

Short communication

Prevalence of lysozyme sensitization in an egg-allergic population

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An egg protein, lysozyme, is a still unlabeled additive currently used in cheese preparation. Furthermore, the WHO-FAO committee considers it innocuous. However, 31% of children and 8% of adults with food allergies are allergic to eggs. This work aimed to determine the percentage of patients sensitized to lysozyme from a population of egg-allergic patients. Specific IgE was determined with Cap RAST in 52 patients clinically allergic to egg. Thirty-five percent of egg-allergic patients had antilysozyme IgE. Given this high incidence of lysozyme sensitization, it seems that the presence of lysozyme should be indicated on food labels.

Both the frequency and severity of food allergies have increased considerably in recent years (1–3). Attention has focused on certain food proteins that are used as additives and that can act as hidden allergens, unknown to the consumer. Worthy of note, among others, are the following substances: caseinates, which are used as texturing agents (4, 5); papain, a clarifying agent added to beer; cochineal carmine, a food dye found in yogurt; and α -amylase, which is used to improve the rising of dough and is present in bread and other dough products.

Hen's egg-white lysozyme is used in numerous foods, especially in Japan, as a bactericidal additive. For example, it is used in France in the production of cheese to prevent "late blowing" (6, 7). As a food additive, lysozyme is not considered to be a health hazard (6). However, 31% of children and 8% of adults with food allergies are allergic to eggs, which are a major food (8, 9). Although the major egg allergens are ovalbumin and ovomucoid, publications have shown that lysozyme is indeed aller-

genic (10–13). One is now forced to reconsider the eventual risk to egg-sensitive patients when they ingest food containing lysozyme. Indeed, severe allergic reactions to drugs containing lysozyme have been observed in patients allergic to eggs (14–16). In addition, a case of occupational asthma due to the inhalation of lysozyme has been published (17). The present study was carried out to evaluate the high prevalence of immunologic hypersensitivity to this additive. The high frequency of sensitization to lysozyme, as well as some positive oral challenges, argue that there is an actual allergic risk when lysozyme is used as a food additive.

Material and methods

Patients

Serum was obtained from 52 patients, aged from 6 months to 45 years, who were clinically allergic to eggs: 32 were under 6 years old, 18 were 7–14 years old, and two were adults. Food hypersensitivity was

documented by skin prick tests and by measurement of specific IgE antibodies. The food allergy was then confirmed by oral challenge test.

Skin tests

Prick tests were carried out with hen's egg-white extract and hen's egg-yolk extract (Stallergènes, Fresnes, France). Reactions with a wheal diameter greater than or equal to 3 mm were considered positive if the positive control (codeine) was normal and the diluent control was negative.

Specific IgE

Sera from all 50 patients were assayed for specific serum IgE antibodies to hen's egg white and hen's egg lysozyme with the CAP-RAST System (Pharmacia, Uppsala, Sweden). The result was considered positive when the antibody level was ≥ 0.35 kU/l.

RAST-inhibition assays

The lysozyme CAP-RAST assay was inhibited with a 10 mg/ml solution of α -lactalbumin (L 6010, Sigma, St Louis, MO, USA) to detect possible cross-reactivity between these two proteins, as has been previously suggested (18, 19). The inhibition mixture, 25 μ l of serum added to 25 μ l of α -lactalbumin (10 mg/ml in 0.1 M phosphate buffer, pH 7.4), was stirred gently for 30 min. A control mixture was prepared of 25 μ l serum and 25 μ l phosphate buffer. Each mixture was incubated with CAP lysozyme (RK 208) and assayed in the normal way (20). The percent inhibition (I) was calculated as follows:

$$I = 1 - \frac{(\text{cpm bound with inhibition mixture})}{(\text{cpm bound with control mixture})}$$

A sufficient quantity of serum was required for RAST-inhibition assays, and only 12 sera could be screened.

