

Allergenic activity of heated and ovomucoid-depleted egg white

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Background: No egg white products have been clearly proven to be hypoallergenic. The role of egg white proteins in allergic reactions to eggs is still debatable.

Objective: This study was designed to determine the importance of ovomucoid, an egg white protein, in the development of allergies to egg white.

Methods: We performed a double-blind, placebo-controlled food challenge in subjects with high levels of IgE antibodies for egg white to compare the allergenicities of heated and ovomucoid-depleted egg white, freeze-dried egg white, and heated egg white. Levels of IgE antibodies for egg white, ovomucoid, ovalbumin, ovotransferrin, and lysozyme were measured in serum by RAST.

Results: Twenty-one of 38 subjects with positive challenge responses to freeze-dried egg white had negative challenge responses to heated egg white, whereas 16 of 17 subjects (94.1%) with positive responses to heated egg white did not respond to the heated and ovomucoid-depleted egg white challenge. The subjects with positive challenge responses to freeze-dried egg white tended to have higher IgE antibody values to ovomucoid than those with negative responses. IgE antibody levels to ovomucoid were significantly higher in subjects with positive responses to a challenge with heated egg white than in those with no response. There were no significant differences in the levels of IgE antibodies to the other proteins, except ovomucoid, in the negative-response and positive-response groups in challenge tests with freeze-dried and heated egg white.

Conclusion: The heated and ovomucoid-depleted egg white preparation was less allergenic than heated or freeze-dried preparations. Ovomucoid has a more important role in the pathogenesis of allergic reactions to egg white than other proteins in egg white. (*J Allergy Clin Immunol* 1997;100:171-6.)

Key words: Egg hypersensitivity, ovomucoid, double-blind, placebo-controlled food challenges, specific IgE antibodies, heated and ovomucoid-depleted egg white

Hen's egg is one of the more frequent causes of food hypersensitivity in infants and young children.^{1,2} Moreover, in a study by Sampson,³ egg hypersensitivity was

Abbreviations used

DBPCFC: Double-blind, placebo-controlled food challenge
SDS-PAGE: Sodium dodecylsulfate-polyacrylamide gel electrophoresis

found in two thirds of children with severe atopic dermatitis. Because eggs are highly nutritious sources of protein and are consumed in large quantities throughout the world, an understanding of which egg components are allergenic will be of immense benefit in finding ways to manage egg sensitivity.

Currently, proteinase treatment is the most popular strategy for reducing the allergenicity of food. Good examples are the commercially available hypoallergenic products derived from casein hydrolysates of cow's milk. However, there is some doubt about whether hypoallergenic egg white products can be produced by such a method. This is because ovomucoid (Gal d III) is relatively resistant to treatment by heat or digestive enzymes; whereas ovalbumin (Gal d I), ovotransferrin (Gal d II), and lysozyme are relatively sensitive to such treatments. Indeed, this might explain why it has been difficult to reduce the allergenicity of egg white through proteinase treatments so far.

Ovomucoid—like ovalbumin, ovotransferrin, and lysozyme—is an allergenic component of egg white. Studies by Bleumink and Young⁴ and Bernhisel-Broadbent et al.⁵ have shown that ovomucoid is a major allergen of egg white, whereas Hoffman⁶ concluded that ovalbumin, ovomucoid, and ovotransferrin are all strong allergens and that lysozyme is a weak allergen. Furthermore, Holen and Elsayed⁷ and Elsayed⁸ have also presented evidence that ovalbumin is a major egg white allergen. Hence, the issue of which protein is the more important allergenic component in egg white is still controversial.

Because ovomucoid is not coagulable by heat but the other potential allergenic components are, we have tried to deplete ovomucoid levels by rinsing heat-treated egg white to reduce the allergenicity. To assess the allergenicity of the heated and ovomucoid-depleted egg white, we have carried out a double-blind, placebo-controlled food challenge (DBPCFC) in subjects with egg hypersensitivity. Furthermore, we have investigated the clinical significance of ovomucoid, ovalbumin, ovotransferrin, and lysozyme in egg hypersensitivity by measuring

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specific IgE antibodies to these egg white proteins in the subjects in this study.

