculated in each subgroup of egg-allergic patients.

Paper radioimmunosorbent test (PRIST). Total IgE-level in each patient serum was determined by means of PRIST, using PRIST-kits (Pharmacia, Sweden) according to the producer's instructions. Results were expressed in kU/l (1 U corresponding to 2.4 ng).

Crossed immunoelectrophoresis (CIE) and crossed radioimmuno-electrophoresis (CRIE) were performed in order to determine the relative amount of IgE antibodies to each of the allergens in hen's egg white in each of the 68 sera from egg-allergic patients included in this study. Equipment, reagents and experiment conditions were as described previously (8). Basically, in CRIE an incubation of CIE slides with single patient sera (400 µl serum in 7 ml total volume) was followed by an incubation with approximately 250,000 cpm ¹²⁵I-anti IgE (Phadebas, Pharmacia, Sweden) in 7 ml total volume (8). Subsequently, the slides were placed face-down on X-ray film for autoradiography. The autoradiography was carried out six times with each glass slide, varying the duration of exposure from 12 h to 32 days for a semiquantification of the radiostaining. The degree of radiostaining obtained with the various sera to each of the allergens in the egg white, was expressed by means of CRIE-classes (A-F), according to the CRIE reference system of Aukrust & Aas (2).

Egg-allergic patients

The study comprised 68 egg-allergic patients (aged 1–15 years) admitted to the Allergy Institute, Voksentoppen. All patients were hypersensitive to egg white, the diagnosis in each case being based on a combination of the data obtained by history, RAST and skin prick test (SPT) as previously outlined (7). In some cases, where it was considered safe and necessary, challenge tests were performed (1).

The adverse reactions observed by egg ingestion were recorded in each patient (7).

Analyses of CRIE results in various groups of egg-allergic patients

The following symptoms provoked by egg

white were used in subgrouping the egg-allergic patients: Pruritus and exacerbation of atopic dermatitis (28 patients), urticaria (15 patients), bronchial asthma (16 patients), vomiting (17 patients), and reactions to egg white by inhalation (12 patients). The remaining egg-allergic patients, not reacting with the symptoms in question, served as controls.

Furthermore, the following pairs of subgroups were compared: 17 patients with RAST to egg white $\geq 32\%$ and 17 patients with RAST $\leq 12\%$, 20 patients with total IgE more than 2000 kU/l and 24 patients with total IgE below 500 kU/l, eight patients aged 1–2 years and 11 patients aged 10–15 years.

Allergens 6, 12, 22 (major allergens) and 5, 10, 13 and 16 (intermediate allergens) were studied, whereas the minor allergens and allergen 15 were excluded, due to a low number of CRIE reactions to these allergens (less than 10/68). In each pair of subgroups the number of various CRIE reactions (A–F and 0 (no reaction)) to each allergen was recorded.

Statistical analyses. When comparing CRIE reactions in the various pairs of subgroups, a two-sided Wilcoxon rank-sum test, with correction for ties, was applied on the individual CRIE reactions in each group, in order to decide whether observed differences were statistically significant.

RESULTS

Fig. 1 presents clinical symptoms, age group, results of PRIST, RAST and CRIE analysis for each of the 68 study patients. Allergen 6, 12 and 22, being classified as major allergens, have previously been identified as ovalbumin, ovomucoid and ovotransferrin, respectively (8, 9). The mean RAST values for each subgroup are presented in Table 1.

The following statistically significant differences in CRIE scores between pairs of subgroups were found:

The most pronounced differences in CRIE scores were found in the comparison of the two subgroups with high ($\geq 32\%$) and low ($\leq 12\%$)

Table 1
Mean RAST values to egg white in subgroups of egg-allergic patients

Subgroups	RAST value (mean) (%)	Control groups	RAST value (mean) (%)
Symptoms of egg allergy			
Exacerbation of atopic dermatitis	19	Not reacting with exacerbation of atopic dermatitis	23
Urticaria	26	Not reacting with urticaria	20
Asthma	24	Not reacting with asthma	21
Vomiting	23	Not reacting with vomiting	21
Reaction from egg inhalation	26	No reaction from egg inhalation	21
< 2 years	21	10-15 years	19
Total IgE > 2000	24	Total IgE < 500	19
RAST $\geq 32\%$	36	RAST $\leq 12\%$	10

Table 2
CRIE reactions to individual allergens in the subgroups with high and low RAST values to hen's egg white