Oral food challenge test

Prior informed consent of patients or their parents was obtained for double-blind, placebo-controlled food challenge (DBPCFC) using raw egg white, according to Bock & Atkins (21). Raw egg white was hidden in 12 g of mashed potatoes. The doses were administered every 20 min in accordance with the following progression: 5, 10, 50, 200, and 700 mg (cumulative doses: 965 mg). The placebo comprised the same amount of potatoes, repeated five times. The criteria used to define a positive

DBPCFC were angioedema; urticaria; rash on the eczematous flexures; wheezing; and modifications of the pulse, blood pressure, and peak-flow rate. A single subjective sign was taken into consideration – abdominal pain – since patients presenting with this symptom and receiving a greater quantity of food showed objective digestive symptoms such as vomiting or diarrhea.

Six patients gave their informed consent to DBPCFC using lysozyme. Increasing quantities of raw egg white and/or lysozyme were tested: from 10 to 7110 mg (egg white) and from 3 to 230 mg (lysozyme). In one case, a labial challenge was carried out with one drop of lysozyme solution (1 mg/ml) applied to the lower lip of the half-opened mouth.

Results

Of the 52 patients, three experienced allergic reactions to Gruyère cheese. Patient 52 is detailed. D'A. A., 19 months old, was RAST positive to egg white at 51.2 kU/l, and to lysozyme at 8.5 kU/l. DBPCFC was positive to 65 mg of egg white. Egg was removed completely from the diet, and then atopic dermatitis clearly improved. He was referred some months later for an acute attack of facial and laryngeal angioedema occurring some minutes after ingestion of grated Gruyère. Allergy to milk proteins was ruled out. The labial test was positive for lysozyme, rash spreading on the eczematous skin (Fig. 1), and the manufacturer confirmed the presence of lysozyme.

All 52 patients had positive skin prick test, and positive RAST to egg white from 0.56 to more than 100 kU/l (Table 1).

The oral challenge was always positive to raw egg white with a cumulative dose ranging from 10 mg to 10 g. Of seven challenges to lysozyme, three were positive: one after ingestion of 3 mg of



Fig. 1. Positive labial test to lysozyme, performed on child D'A. A. Rash spreading on eczematous skin.

Table 1. Results of RAST to egg white and to lysozyme in serum of 50 patients clinically allergic to egg. Inhibition of RAST to lysozyme by α -lactalbumin was always negative for 12 sera tested. Two positive DBPCFC to lysozyme were related to high values of RAST to lysozyme

Patient no.	RAST to egg kU/l (class)	RAST to lysozyme kU/l (class)	Inhibition of RAST to lysozyme by α -lactalbumin (%)	DBPCFC using lysozyme
1	10.21 (3)	0.35 (0)		Negative (230 mg)
2	10.00 (3)	0.35 (0)		
3	5.00 (3)	0.35 (0)		
4	7.50 (3)	0.35 (0)		
5	2.30 (2)	0.35 (0)		
6	1.25 (2)	0.35 (0)		
7	32.70 (4)	3.19 (2)	0%	
8	10.10 (3)	1.11 (2)	0%	
9	33.70 (4)	17.60 (4)	0%	
10	2.68 (2)	0.35 (0)		
11	3.70 (3)	0.35 (0)		
12	10.50 (3)	0.35 (0)		
13	1.24 (2)	0.35 (0)		
14	2.84 (2)	0.35 (0)		
15	2.03 (2)	0.35 (0)		
16	14.00 (3)	1.53 (2)	0%	
17	26.20 (4)	2.53 (2)	0%	
18	1.99 (2)	0.35 (0)		
19	97.40 (5)	2.43 (2)	0%	
20	1.67 (2)	0.35 (0)		
21	2.21 (2)	0.35 (0)		
22	79.2 (5)	1.8 (2)		
23	2.18 (2)	0.35 (0)		
24	2.32 (2)	0.35 (0)		
25	1.84 (2)	0.35 (0)		
26	1.22 (2)	0.35 (0)		Negative (36 mg)
27	8.5 (3)	0.35 (0)		
28	1.1 (2)	0.35 (0)		
29	6.00 (3)	7.90 (3)	0%	
30	1.99 (2)	0.35 (0)		
31	30.60 (4)	33.40 (4)	0%	Positive (3 mg)
32	2.70 (2)	0.35 (0)		
33	7.5 (3)	0.35 (0)		
34	76.00 (4)	13.4 (3)	0%	Negative (230 mg) Positive (labial test 1 mg/ml)
35	3.7 (3)	1.96 (2)	0%	
36	13.40 (3)	0.35 (0)		
37	20.10 (4)	0.35 (0)		
38	24.8 (4)	0.35 (0)		
39	38.00 (4)	9.6 (3)	0%	
40	0.56 (1)	0.35 (0)		
41	46.70 (4)	0.35 (0)		
42	10.30 (3)	0.35 (0)		Negative (230 mg)
43	4.90 (3)	0.35 (0)		
44	> 100 (4)	34.2 (4)	0%	
45	60.6 (4)	5.62 (3)		
46	24.9 (4)	< 0.35 (0)		
47	4.27 (3)	< 0.35 (0)		
48	> 100 (4)	1.31 (2)		
49	46.8 (4)	< 0.35 (0)		
50	4.78 (3)	4.23 (3)		
51	> 100 (4)	20 (4)		
52	51.2 (4)	8.5 (3)		

