

Allergenic Cross-Reactivity of Egg-White and Egg-Yolk Proteins

An in vitro Study

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Abstract. The radioallergosorbent test (RAST) and RAST inhibition test were used to examine cross-allergenicity amongst the major hen's egg-white and egg-yolk proteins. Using ovalbumin as a reference allergen to compare cross-reactivity, it was apparent that the proteins conalbumin, ovomucoid and lysozyme substantially inhibited binding to ovalbumin discs of IgE in the sera of patients clinically hypersensitive to egg. The converse situation with conalbumin, ovomucoid and lysozyme on the discs and ovalbumin as the inhibitor also resulted in significantly decreased levels of IgE binding to the proteins on the discs. It was also demonstrated that cross-reactions occurred between ovalbumin and the yolk protein, apovitellenin I. Cross-reaction was also observed surprisingly when egg lysozyme was on the disc and the milk protein allergen alpha-lactalbumin was used as the inhibitor. The demonstration of cross-reaction between all of these proteins may signify that there are a number of common allergenic determinants on these egg proteins, thus providing a molecular basis for the phenomenon of cross-reactivity.

Introduction

It has been known for some time that several proteins present in hens' egg-white produce immediate allergic reactions (type I) in susceptible individuals [26]. Ovalbumin, ovomucoid and conalbumin from egg-white have all been implicated in egg allergy by both in vivo and in vitro investigation [19, 21-24]. All of these egg-white proteins, and a number of others, have been well characterised chemically but their allergenic determinants (IgE-binding sites) have not so far been elucidated [4, 5, 11, 12, 21, 27]. Recently, work has begun in determining the sequence of the allergenic determinant(s) in ovalbumin [15]. The question of whether each particular egg-white protein has several different allergenic determinants or whether there are a smaller number of common allergenic determinants on the various proteins is of interest. This possibility would form the molecular basis for any observable cross-reaction. Little has been published on cross-reaction amongst allergens of food origin, either among foods from different sources or among

proteins from the same food [16, 25]. The occurrence of multiple food allergies has been noted [18, 28], but to our knowledge only cross-reaction among legume species has been studied in any detail [9]. We have previously demonstrated cross-reaction between whole egg-white and egg-yolk fractions by radioallergosorbent test (RAST) inhibition [2]. It is not known which particular proteins contribute to this apparent cross-reaction. If this were to be determined, and since the amino acid sequences of many egg proteins are known, then similar features may be detected which could be the source of the cross-reactivity. This paper reports a more detailed RAST inhibition study of the possible cross-reactions between some of the individual egg-white and egg-yolk proteins.

Three new allergens from egg-yolk, namely apovitellenin I, apovitellenin VI and phosvitin, have recently been studied [unpubl. results]. In the present paper the question of cross-reaction between some of these proteins and another major egg allergen, ovalbumin, has been examined. By using ovalbumin as a reference allergen in RAST inhibition we have stud-

ied cross-reactivity between (a) the major egg-white proteins, ovomucoid, conalbumin and lysozyme, and (b) the white and yolk proteins, ovalbumin and apovitellenin I, respectively.

Further, it is known that there is a high degree of sequence homology between lysozyme and bovine alpha-lactalbumin [10], although they do not seem to have similar antigenic sites [20]. The latter is a major allergen of cow's milk [7]. However, since it has been reported that lysozyme is allergenic for some patients [2], we have now examined cross-reactivity between lysozyme and alpha-lactalbumin to determine if there is a relationship between their allergenicities.

Materials and Methods

Eggs

Australorp hens kept at the Division of Animal Production, CSIRO (North Ryde, NSW, Australia) were the source of eggs. The hens were maintained on a commercial mash diet and unfertilised eggs were used within 2 days of laying.