METHODS

Preparation of heated and ovomucoid-depleted egg white

Five kilograms of liquid egg white was heated at 90° C for 60 minutes. The treated egg white was rinsed in 20 L of cold saline solution or distilled water for 120 minutes and then squeezed through a bag made of cotton cloth to remove ovomucoid. Saline solution was used for the first three rinses, and distilled water was used for the remaining three rinses. After freeze-drying, the product was milled into a powder by using a homogenizer. For comparison, a freeze-dried or heat-treated egg white product was also prepared.

Electrophoresis and immunoblotting

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by using a 14% separating gel and a 4% stacking gel. Twenty microliters of each preparation (1 mg/ml solution) was applied to each well. Electrophoresis was performed for approximately 4 hours at a constant current of 25 mA. To assure proper protein separation and visualization, gels were stained with Coomassie Brilliant Blue (Bio-Rad Laboratories, Richmond, Calif.). The molecular weight standards (Pharmacia AB, Uppsala, Sweden) were phosphorylase b (94.0 kd), bovine serum albumin (67.0 kd), carbonic anhydrase (30.0 kd), soybean trypsin inhibitor (20.1 kd), and α -lactalbumin (14.4 kd).

Specific IgE antibody values to egg white and egg white components

Freeze-dried egg white, ovomucoid, ovalbumin, ovotransferrin (Gal d II), and lysozyme were conjugated to a 5 mm diameter CNBr-activated paper disk (Toyo Roshi K.K., Tokyo, Japan). Ovomucoid was purified according to the modified method described by Waheed and Salahuddin⁹; ovotransferrin was purchased from Wako Junyaku KK (Osaka, Japan); ovalbumin, re-purified by high-performance liquid chromatography, was a gift from Prof. Ryo Nakamura of Nagoya University; and lysozyme was a gift from Eizai Pharmaceutical Company (Tokyo, Japan). RAST assays were performed according to the procedures for the Phadebas RAST kit (Pharmacia Diagnostics AB). Percent binding was calculated by using this formula: Bound isotope count $\div$ Added total count isotope of iodine 125-labeled anti-human IgE $\times$ 100.

DBPCFC

DBPCFC with freeze-dried, heated, and heated and ovomucoid-depleted egg white was carried out with modifications of the method described by Bock et al.¹⁰ Briefly, oral provocations were conducted by administering, in a titrated manner, up to 8 gm (equivalent to approximately 1 egg) of each egg white preparation mixed in 50 ml of a thick liquid vehicle consisting of the juices from an assortment of fruits. Glucose in the same juice was used as a placebo. After the administration of the initial dose of 0.1 ml of egg white preparation, the dose was increased sequentially every 30 minutes to 1 ml, 10 ml, and then the remainder until objective symptoms developed or until the entire challenge dose was ingested. Reactions that were observed within 2 hours of the final administration were deemed to be immediate, and reactions that occurred more than 2 hours after the final administration were considered to be nonimmediate.

Subjects

Seventy-two subjects with high IgE antibody titers to egg white were enrolled for the DBPCFC (Tables I and II). A high IgE antibody titer was defined as a percent binding value that was higher than 3.1% in the RAST assay. This cutoff titer was the mean plus two standard deviations of the percent binding to egg white disks of 19 control subjects with bronchial asthma and/or atopic dermatitis but without egg hypersensitivity. These subjects were divided into four groups on the basis of their immediate reactions to oral provocation tests. The first group ($n = 1$, male) showed a positive challenge result for heated and ovomucoid-depleted egg white. The second group ($n = 16$, male/female ratio = 8:8) consisted of subjects who showed positive reactions to heated egg white but negative reactions to heated and ovomucoid-depleted egg white. The third group ($n = 21$, male/female ratio = 14:7) was composed of subjects who responded positively to freeze-dried egg white and negatively to heated egg white. In the last group ($n = 34$, male/female ratio = 19:15), subjects had negative responses to freeze-dried egg white.

