

Original article

Egg white proteins as inhalant allergens associated with baker's asthma

Background: Bakery workers may develop IgE-mediated allergy to liquid and aerosolized hen's egg proteins that are commonly used in the baking and confectionery industries.

Methods: We studied four bakery workers who had work-related allergic respiratory symptoms upon exposure to egg aerosols. The causative role of egg proteins in their respiratory symptoms was investigated by immunologic and specific inhalation challenge (SIC) tests.

Results: Skin prick tests to egg white extract and to lysozyme gave positive responses in all the subjects, to ovalbumin in two, to ovomucoid in one and to egg yolk in two subjects. They were also sensitized to wheat, rye and barley flours. Specific IgE determinations to egg white were positive in all patients, to lysozyme in two, to ovalbumin in three, to ovomucoid in two and to egg yolk in two of them. Methacholine inhalation tests revealed bronchial hyperresponsiveness in all workers ($PC_{20} < 16$ mg/ml). SICs were performed with aqueous extracts of lysozyme ($n = 4$), ovalbumin ($n = 2$) and ovomucoid ($n = 1$), which elicited isolated early asthmatic reactions in all subjects. Double-blind, placebo-controlled, oral challenge tests with raw egg white were positive in three subjects.

Conclusions: These bakery workers had developed IgE-mediated occupational asthma to hen's egg white proteins.

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Hen's egg white proteins have been classically implicated in the development of food allergy. The major allergens reside in egg white fraction, i.e. ovomucoid (Gal d 1), ovalbumin (Gal d 2), conalbumin (Gal d 3) and lysozyme (Gal d 4) (1, 2). Occupational asthma because of egg white aerosols has also been reported (3, 4). Bernstein et al. (5) and Smith et al. (6) described in the late 1980s an association between exposure to egg proteins and the development of IgE-mediated sensitization and work-related respiratory symptoms among employees at several egg-processing plants. Since then, several investigators have reported that bakery workers, confectioners, pharmaceutical plant workers and homemakers may develop IgE-mediated allergy and asthma to egg white aeroallergens from liquid or powdered egg white aerosols (7–13). However, the causative role of egg white proteins in asthma symptoms has been scantily documented by means of specific inhalation challenge (SIC) (7, 8, 10, 11).

In this article, we describe four employees in the baking industry who developed work-related respiratory symptoms upon exposure to liquid egg aerosols. In addition, two of them had developed symptoms of food allergy to egg. The causative role of purified egg white proteins in the symptoms of these workers was investigated by means

of clinical and immunologic tests and confirmed by specific inhalation and oral challenge studies.

Materials and methods

Subjects

We evaluated three confectioners and one baker. The four workers were male and their age range was between 27 and 54 years. At work, they all shaped the cakes, and used an air compressor to aerosolize liquid hen's egg on them. In addition, one confectioner used a brush to spread the liquid egg.

These subjects had developed work-related symptoms of cough, chest tightness, shortness of breath and wheezing. Subjects 1 and 2 had previously developed IgE-mediated occupational asthma to cereal flours. Sneezing, itching, and runny nose preceded the development of asthma symptoms in all patients. All of them improved on weekends and particularly during long holidays. These patients were exposed routinely to egg aerosols. They all established a causal relationship between exposure to high concentrations of egg aerosols (like a 'cloud') while using an air compressor in the workplace and immediate worsening of nose and asthma symptoms. They had been working in the baking industry between 120 and 300 months. The mean duration of asthma symptoms in these patients was 123 months (range 48–216 months). Patients' characteristics and demographic data

are presented in Table 1. Two workers were still working at the time of evaluation. They all were taking inhaled corticosteroids on a regular basis and short-acting and/or long-acting beta-agonists at the time of the study. The four patients had needed emergency treatment because of their asthma at least on one occasion, and subjects 3 and 4 had been admitted into the intensive care unit because of severe asthma attacks. Both patients were on a sick leave and could not return to work because of the severity of these episodes. All the patients could eat bread and pastries containing egg without any symptoms. Two subjects suffered oral itching when they ate raw or undercooked egg (e.g. fried egg, omelette, etc.) and one of them had contact urticaria with raw egg. The two patients with food allergy to egg had developed these symptoms months after the onset of the respiratory manifestations. Patients 2 and 4 were atopic as determined by positive skin tests to common inhalant allergens.

Preparation of allergenic extracts

Ovalbumin, ovomucoid, conalbumin and lysozyme were purchased from Sigma (Sigma Chemical Co., St Louis, MO, USA). These proteins were prepared at concentration (dry weight) of 10 mg/ml by adding phosphate-buffered saline (PBS). All the extracts were filtered through a 0.22-µm membrane (Millipore Corp., Bedford, MA, USA) and aliquoted. Several twofold dilutions of the extracts in PBS were done for skin and inhalation tests.