Protein Fractions

Ovalbumin was prepared by three times recrystallisation of egg-white extract from an ammonium sulphate solution. The solution of impure ovalbumin was then purified on a diethylaminoethyl (DEAE)-Sephacel column equilibrated with 0.05 M Tris-HCl buffer (pH 8.0) containing 0.05 M sodium chloride. Ovalbumin was eluted from the column using a linear gradient of 0–1 M sodium chloride in 0.05 M Tris-HCl (pH 8.0). Lysozyme was prepared by the method of Alderton and Fevold [1]. The crystals were then further purified on a DEAE-Sephacel column equilibrated with 0.05 M Tris-HCl buffer (pH 8.0) containing 0.05 M sodium chloride. Lysozyme was eluted from the column using a linear gradient of 0–2 M sodium chloride in 0.05 M Tris-HCl (pH 8.0). Conalbumin (98% pure) was purchased from Sigma Chemical Co., (Miss.). This was further purified by ion-exchange high-performance liquid chromatography (HPLC) on a Brownlee X03 MP aquapore cation column. The sample was injected and washed with 0.015 M NH_4HCO_3 (pH 4.7) until all unbound material was eluted. The conalbumin was then eluted with a 0.015 M (pH 4.7) to 0.35 M (pH 6.5) linear gradient of ammonium hydrogen carbonate. The material thus eluted was finally purified by HPLC on a Water's Associates reverse-phase C18 μ Bondapak column using a 30–70% linear gradient of acetonitrile in water. Ovomucoid was prepared according to the method of Kurisaki et al. [21]. It was further purified by ion-exchange HPLC on a LKB DEAE-TSK column using a linear gradient of 0–0.01 M sodium chloride in 50 mM Tris-HCl (pH 6.2). Final purification was on a Water's Associates reverse-phase C18 μ Bondapak column using a linear gradient of 20–50% acetonitrile in water. Apovitellenin I was isolated and purified by the method of Burley and Davies [11] from the low-density lipoprotein of egg-yolk. Alpha-lactalbumin (grade II from bovine milk) was obtained from Sigma Chemical Co., and was approximately 90% pure. A sample was purified by chromatography on Biogel P100 pre-equili-

Table I. Patients history of sensitivity to egg

Patient code	Sex	Age years	Symptoms on ingestion of egg
Goa	M	41	laryngeal oedema, vomiting, nausea
Hor	F	5	eczema
Kas	F	5	angioedema, vomiting
Mar	F	10	facial oedema, blotchy skin
McL	F	8	eczema
Squ	F	14	nausea, vomiting, diarrhoea

brated with 0.050 M ammonium hydrogen carbonate buffer and eluted with the same solution. The eluent was monitored at 280 nm and the major peak of absorbance collected.

Purity for each fraction was determined by sodium dodecyl sulphate-gel electrophoresis on a 10% T gel [14].

Preparation of RAST Discs and RAST Procedure

Filter paper discs (6 mm) were activated with cyanogen bromide (CNBr) by the method of Ceska et al. [13]. Since apovitellenin I is poorly soluble in the NH_4HCO_3 buffer used for coupling, any experiments using this protein were done utilising nitrocellulose as the solid-phase support [29]. The optimal amount of each protein to be coupled was determined by constructing a binding curve in which increasing amounts of the various allergen were added to CNBr-activated or nitrocellulose discs. RAST was then performed using these discs and one serum sample with high levels of IgE specific to the particular protein. At least 10 $\mu\text{g}/\text{disc}$ was sufficient to give maximal binding except in the case of apovitellenin I which required 50 $\mu\text{g}/\text{disc}$. A standard amount of 50 $\mu\text{g}/\text{disc}$ was used for each of the allergens. The RAST procedure followed the manufacturer's instructions (Pharmacia AB, Sweden).

Sera

Serum was obtained as venous blood from individuals presenting at various clinics and who were clinically sensitive to egg by skin-prick test or oral challenge. Table I gives details of clinical histories for some of the patients.

RAST Inhibition

The method of Gleich et al. [17] as modified by Barnett et al. [8] was used for the RAST inhibitions. Sera (50 μl) were incubated with 50 μl RAST buffer (0.9% sodium chloride and 0.01% Tween 20) containing 50 μg of the appropriate protein. After 3–4 h 50 μl of the inhibition mixture was applied to the appropriate discs and incubated for 4 h. The discs were then washed in RAST wash solution (Pharmacia AB, Sweden) and incubated with 50 μl of ^{125}I -anti-IgE overnight. The discs were again washed as above and the amount of radiolabelled anti-IgE bound to the discs was estimated by gamma counting. If the percent radioactive uptake of an allergen disc without inhibition was low (less than 3%) the results of the inhibition were not regarded as reliable. This occurred only with patients Coa, Nas, Rey and Nim when lysozyme was the allergen. Two other patients (Goa and Kas) with higher radioactive uptakes to lysozyme were therefore studied (table II).