We compared the allergenicity of three egg white preparations in the immediate and nonimmediate hypersensitive reactions using the following assumptions. If subjects had positive responses to challenge tests with heated and ovomucoid-depleted egg white, they were regarded as having positive responses to the other preparations. Similarly, subjects with a positive reaction to challenge tests with heated egg white were assumed to have positive reactions to freeze-dried egg white, and subjects with negative responses to heated egg white were assumed to have negative responses to heated and ovomucoid-depleted egg white. Subjects with negative reactions to freeze-dried egg white were regarded as having negative reactions to the other preparations.

Informed consent was obtained from children and/or parents. The study was approved by the Ethics Committee of Fujita Health University School of Medicine.

Statistical analysis

An unpaired t test or the Wilcoxon signed-rank test was used for determining the statistical significance ($p < 0.05$) of specific IgE antibody values after logarithmic transformation.

RESULTS

SDS-PAGE

The proteins of freeze-dried egg white, heated egg white, and heated and ovomucoid-depleted egg white were separated and visualized by using SDS-PAGE as shown in Fig. 1. A decrease in the intensity of the band corresponding to ovomucoid can be clearly seen in the lane for heated and ovomucoid-depleted egg white. An immunoblotting study also showed that a band with mobility similar to that of ovomucoid, which reacted to IgE antibodies from patients allergic to eggs, was efficiently reduced as shown in Fig. 2.

DBPCFC

Tables I and II summarize the results from the DBPCFC. Thirty-eight of 72 subjects (groups 1, 2, and 3) were judged to be positive responders to provocation tests with freeze-dried egg white. Twenty-one (group 3, 55.3%) of 38 subjects (groups 1, 2, and 3) with positive immediate reactions to freeze-dried egg white showed

TABLE I. Characterizations of patients who had oral egg white challenge

Group	Patient	Sex	Age (yr)	History of egg allergy		Complicating disease	Oral challenge test					
				Symptom	Age (yr)		Freeze-dried		Heated		Heated and ovomucoid-depleted	
							IHR	Non-IHR	IHR	Non-IHR	IHR	Non-IHR
1	KN	M	6.2	Anaphylaxis	2	AD, BA, FA	ND	ND	ND	ND	+	+
	RK	M	1.6	Urt	1.3	AD, FA	ND	ND	+	+	-	+
	MS	F	1.1	No		AD	ND	ND	+	+	-	-
	TO	F	1.3	No		AD, BA	ND	ND	+	+	-	-
	YT	F	1.4	Urt	1	AD, FA	ND	ND	+	+	-	-
	KS	M	2.0	No		AD, BA	ND	ND	+	+	-	-
	AM	F	2.3	Urt, Ery	0.5	AD, FA	ND	ND	+	+	-	-
	HS	M	10.0	Urt	7.1	AD, FA	ND	ND	+	+	-	-
2	RI	M	1.2	Ery	0.7	BA, AD, FA	+	-	+	-	-	-
	YW-1	M	2.4	No		AD	+	-	+	-	-	-
	YN	F	1.4	Unknown		AD	ND	ND	+	-	-	-
	AO	F	2.0	No		AD	ND	ND	+	-	-	-
	KT-1	M	2.1	Diarrhea	1	AD, FA	ND	ND	+	-	-	-
	AT	F	2.4	No		BA, AD	ND	ND	+	-	-	-
	ST-1	M	3.2	Ery	1	AD, FA	ND	ND	+	-	-	-
	YM-1	F	5.7	Urt	1	AD, BA, FA	ND	ND	+	-	-	-
	KH	M	10.0	Urt	8	BA, FA	ND	ND	+	-	-	-
	ST-2	M	1.9	Urt	0.5	AD, FA	+	+	-	-	-	-
	MW	M	2.5	Ery	1	AD, FA	+	+	-	-	-	-
	NH	M	3.1	No		AD	+	+	-	-	-	-
	YH	M	3.1	Wheeze	3	AD, BA, FA	+	+	-	-	-	-
	SS	M	1.8	Ery	0.4	AD, FA	+	+	-	-	ND	ND
	RO	M	2.8	Urt	1.3	AD, FA	+	+	-	-	ND	ND
	FW	F	2.2	No		AD	+	-	-	-	-	-
	SY	F	5.0	No		AD	+	-	-	-	-	-
	TH	F	5.2	Wheeze	2	AD, FA	+	-	-	-	-	-
	TT	M	1.7	Vomiting	0.8	AD, FA	+	-	-	-	ND	ND
3	KN	M	1.7	No		AD	+	-	-	-	ND	ND
	KK	F	1.8	No		BA	+	-	-	-	ND	ND
	NY	F	2.0	Wheeze	1	BA, FA	+	-	-	-	ND	ND
	MK	F	2.0	Urt	1	AD, FA	+	-	-	-	ND	ND
	HI	M	2.3	No		AD	+	-	-	-	ND	ND
	NM	M	3.1	No		AD	+	-	-	-	ND	ND
	YW-2	M	3.2	No		AD	+	-	-	-	ND	ND
	KT-2	M	3.8	Ery	0.9	AD, FA	+	-	-	-	ND	ND
	TS	M	4.2	Ery	0.6	BA, AD, FA	+	-	-	-	ND	ND
	YM-2	F	4.7	Ery	2.2	AD, AR, FA	+	-	-	-	ND	ND
DF	M	6.3	Urt	1.5	AD, FA	+	-	-	-	ND	ND	