Skin tests

Skin prick tests (SPTs) were performed by the prick method with commercially available extracts of whole egg, egg white and egg yolk 5% w/v (ALK-Abelló, Madrid, Spain), as well as with purified ovomucoid, ovalbumin, lysozyme and conalbumin (Sigma). In addition, skin end-point titration with the purified egg white proteins was done by testing decreasing twofold concentrations of the extracts using the skin-prick method. Patients were tested with extracts of wheat, rye, barley, oats, corn, rice flour extracts at 5% w/v (ALK-Abelló) and soybean flour and alpha-amylase at 10 mg/ml (CBF Leti, Madrid, Spain). Moreover, skin tests were performed with a panel of common inhalant allergens (ALK-Abelló), including pollens (grass, trees and weeds), mites (*Dermatophagoides pteronyssinus* and *D. farinae*), storage mites (*Acarus siro*, *Lepidoglyphus destructor* and *Tyrophagus putrescentiae*), cockroach (*Blatella orientalis* and *B. germanica*), animal dander (cat and dog) and moulds (*Alternaria*, *Aspergillus* and *Penicillium*).

Histamine phosphate at 10 mg/ml and normal saline were used as positive and negative controls, respectively. The response was read 15 min after puncture, and the results were expressed as the mean wheal diameter (mm). A wheal diameter 3 mm or greater accompanied by erythema, compared with the saline control, was defined as a positive reaction. Atopy was defined as the presence

of a positive skin reaction to at least one of the common aeroallergens.

Serum IgE determinations

Total serum IgE was measured by Pharmacia CAP system (Pharmacia Diagnostics, Uppsala, Sweden) IgE fluoroenzyme immunoassay (FEIA). The determination of specific IgE antibodies to whole egg, egg white, egg yolk, ovalbumin, ovomucoid, conalbumin and lysozyme was performed using Pharmacia CAP system FEIA. IgE levels higher than 0.35 kU/l were regarded as positive, as recommended by the manufacturer.

Inhalation challenge tests

Methacholine inhalation test was performed according to Cockcroft et al. (14) with some modifications (15) before SICs. The aerosolized particles were generated by a continuous pressurized nebulizer model DeVilbiss 646 (DeVilbiss Co., Somerset, PA, USA) with an output of 0.28 ml/min. The result of this test was expressed as the provocative concentration of methacholine causing a 20% fall in forced expiratory volume in 1 s (PC₂₀) and it was determined by interpolation of the last two concentrations. Methacholine PC₂₀ values of less than 16 mg/ml were considered to reflect significant bronchial hyperresponsiveness.

SICs were performed with the egg white proteins to which the patients showed a wheal diameter greater than 3 mm on SPTs. SICs to ovalbumin, ovomucoid, conalbumin and/or lysozyme was carried out in the four patients who gave informed consent. SICs were also performed to confirm the diagnosis of occupational asthma in patients 3 and 4 who had suffered severe asthma attacks in the workplace, as recommended for patients who cannot return to work for safety reasons (16). The patient inhaled the aerosolized allergen (aqueous extracts of ovalbumin, ovomucoid, conalbumin and/or lysozyme) using the nebulizer method mentioned above in progressive concentrations at tidal breathing for 2 min. A control challenge with PBS was carried out between 24 and 48 h before antigen provocation. Increasing concentrations of the extract were given by inhalation starting with a concentration that induced a 2-mm wheal on end-point titration by skin prick testing. The dose was increased in twofold increments at intervals of 10 min and FEV₁ was measured at 5 and 10 min after inhalation of each concentration. Inhalation challenge test was discontinued when there was a fall in FEV₁ of 20% or greater from the lowest postsaline value or when the highest concentration (10 mg/ml) had been given. At the end of the inhalation test, spirometry was performed at 20, 30, 40 and 60 min after challenge, and again the following day. From that moment PEF and FEV₁ measurements were done hourly with a computerized flowmeter (VM Plus, Clement Clarke Ltd., Harlow, UK) for 24 h after challenge, respecting sleeping time. A fall in FEV₁ of 20% or more from the lowest postsaline within 60 min of challenge was considered an early asthmatic reaction, and

Table 1. Clinical and demographic characteristics of the study patients

Patient no.	Age (years)	Smoking	Job	Duration of exposure (months)	Duration of symptoms (months)	FVC (l) (% pred)	FEV ₁ (l) (% pred)	IgE total (kU/l)
1	45	Ex-smoker	Baker	240	108	5.32 (112%)	3.35 (86%)	353
2	38	Ex-smoker	Confectioner	240	120	3.9 (111%)	2.73 (77%)	2180
3	54	Nonsmoker	Confectioner	300	216	4.07 (101%)	3.19 (106%)	321
4	27	Ex-smoker	Confectioner	120	48	5.49 (109%)	4.67 (109%)	480