Table II. Percent RAST uptake for the sera of the patients used in the following three tables to various hen egg proteins

Patient code	Percent radioactive uptake with allergen					
	ovalbu-min	ovomu-coid	conalbu-min	lyso-zyne	Apo I	milk
Cad	NM	NM	NM	29.0	NM	4.7
Ced	NM	NM	NM	9.0	NM	14.8
Cia	NM	NM	NM	3.8	NM	3.8
Coa	8.6	28.6	20.6	NM	22.6	NM
Coo	15.4	NM	NM	NM	18.2	NM
Dah	NM	NM	NM	5.4	NM	5.3
Dai	NM	NM	NM	5.0	NM	17.3
Goa	3.4	NM	NM	5.4	NM	NM
Kas	25.6	NM	NM	13.8	NM	NM
Mar	25.7	NM	NM	3.1	NM	NM
McC	1.4	NM	NM	NM	10.0	NM
McL	NM	NM	NM	4.3	NM	NM
Nas	25.9	4.7	3.5	NM	NM	NM
Nim	38.7	43.7	47.0	NM	NM	NM
Rao	NM	NM	NM	7.4	NM	17.6
Rey	22.7	8.3	12.9	NM	NM	NM
Sim	5.3	NM	NM	NM	8.3	NM

Apo I = Apovitellenin I; NM = not measured.

Results

Cross-Reactivity of Egg-White Proteins

Studies have shown that egg-white-sensitive patients are not all allergic to the same set of proteins [16]. RAST inhibition experiments were performed to detect similarities in the cross-reactivity of the major egg-white proteins compared to ovalbumin. The relative IgE-binding capacities of the major egg-white proteins compared to ovalbumin were also measured. Table III shows the ability of ovalbumin to inhibit IgE binding to the other major egg-white proteins and conversely the ability of these other proteins to inhibit IgE binding to ovalbumin. Fifty micrograms of each protein were used in this study as this resulted in at least 50% self-inhibition in most sera. Although the results differ between patients and proteins, each protein showed some cross-reactivity with ovalbumin as each was able to inhibit IgE binding to some degree and conversely ovalbumin inhibited IgE binding to the other proteins.

Cross-Reactivity Study of Apovitellenin I and Ovalbumin

It has been demonstrated that whole egg-white and

Table III. Percent RAST inhibitions for the sera of six patients sensitive to hen egg against 50 µg each of the major egg-white proteins

Disc	Inhibitor	Percent RAST inhibition in patient					
		Coa	Nas	Rey	Nim	Goa	Kas
Ovalbumin	ovalbumin	43.0	90.0	95.5	72.8	64.7	81.3
	ovomucoid	14.0	23.0	28.0	9.8	NM	NM
	lysozyme	0	35.5	87.0	13.0	11.8	24.2
	conalbumin	0	1.0	30.0	15.0	NM	NM
Ovomucoid	lysozyme	0	35.5	87.0	13.0	11.8	24.2
	conalbumin	0	1.0	30.0	15.0	NM	NM
Ovomucoid	ovalbumin	19.2	46.8	45.8	3.7	NM	NM
	ovomucoid	96.2	55.3	60.2	39.1	NM	NM
Lysozyme	ovalbumin	low ¹	low ¹	low ¹	low ¹	18.5	13.8
	lysozyme	low ¹	low ¹	low ¹	low ¹	88.5	85.5
Conalbumin	ovalbumin	15.5	54.2	34.1	0.0	NM	NM
	conalbumin	50.0	71.4	89.9	79.1	NM	NM

First, the table lists their inhibition of sera reacting with ovalbumin and then their inhibition by ovalbumin. In all cases the sera were also separately pre-incubated with the proteins present on the discs as a control. Percent radioactive uptake for controls was an average of $0.6 \pm 0.2\%$ for foetal cord serum and $0.7 \pm 0.4\%$ for non-egg-white-sensitive but high total IgE serum (Pharmacia PRIST 100 kU IgE/l standard). NM = Not measured.