	CRIE- classes	Ag 5	Ag 6	Ag 10	Ag 12	Ag 13	Ag 16	Ag 22
17 patients with RAST $\geq 32\%$	A	0	5	0	2	0	0	4
	B	0	6	0	6	0	0	2
	C	1	4	1	3	4	2	2
	D	0	1	0	4	5	3	2
	E	1	0	2	0	2	1	2
	F	2	1	3	0	3	0	2
	0	13	0	11	2	3	11	3
17 patients with RAST $\leq 12\%$	A	0	0	0	0	0	0	0
	B	0	1	0	0	0	0	0
	C	0	3	0	0	0	0	0
	D	0	2	0	3	0	0	0
	E	0	7	3	2	1	0	4
	F	0	4	4	1	4	1	2
	0	17	0	10	11	12	16	11
		n.s.	$P < 0.001$	n.s.	< 0.001	< 0.001	n.s.	< 0.001

The number of reactions in the various CRIE classes (A-F and 0) has been recorded for each of the allergens in both groups. Results of the group with low RAST values are placed below the corresponding results in the group with high RAST values.

RAST values to egg white. The group with high RAST values had stronger reactions to allergens 6, 12, 13 and 22 ($P > 0.001$), whereas the observed differences were not statistically significant with respect to allergens 5, 10 and 16. The individual CRIE reactions for each of the aller-

gens in these two subgroups are shown in Table 2.

The group of patients with total IgE below 500 kU/l showed stronger CRIE reactions to allergen 10 than the group whose total IgE was higher than 2000 kU/l ($P = 0.01$).

Patients reacting to egg ingestion with pruritus, and exacerbation of atopic dermatitis, had weaker CRIE reactions to allergen 6 (ovalbumin) than the corresponding control group ($P = 0.02$).

The subgroup of patients reacting with asthma on egg ingestion, showed stronger CRIE reactions to allergen 22 (ovotransferrin) than the control group ($P = 0.02$).

Patients reacting to inhalation of egg products revealed stronger CRIE reactions to allergen 12 (ovomucoid) than did the control group ($P = 0.04$). Egg-allergic patients under 2 years old had stronger CRIE reactions to allergen 12 (ovomucoid) and allergen 13 than egg-allergic patients between 10 and 15 years old ($P = 0.02$).

DISCUSSION

The present study was restricted to patients with obvious egg allergy, judged on the basis of a combination of history, RAST and SPT. RAST and SPT are useful supplements to history in the diagnosis of egg allergy (1). At Voksentoppen, any doubt as to diagnosis is confirmed or dismissed by means of challenge tests. Accordingly, the clinical data used in this analysis should be sufficiently reliable for the present purpose.

CRIE is a semiquantitative method (11). The CRIE reference system is thus designed that on going from one CRIE class to a higher, the amount of ^{125}I -anti-human IgE bound to a precipitate will be approximately doubled (2). A linear relationship has been found between the amount of human serum IgE-antibody used in CRIE, and the degree of radiostaining obtained (3). Therefore differences in CRIE classes between various sera will reflect differences in the relative titers of specific IgE-antibodies in these sera to the allergen in question. Certain reservations should be mentioned in this connection. Firstly, the allocation of the CRIE class of a particular radiostaining, obtained with serum from an allergic patient, is based on the reading of X-ray films. The accuracy of this reading has been estimated to be

($\pm$) one CRIE class (2). Secondly, rabbit antibodies may cause a different degree of blocking of IgE-binding sites of the various allergens (4). This problem will probably be of no significance in the present study, because the CRIE reactions being compared were related to the same allergens. Another potentially disturbing factor in CRIE is "blocking" antibodies in patients' sera. As the significance of this factor had not been fully studied in CRIE, we investigated this problem by incubating an ovalbumin precipitate with an egg-allergic serum pool, with various amounts of human IgG-antibodies against ovalbumin (determined by means of enzyme-linked immunosorbent assay (ELISA)) simultaneously added to the serum incubation solution. Inhibition of the IgE-binding to the precipitate was not observed, even when adding high amounts of IgG anti-ovalbumin to the serum incubation solution, indicating that "blocking" antibodies in patients' sera will be of little significance in CRIE (10).

The RAST value to egg white was the factor found to exert the strongest influence on the CRIE reactions. This was in accordance with the correlation found between RAST and CRIE results in other studies concerning inhalatory allergens, such as timothy and various danders (11). The observation that the major allergens (6, 12 and 22) most clearly reflected the difference in the RAST values was expected, because these allergens probably bind a high proportion of the total IgE-antibody to egg white in various sera. The lack of statistical significance between these two groups in their reactions to allergen 5, 10 and 16 may be due to low numbers of CRIE reactions to these allergens (Table 2).