IHR, Immediate hypersensitivity reaction; AD, atopic dermatitis; BA, bronchial asthma; FA, food allergy; ND, not done; Urt, urticaria; Ery, erythema; AR, allergic rhinitis.

negative responses to heated egg white, and 16 (group 2, 94.1%) of 17 subjects (groups 1 and 2) with positive responses to heated egg white had negative challenge results for heated and ovomucoid-depleted egg white. Also, 37 (groups 2 and 3, 97.4%) of 38 subjects (groups 1, 2, and 3) with positive responses to the freeze-dried product had negative challenge test responses to the heated and ovomucoid-depleted product.

Assessment of the results of nonimmediate hypersensitive reactions to oral provocation tests revealed that six of 14 subjects (42.9%) with positive nonimmediate reactions to freeze-dried egg white had a negative reaction to challenge tests with heated egg white. In contrast, six of eight

(75.0%) subjects with positive nonimmediate reactions to heated egg white had a negative reaction to challenge tests with heated and ovomucoid-depleted egg white.

Specific IgE antibody values to egg white and egg white components

The subjects with positive immediate hypersensitive reactions to freeze-dried egg white (groups 1, 2, and 3) had significantly higher IgE antibody values to egg white ($p = 0.013$) and a tendency toward higher IgE antibody values to ovomucoid ($p = 0.091$) than subjects with a negative reaction (group 4) as shown in Fig. 3. However, there was an overlap in the levels of IgE antibodies to