¹ Low RAST and therefore rejected.

egg-yolk extracts cross-react [2]. The available quantities of sera limited our ability to test cross-reactivity amongst all of the individual white and yolk proteins. Ovalbumin and apovitellenin I were chosen as representatives of each fraction. The amino acid sequence of both of these proteins is known and the aim of this part of the study was, at this stage, to establish whether cross-reaction existed between the two individual proteins. Table IV shows the results of this set of inhibitions. There is clearly a cross-reaction between apovitellenin I when reactions with sera were inhibited by ovalbumin. Ovalbumin was also inhibited by apovitellenin I for a majority of sera.

Cross-Reactivity Study of Alpha-Lactalbumin and Lysozyme

Table V shows the results for RAST inhibition of egg lysozyme by the milk protein alpha-lactalbumin. It is apparent that alpha-lactalbumin inhibited IgE binding of lysozyme in the sera of many patients.

Table IV. Percent RAST inhibition using ovalbumin and apovitellenin I (Apo I) discs and inhibiting the sera of five patients with 50 µg of each of the proteins

Disc	Inhibitor	Percent RAST inhibition in patient			
		Coa	Coo	McC	Sim
Ovalbumin	Apo I	25.0	44.2	0	69.8
	ovalbumin	13.2	42.2	0	66.0
Apo I	ovalbumin	32.7	37.4	25.0	67.5
	Apo I	61.9	55.6	24.0	68.9

Percent radioactive uptake for controls was an average of $0.3 \pm 0.2\%$ for foetal cord serum and $0.4 \pm 0.4\%$ for non-egg-white-sensitive but high total IgE serum (house dust mite-allergic serum sample).

Table V. Percent RAST inhibition for lysozyme discs and sera inhibited by 50 µg of lysozyme or alpha-lactalbumin

Patient code	Lysozyme	Alpha-lactalbumin	Milk, % radioactive uptake
Cad	70.3	0	4.7
Ced	24.4	0	14.8
Cia	34.2	36.8	3.8
Dai	6.0	12.0	17.3
Dah	0	0	5.3
Goa	92.4	48.6	0.9
Mar	3.3	16.7	NM
McL	a	22.5	NM
Rao	50.0	25.7	17.6

NM = Not measured.

Discussion

The study of cross-reaction amongst egg-white and egg-yolk proteins was as comprehensive as possible given the volumes of sera available to us. The results in table III indicate that the egg-white proteins have a degree of cross-reactivity that varies with the serum of the individual being studied and the protein being tested. This may imply that similarities exist between the sequences of the allergenic determinants of the egg-white proteins. The variability in the results among patients implies that more than one allergenic determinant exists in each protein, and it is conceivable that there is a varied population of antibodies present in each serum. Since the serum of each indi-

vidual displays differing levels of inhibition, this may be a reflection of one individual's population of antibodies to egg-white protein determinants being different to another individual's. It is not unreasonable to expect multiple allergenic determinants on molecules of the size and molecular weights present in eggs (12,000–80,000 daltons), as is known to be the case with antigenic determinants [3]. In a few cases, inhibition occurred in one way but not in the other: for example, for patient Nas in table II, for which conalbumin gave a low inhibition when ovalbumin was on the disc but ovalbumin gave a high inhibition when conalbumin was on the disc. We do not know why this is so but have observed this phenomenon previously with various food and inhalant allergens [6, 9].

Cross-reaction was also demonstrated between the egg-white protein ovalbumin and the egg-yolk protein apovitellenin I. Thus here also, there exists a determinant on apovitellenin I capable of binding IgE directed against ovalbumin. The only result which is inconsistent with the finding that the two proteins cross-react is for patient McC who showed a low RAST result when ovalbumin was on the disc, however, ovalbumin was still able to inhibit reactions to sera with apovitellenin I.

Table V demonstrates the cross-reaction between lysozyme and a milk protein, alpha-lactalbumin, which has a high amino acid sequence homology. This result contrasts with the reported lack of cross-antigenicity between them [20]. It would appear that inhibition of IgE binding to lysozyme by alpha-lactalbumin does not depend on milk sensitivity. We did not have milk-allergic sera to test inhibition of them by lysozyme to see if the converse was true.

We are conducting further studies of the molecular bases of cross-reactions amongst egg proteins as suggested by these results. This task is made easier by the fact that the amino acid sequence (and even secondary and tertiary structural information in some cases) is known for all of the individual proteins examined in this work.

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