The mean total IgE levels were similar in both of these groups (~ 2500 kU/l), indicating that unspecific IgE-binding to the paper discs due to high total IgE levels, may have been of little significance in this RAST series.

Due to the strong correlation between RAST values and CRIE reactions, the mean RAST value was calculated in each subgroup in order to consider differences in CRIE reactions in the light of possible differences in RAST values between two subgroups.

The amount of total IgE has been shown to have little significance for the radiostaining in CRIE (3). In accordance with this, the two groups with high and low total IgE values were similar in their CRIE reactions, except that the intermediate allergen 10 gave rise to stronger radiostaining with sera from patients in the group with low total IgE. A reasonable explanation for this could not be found.

It is clinical experience that unrelated foods, such as hen's egg and cow's milk, often provoke the same immediate-type reaction in a hypersensitive patient, indicating that the clinical consequences of an immediate-type reaction will be decided by the constitution of the individual, more than by the molecular properties of the allergen provoking the reactions. Accordingly, it could be expected that the symptoms of egg allergy would not influence the CRIE results. CRIE reactions in the various subgroups established on the basis of various symptoms of egg allergy, were mostly similar, yet, a few statistically significant differences were observed, which should be commented.

Patients reacting with pruritus and exacerbation of atopic dermatitis presented weaker CRIE reactions to allergen 6 (ovalbumin) and lower mean RAST values to egg white than the corresponding control group (Table 1). By excluding the eight patients with the lowest RAST values in the group reacting with exacerbation of atopic dermatitis, the mean RAST value for the remaining 20 patients will be 23%, which is the same as in the control group. A comparison of CRIE reactions to allergen 6 and other allergens between these two groups revealed no statistically significant differences. This observation may indicate that the weaker CRIE reactions to ovalbumin in sera from patients reacting with exacerbation of atopic dermatitis simply reflect a tendency to lower RAST values in this group than in the control group. A possible explanation for this could be that children suffering from atopic dermatitis would more easily present symptoms of egg allergy (pruritus and exacerbation of atopic dermatitis) than children without this skin disorder. As clinical symptoms of egg allergy were required for inclusion in this study, this may be

the reason for the predominance of patients with atopic dermatitis among the egg-allergic patients with the lowest RAST values to egg white.

Patients reacting to egg ingestion with bronchial asthma, revealed stronger CRIE reactions to allergen 22 (ovotransferrin) and a higher mean RAST value to egg white than the corresponding control group. The difference in CRIE reactions to ovotransferrin was not statistically significant if patients 9, 10 and 11 were excluded. Thus, the group reacting with asthma consisted of 13 patients with a mean RAST value of 21%, which is the same as in the control group. Yet, the tendency of a stronger reaction to ovotransferrin was still present ($P = 0.08$). Whether ovotransferrin is particularly capable of provoking bronchial obstructions in predisposed individuals, or whether other non-causal factors account for the stronger CRIE reactions to this protein in the group reacting with asthma is unknown.

Inhalatory reactions to egg white could possibly be based on one of the following three factors or their combinations: A high reactivity in the shock organ, namely the mucosa of the respiratory tract, a high degree of egg allergy, or allergy to proteins with the capacity of acting as airborne allergens. The group presenting inhalatory reactions had a higher IgE-antibody titer against ovomucoid and a higher RAST value to egg white than the control group. As a correlation exists between the RAST value and the IgE-antibody titer to ovomucoid (Table 1), further division of these two subgroups would have been necessary to elucidate the individual significance of each of these two factors. However, due to the low number of patients in the group with inhalatory reactions, this was not possible in the present study.

Food allergy tends to subside with age, the tendency appears to vary for different foods (5, 6). It could thus be expected that individual proteins within one food item also could vary with respect to their ability to induce tolerance in sensitized children. The observation that children aged 10–15 years present weaker CRIE reactions to allergens 12 and 13 could possibly reflect such a phenomenon. To study this fur-

ther it would be desirable to repeat CRIE analysis of sera from the same egg-allergic children during their growing-up years.

Although some statistically significant differences in CRIE reactions between various subgroups were observed in this study, the allergens causing strongest and most frequent CRIE reactions tended to be the same in all subgroups. For instance, the major allergens appeared to be most important in all subgroups. Fig. 1 shows that a number of allergens would have been missed, and the classification of allergens been different, had the CRIE study not included a sufficient number of patients with high RAST values to egg white. Apart from this the data of this study suggest that an allergogram would be similar and subsequent allergen classification the same, independent of the characteristics of the group of patients whose sera were used to establish the allergogram.

ACKNOWLEDGEMENT

Leiv Sandvik is acknowledged for skilful assistance with the statistical analyses.

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