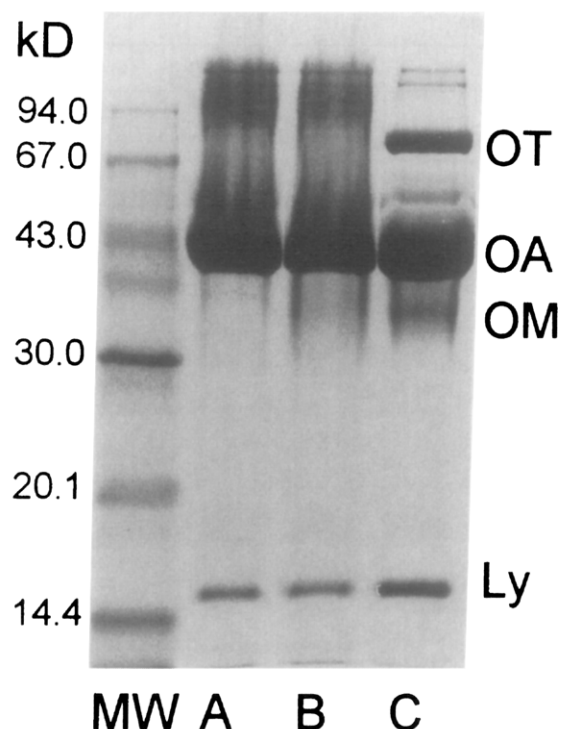


FIG. 1. SDS-PAGE of heated and ovomucoid-depleted egg white (lane A), heated egg white (lane B), and freeze-dried egg white (lane C). OT, Ovotransferrin; OA, ovalbumin; OM, ovomucoid; Ly, lysozyme.

TABLE II. Immediate hypersensitive reactions to oral challenge test with freeze-dried, heated, and heated and ovomucoid-depleted egg white antigens

Group	Egg white			No.
	Freeze-dried	Heated	Heated and ovomucoid-depleted	
1	Positive	Positive	Positive	1
2	Positive	Positive	Negative	16
3	Positive	Negative	Negative	21
4	Negative	Negative	Negative	34
Sum				72

The allergenicity of three egg white preparations is compared on the basis of the following assumptions. A subject with positive reactions to a challenge test with heated and ovomucoid-depleted egg white will have positive reactions to the other preparations. Subjects with positive reactions to challenge tests with heated egg white will have positive reactions to freeze-dried egg white. Subjects with negative reactions to heated egg white will have negative reactions to heated and ovomucoid-depleted egg white. Subjects with negative reactions to freeze-dried egg white will have negative reactions to the other preparations.

Rate of change to negative challenge responses:

Negative for heated (group 3)/positive for freeze-dried (groups 1, 2 and 3) $\times 100 = 21/38 \times 100 = 55.3\%$.

Negative for heated and ovomucoid-depleted (group 2)/positive for heated (groups 1 and 2) $\times 100 = 16/17 \times 100 = 94.1\%$.

Negative for heated and ovomucoid-depleted (groups 2 and 3)/positive for freeze-dried (groups 1, 2, and 3) $\times 100 = 37/38 \times 100 = 97.4\%$.

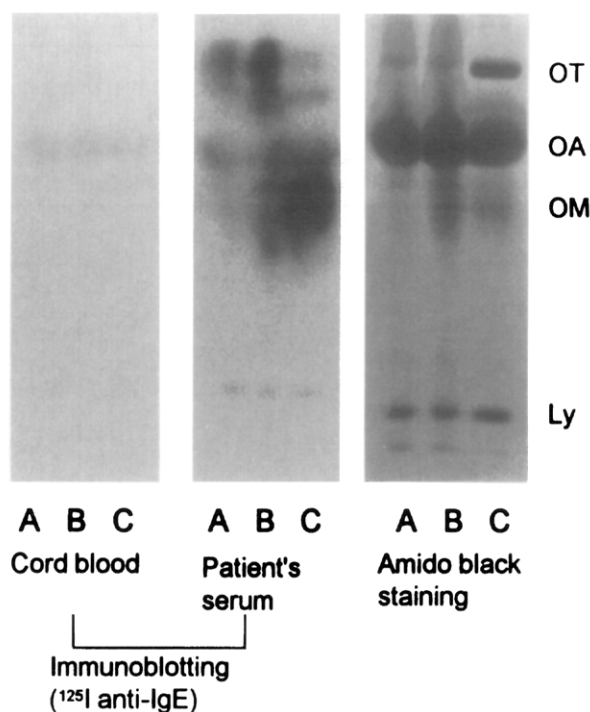


FIG. 2. Evaluation of IgE-binding activity to heated and ovomucoid-depleted egg white (lane A), heated egg white (lane B), and freeze-dried egg white (lane C) by immunoblotting. OT, Ovotransferrin; OA, ovalbumin; OM, ovomucoid; Ly, lysozyme.