Table 2. Results of skin prick tests (SPT), specific IgE determinations, specific inhalation challenges (SIC) and double blind, placebo-controlled, oral challenge tests (OC)

	Patient 1				Patient 2				Patient 3				Patient 4			
	SPT	IgE	SIC	OC	SPT	IgE	SIC	OC	SPT	IgE	SIC	OC	SPT	IgE	SIC	OC
Whole egg (5% w/v)	4	ND			7	ND			6	ND			10	ND		
Egg white (5% w/v)	4	0.60		+ (OAS)	7	51.3		+ (OAS)	5	4.51		–	8	1.26		+ (OAS)
Egg yolk (5% w/v)	0	<0.35		–	5	6.0		–	0	0.99		–	3	<0.35		–
Ovalbumin (10 mg/ml)	0	<0.35			6	29.2	EAR		5	5.3	EAR		2	0.45		
Ovomucoid (10 mg/ml)	0	0.38			0	0.95			6	<0.35	EAR		0	<0.35		
Lysozyme (10 mg/ml)	10	0.76	EAR		5	<0.35	EAR		6	1.66	EAR		6	<0.35	EAR	
Conalbumin (10 mg/ml)	0	<0.35			0	<0.35			0	<0.35			0	0.63		

SPT (mm weal); IgE (kU/l); SIC: EAR, early asthmatic response; DBPCOC: OAS, oral allergy syndrome; ND, not done.

a similar fall between 2 and 24 h after challenge was considered a late reaction if no change was observed during the control day. PC₂₀ allergen was calculated as described above. The subjects were told to refrain from taking inhaled bronchodilators for at least 12 h before inhalation testing and the dose of inhaled corticosteroids was taken the night before bronchial inhalation test. The tests were undertaken when the patients were absent from work (for at least 48 h). SICs with different allergenic extracts were performed at least 1 week apart.

Oral challenge tests

Double-blind, placebo-controlled, oral challenge (DBPCOC) tests were carried out with raw egg white and egg yolk after obtaining written informed consent. Increasing doses of a regular raw egg white and egg yolk or placebo (milkshake with vanilla) were given at 30-min intervals. PEF and FEV₁ measurements were done 5, 15 and 30 min after challenge with the computerized flowmeter described above. Oral challenge test was discontinued when the subject experienced symptoms or when the highest amount of raw egg white or egg yolk had been given (a regular hen's egg).

Results

Skin tests

Subjects 1, 2 and 4 had positive SPT to common inhalant allergens. All the subjects showed positive SPT to wheat, rye and barley flour, as well as to egg white and lysozyme, two subjects reacted to ovalbumin and one to ovomucoid. In addition, subjects 1 and 2 were sensitized to alpha-amylase. Skin test results with egg extracts are shown in Table 2. Skin prick tests to these egg proteins in 10 atopic nonexposed subjects elicited no reaction.

Serum IgE determinations

Total serum IgE levels were elevated in all the patients (Table 1). Specific IgE to wheat flour was positive in three subjects. Specific IgE to egg white was positive in all the patients, three subjects had positive IgE to ovalbumin, two subjects to lysozyme, two subjects to ovomucoid and two to egg yolk. Results of specific IgE determinations against egg proteins are shown in Table 2.

Table 3. Results of inhalation challenge tests

Patient no.	Methacholine inhalation test	Specific inhalation challenge			
	PC ₂₀ (mg/ml)	PC ₂₀ ovalbumin (mg/ml)	PC ₂₀ ovomucoid (mg/ml)	PC ₂₀ lysozyme (mg/ml)	
1	0.25	ND	ND	0.020	
2	0.29	0.015	ND	0.260	
3	0.12	0.019	0.001	0.004	
4	0.79	ND	ND	0.031	

ND, not done.

Inhalation challenge tests

All patients had bronchial hyperresponsiveness as measured by methacholine inhalation test.

SIC with ovomucoid in patient 3 elicited an isolated early asthmatic reaction, with a fall in FEV₁ of 20%. SIC with ovalbumin in patients 2 and 3 elicited isolated early asthmatic reactions in both patients, with maximum falls in FEV₁ of 25 and 21%, respectively. SIC with lysozyme elicited early asthmatic reactions in all patients, with drops in FEV₁ that ranged between 20 and 30%. No late asthmatic reactions were observed.

The results of inhalation challenge tests are shown in Tables 2 and 3, and Fig. 1.

Oral challenge tests

Three subjects experienced oral allergy syndrome within 15 min after eating raw egg white, and in two cases they also had abdominal pain and nausea. All subjects could eat egg yolk without any ill effect. The results of oral challenge tests are shown in Table 2.