lysozyme and two after labial challenge with one drop of lysozyme solution (1 mg/ml).

Antilysozyme specific IgE ranging from 1.1 to 34.2 kU/l was detected in 18 of the 52 sera tested (35%) (Table 1). There was a weak correlation

between the levels of specific IgE to egg white and to lysozyme ($r^2=0.22$, $P<0.03$) (Fig. 2).

There was no significant inhibition of the lysozyme CAP-RAST by α -lactalbumin in any of the sera assayed.

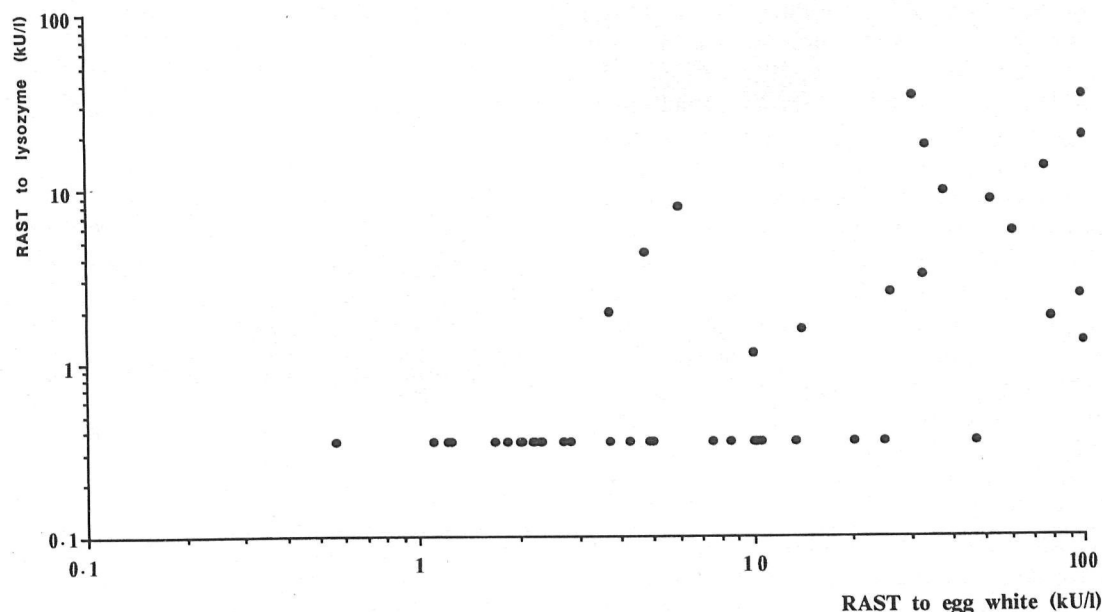


Fig. 2. RAST to egg white and to lysozyme measured in sera of 52 patients clinically allergic to egg (log scale).