ovomucoid between the two groups. In contrast, the challenge tests with heated egg white resulted in significantly higher IgE antibody values to both egg white ($p = 0.022$) and ovomucoid ($p = 0.002$) in the positive responders (groups 1 and 2) compared with the negative responders (groups 3 and 4). All subjects, except one who had IgE antibody levels below the mean minus one standard deviation of the ovomucoid-specific IgE antibody values of groups 1 and 2, had negative responses to the heated egg white. There were no significant differences in the levels of IgE antibodies to ovalbumin, ovotransferrin, or lysozyme between subjects with positive reactions and those with negative reactions to the challenge tests with freeze-dried or heated egg white.

Finally, we compared the levels of IgE antibodies between two groups of subjects who had positive challenge responses to freeze-dried egg white with those who had positive reactions to heated egg white (groups 1 and 2) and those who had negative reactions to heated egg white (group 3). The values for IgE antibodies to ovomucoid in group 3 were significantly ($p = 0.01$) lower than values in groups 1 and 2. No significant differences were found in the values for IgE antibodies to the other egg white proteins, except ovomucoid, between these groups.

DISCUSSION

Depletion in ovomucoid from egg white was efficiently completed by rinsing in saline solution and distilled

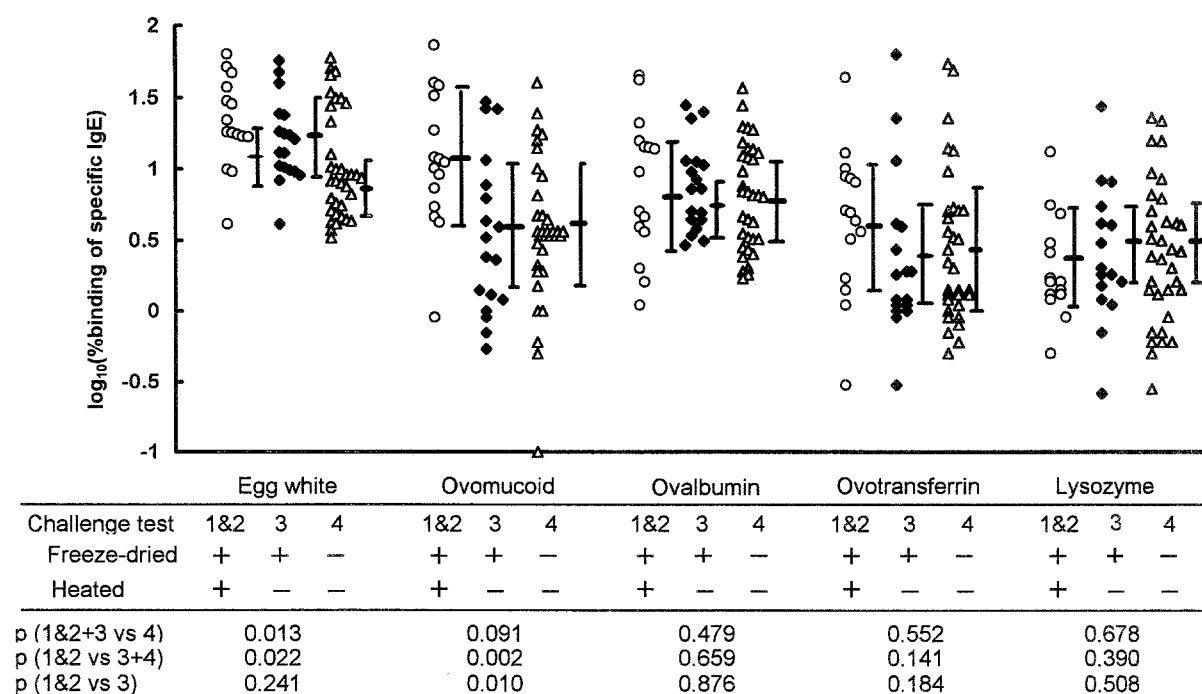


FIG. 3. Comparison of specific IgE antibody values for egg white and the components between subjects with positive and negative responses to oral challenge test with freeze-dried and heated egg white. *Open circles*, Groups 1 and 2 with positive reactions to challenge tests with both freeze-dried and heated egg white; *filled diamonds*, group 3 with positive reactions to challenge tests with freeze-dried egg white but negative reactions to heated egg white; *open triangles*, group 4 with negative reactions to challenge tests with both freeze-dried and heated egg white. Error bar represents one standard deviation.

water after heat treatment as shown in Figs. 1 and 2. Oral challenge tests were performed in subjects with high IgE antibody values to egg white to compare the allergenicity of three different egg white preparations. First, heat treatment reduced the allergenic activity of egg white. This heated preparation was tolerated in approximately half of the patients who were sensitive to freeze-dried egg white and was also well tolerated by the subjects with relatively lower titers for ovomucoid. Furthermore, heated and ovomucoid-depleted egg white was much more hypoallergenic than heat-treated egg white. Even subjects with high titers of antibodies to ovomucoid showed negative challenge responses to heated and ovomucoid-depleted egg white. The reduction in allergenicity of these products was also confirmed by skin prick tests (data not shown). Hence, these data from oral challenge tests and skin tests indicate the importance of ovomucoid and heat-unstable antigens such as ovalbumin, ovotransferrin, and lysozyme in the pathogenesis of egg allergy.