Discussion

Baker's asthma is still a frequent occupational respiratory disease (17). This disorder has been classically considered a form of allergic asthma mediated by IgE antibodies

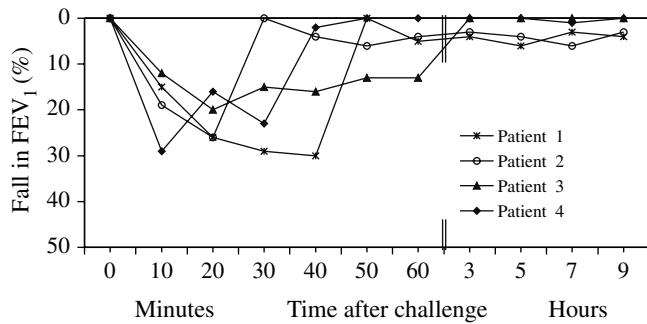


Figure 1. SIC with lysozyme in the four bakery workers.

specific to cereal flour antigens (18). However, the list of causative agents of baker's asthma has been expanded with the demonstration that flours from different sources (soybean, buckwheat), enzymes, egg proteins and organic contaminants such as storage mites, fungi and insects are also capable of causing IgE-mediated occupational asthma (19).

Type I hypersensitivity to hen's egg white proteins is a common cause of food allergy that usually affects atopic children. Moreover, aerosolized egg white proteins may also act as aeroallergens giving rise to rhino-conjunctivitis and asthma symptoms that are usually work-related. Liquid and aerosolized hen's eggs are commonly used in the baking and confectionery industries. Boeniger et al. (20) evaluated exposure to airborne egg protein concentrations in an egg breaking and processing plant that had cases of occupational asthma. They analysed personal air samples for egg protein. The highest concentrations were found in the washing room and breaking room, which were also the areas where the risk of being sensitized was the greatest (20). Smith et al. (6) examined the workers from two different egg-processing plants and, among the 86 workers in whom skin tests were performed, there were 29 (34%) with cutaneous sensitization to one or more egg allergens. Of these workers with positive skin tests to at least one egg allergen, 55% had a positive egg-specific IgE determination. Sixteen out of the 29 workers (55%) with cutaneous sensitization to egg had criteria for diagnosis of occupational asthma (6).

Specific conjunctival and inhalation challenges have confirmed that aerosolized egg proteins may give rise to respiratory allergy and occupational asthma (7–13). Bronchial inhalation challenges with purified egg proteins have been performed with lysozyme (8, 10, 11) and chicken serum albumin (12, 21). Respiratory allergy to lysozyme was confirmed by inhalation challenges in one

employee at a pharmaceutical company (8), in a cheese worker (10) and in a homemaker who had rhinoconjunctivitis upon beating or breaking eggs at home (12). The role of these egg proteins, however, has not been previously documented among bakery or confectionery workers using SICs with purified egg proteins. In our patients, we could demonstrate by means of skin tests, IgE determinations and SIC that egg white lysozyme (in all subjects), ovalbumin (in two subjects) and ovomucoid (in one subject) may give rise to occupational asthma among these bakery and confectionery workers through an IgE-dependent mechanism. In fact, these proteins appear to be potent aeroallergens that may induce near-fatal asthma attacks in sensitized subjects, as it happened to subjects 3 and 4.

The association between respiratory allergy and food hypersensitivity is increasingly being reported (22). In the last years, a relationship between food allergy to egg yolk and inhalational type I hypersensitivity to bird antigens has been described, which is named as 'bird-egg syndrome' (21–25). More recently, an association has been reported between respiratory allergy caused by egg white proteins and food allergy to raw egg (12, 13), which has been referred as the 'egg-egg syndrome' (13). However, food allergy to egg in the aforementioned study was not demonstrated by oral challenge tests. In our study, two subjects had oral allergy symptoms upon ingestion of raw or undercook egg. The food allergy symptoms developed several months after the onset of the respiratory manifestations induced by liquid egg aerosols. We demonstrated by a DBPCOC with raw egg white and egg yolk that three subjects had clinical allergy to egg white but not to egg yolk. However, subject 1 was not aware of his food allergy to egg white, possibly because he did not consume raw or undercook egg. It has been previously reported that some sensitized patients can tolerate well cooked, but not raw eggs (26).

In conclusion, egg proteins may act not only as food allergens but also as inhalant allergens that induce IgE-mediated asthma among bakery and confectionery workers who are exposed to egg aerosols, and therefore should be considered in the diagnosis of baker's asthma. Poly-sensitization to different baking ingredients seems to be common among symptomatic bakers. This is possibly due to the fact that the initial IgE sensitization, either to cereal antigens or egg proteins, might have facilitated further atopic sensitizations to other allergens found in the work environment, as a consequence of the ongoing inflammatory reaction in the airways.

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