Discussion

As reported by Langeland (12), the major allergens of hen's egg white are ovalbumin and ovomucoid, although ovotransferrin appears to characterize egg-sensitive asthmatic patients (13). Patients of this sort may also have bird-egg syndrome, in which case they are mainly allergic to α -livetins, hen serum albumin, and sometimes to ovotransferrin (22).

Lysozyme is one of the proteins of hen's egg white (3.5% of total proteins). Its molecular mass is 14 kDa and its amino acid sequence is entirely known. As it is one of the hen's egg allergens, the current allergen nomenclature refers to lysozyme as Gal d 4 (23). Lysozyme had been suspected of being allergenic but was not considered to be an important egg allergen before 1950 (24). In 1983, Hoffman (11) reported that 66% of the sera from egg-sensitive subjects contained lysozyme-specific IgE. The presence of lysozyme-specific IgE was detected by Western blots in sera from 10 egg-sensitive patients examined by Anet et al. (10). Holen & Elsayed (25) consider lysozyme a major hen's egg white allergen, along with ovalbumin, ovomucoid, and ovotransferrin. The frequency of cutaneous sensitivity to lysozyme (at a concentration of 0.1 mg/ml) is not negligible, being found, for example, in 11 (61%) of 18 egg-sensitive patients (26). We consider that the lysozyme sensitization frequency of 32% found in the present study can be taken as a minimum.

There is a high degree of sequence homology between hen's egg lysozyme and bovine α -lactalbumin (18). Walsh et al. (19) observed cross-

reactivity between these proteins in half the sera tested. In contrast, in tests on 12 sera, we observed no inhibition of the lysozyme CAP-RAST by α -lactalbumin, in agreement with available data indicating that these two proteins have no common antigenic sites (27).

The full consequences of allergic sensitivity to lysozyme are still not fully understood. Lysozyme used as medication has been reported to cause severe and even fatal reactions (14, 15), as for example, in vaginal suppositories (16). In the report concerning suppositories, five out of seven of the allergic subjects had a positive skin test to lysozyme (0.1 mg/ml), and all seven had a vigorous T-cell response to this protein. Additional evidence of its allergenicity can be found in a recent report on occupational allergy in an egg-processing factory where a number of workers were found to be allergic to both lysozyme and other egg proteins (17, 28). Worthy of note is that the four negative DBPCFC to lysozyme correspond to a negative RAST to lysozyme, whereas the two positive provocation tests are related to high values of RAST to lysozyme (classes 3 and 4) (Table 1). This demonstrated that even very small amounts of lysozyme can provoke allergic reactions (oral test with 3 mg or labial test with 1 mg/ml).

Nevertheless, in spite of such evidence, the product was approved in 1992, as a cheese additive by the FAO/WHO Expert Committee on Food Additives. The committee concluded that, on the basis of the available data, the low additional intake of lysozyme via cheese was not a hazard to consumer health (6).

Lysozyme is authorized in foods as a bactericidal agent to prevent the development of *Clostridium tyrobutyricum*-type anaerobic bacteria. The amount of lysozyme used in foods varies from country to country. It is in widespread use in Japan, where it is found in amounts of up to 20 µg/g in vegetable-based food products such as noodles, biscuits, crackers, miso, and beans (7). The amount that can be added to the milk used in producing cheese is 10–35 g/100 l (100 kg of finished product), meaning that it is possible to consume 35 mg/100 g of cheese. These quantities are not negligible in patients who are allergic to eggs. The proportion of lysozyme-specific IgE, among other egg protein-specific IgE, varies considerably from one individual to another, making the risk of allergy to this product unpredictable.

In the years to come, the regular consumption of this food allergen could provoke increasing hypersensitivity to it and, consequently, cause very serious anaphylactic reactions (29). It would therefore seem reasonable to warn both producers and regulatory bodies of this risk.

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