Although a large number of studies^{7, 11-14} have been performed to characterize egg white proteins, no clear consensus has been reached as to the relative allergenicity of individual protein components. In this study a tendency toward a difference in IgE antibody values to ovomucoid was observed between subjects with positive and negative immediate hypersensitive reactions to freeze-dried egg white, whereas no differences were

found in IgE antibody values to the other egg white components such as ovalbumin, ovotransferrin, and lysozyme. These results imply a more important role for ovomucoid, compared with other proteins, in egg hypersensitivity.

A significant difference in IgE antibody values to ovomucoid was observed between two subjects who showed positive responses to freeze-dried egg white with positive and negative reactions to heated egg white. However, there were no significant differences in the levels of IgE antibodies to egg white or the other proteins, except ovomucoid, between the two groups. These data suggest a clinical usefulness for measuring the levels of IgE antibodies to ovomucoid as a means of distinguishing between patients who can tolerate heat-treated eggs from those who are hypersensitive to both raw and heated eggs.

Recently, Bernhisel-Broadbent et al.⁵ reported the importance of ovomucoid in egg allergy. Unlike previous studies, all subjects enrolled in their study were allergic to eggs at the time of initial oral challenge and were divided into egg-tolerant and egg-intolerant subject groups on the basis of the follow-up challenge results. Also, they introduced the use of highly purified egg white proteins for in vivo and in vitro laboratory studies. In our study we selected subjects whose levels of serum IgE antibodies to egg white were higher than the mean plus two standard deviations of the levels found in

individuals with asthma and/or atopic dermatitis who were not allergic to eggs. We then compared the levels of IgE antibodies to egg white, ovomucoid, ovalbumin, ovotransferrin, and lysozyme in the challenge-positive and challenge-negative groups. Our results, that subjects with negative challenge responses to freeze-dried egg white tended to have lower levels of IgE antibodies to ovomucoid, might explain why no reactions to egg ingestion occur in spite of high levels of IgE antibodies to egg white. Ovomucoid-specific IgE antibodies may have an important role in the development of tolerance to eggs in subjects who are allergic to eggs and might be used to predict the outcome of egg allergy in young children as described by Bernhisel-Broadbent et al.⁵

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