

Inhalant allergy to egg yolk and egg white proteins

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Summary

Background Several egg white and egg yolk and avian proteins have been described as a cause of inhalant allergy. Sometimes inhalational type I hypersensitivity to these proteins is associated with food allergy to egg.

Objective We studied two patients who experienced respiratory and food allergic symptoms upon exposure to egg or avian antigens through the inhalative or digestive routes. Clinical and immunological studies were carried out in order to identify individual allergens from these sources that could be responsible for crossreactivity reactions.

Results Patient 1 showed IgE sensitization to egg yolk livetins, feathers, and chicken serum. Specific bronchial challenge with chicken albumin and livetin extracts elicited a positive early asthmatic response and an increase in serum eosinophil cationic protein. Immunoblot and CAP-inhibition studies in this patient supported that chicken albumin (α -livetins) was the crossreactive antigen present in egg yolk and chicken serum and feathers. Patient 2 showed sensitization to egg white, ovomucoid and lysozyme. However, SDS-PAGE and immunoblot studies demonstrated contaminating lysozyme in the ovomucoid extract and identified lysozyme as the main allergen causing egg sensitization in this patient. Conjunctival challenge test confirmed allergy to lysozyme.

Conclusion Egg yolk and egg white proteins may act not only as ingested allergens but also as aeroallergens. Immunological studies using highly purified preparations of egg proteins are useful for the accurate diagnosis of allergic reactions to egg proteins and to identify individual allergens that may be responsible for crossreactivity reactions.

Keywords: egg allergy, bird–egg syndrome, respiratory hypersensitivity, lysozyme, chicken albumin

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Introduction

The association between respiratory allergy and food hypersensitivity caused by different allergens is increasingly being reported [1]. In the last years, a relationship between inhalational type I hypersensitivity to bird antigens and food allergy to egg has been described [2–6]. Egg yolk livetins have been found to be the crossreacting allergen responsible for the association between sensitization to bird antigens and egg allergy [4].

Several avian and egg proteins have also been described as a cause on inhalant allergy, usually in the workplace. Although hypersensitivity pneumonitis is the most

commonly reported presentation [7,8], asthmatic responses have also been described [9]. Occupational asthma due to inhalation of egg-white aerosols has been reported in bakers [10], confectionery workers [11–13] and among egg processing workers [14,15]. Moreover, poultry mites [16], feed additives such as spiramycin [17], and chicken feathers [18] have been implicated as possible causal agents of occupational asthma in poultry workers.

We describe in this article two patients who experienced respiratory and food allergic symptoms upon exposure to egg or avian antigens or both. Immunological studies revealed sensitization to individual allergens from these sources. Our study further underlines the importance of using purified allergens in the diagnosis of allergy to egg proteins in order to identify individual allergens that may be responsible for crossreactivity reactions.

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Materials and methods

Patients

Patient one A 27-year-old Caucasian woman, a non-smoker, had suffered from allergic rhinoconjunctivitis and asthma symptoms caused by pollen and cat dander during the last 10 years. When she was 17-year-old she developed oropharyngeal itching, hoarseness, cough and wheezy dyspnoea shortly after eating eggs and egg-containing foods, such as mayonnaise, ice creams, etc. She also experienced pruritic weals on her hands and face shortly after handling raw chicken meat. Ingestion or manipulation of cooked chicken, however, produced no symptoms. Moreover she suffered from severe asthma attacks when sleeping in a room where her parents kept a parrot as a house pet. She noticed that symptoms of egg allergy started within a few weeks after acquiring the parrot and she had never before had any symptoms of hypersensitivity to food. When she moved to a different dwelling, where no birds were kept, asthma symptoms subsided but she still had wheezy dyspnoea when visiting her parent's home. Physical examination, spirometry and chest and sinus X-rays at the time of consultation were within normal limits. Blood differential count showed 480 eosinophils/mm³.

Patient two A 36-year-old Caucasian woman, a non-smoker, had a history of seasonal allergic rhinoconjunctivitis due to grass pollen. In the last year she experienced severe ocular and nasal itching, sneezing, runny nose, nasal blockage and watery eyes upon beating eggs or breaking eggs at home. In addition, she presented oropharyngeal itching, swollen throat, as well as dyspnoea within a few minutes after eating home-made mayonnaise. However, she could eat cooked eggs without any ill effect. The patient showed no symptoms when handling raw poultry or after eating chicken. Clinical examination, chest and sinus X-rays and spirometry were within normal limits. Blood test showed 200 eosinophils/mm³.

Allergen extracts

Livetins were prepared from fresh hen's egg yolk as previously described [11], lyophilized and reconstituted at a final concentration of 10% w/v in phosphate buffered saline (PBS). The protein concentration of this extract estimated by Bio-Rad protein assay (Bio-Rad, Munich, Germany) was 22 mg/mL.

Moreover, ovomucoid (T-2011), lysozyme (L-6876) and chicken albumin (fraction V powder, A-3014) were supplied by Sigma Chemical Co. (St Louis, Mo, USA) and prepared at a concentration (dry weight) of 10 mg/mL in PBS. All these extracts were passed through a 22 µm

millipore filter (Millipore Corp., Bedford, MA, USA) for sterilization.

Skin tests

Skin tests were performed with the skin-prick method. A battery of common inhalant allergens was tested, including *Dermatophagoides pteronyssinus*, *Alternaria tenuis*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Penicillium notatum*, mixed grass and *Olea europaea* pollen, dog and cat dander (ALK-Abelló, Madrid, Spain). Commercially available extracts of whole egg (Bencard, Worthing, UK), egg white, egg yolk, ovalbumin, feathers mix (chicken and duck) (ALK-Abelló, Madrid, Spain) and chicken serum (Leti, Madrid, Spain) as well as purified ovomucoid, lysozyme, and chicken albumin (Sigma Chemical Co.) and raw chicken meat were also tested. In addition, skin end-point titration with livetin and chicken albumin extracts was done by testing decreasing concentrations of the extracts using the skin-prick method.

Histamine phosphate at 10 mg/mL and normal saline were used as positive and negative controls, respectively. A weal with a mean diameter 3 mm or greater with erythema, occurring 15 min after testing, was defined as a positive reaction, being negative, at the same time, the skin-prick test with the saline.

Skin-prick tests with the aforementioned egg and avian extracts were also performed in 10 atopic subjects (six with asthma and four with rhinitis) and 10 non-atopic healthy subjects. All atopic subjects had positive skin tests to grass pollen and five of them to *Olea europaea* pollen as well. Total serum IgE (mean ± SD) was 351.3 ± 194 kU/L in atopic subjects and 30 ± 32.2 kU/L in non-atopic subjects.

Bronchial provocation tests

Methacholine inhalation test This test was performed according to Cockcroft [19] with some modifications. The aerosolized particles were generated by a continuous pressurised 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 second (PC₂₀) and it was determined by interpolation of the last two concentrations.

Specific bronchial challenge This test was carried out in patient 1 with livetin and chicken albumin extracts in different days, following the method previously described [20]. The patient inhaled the aerosolised allergen using the nebulizer method mentioned above in progressive concentrations at tidal breathing for 2 min. A control challenge with PBS was carried out before antigen provocation.

Increasing concentrations of livetin or chicken albumin extract were given by inhalation starting with a concentration that induced a 2–3 mm weal on skin-prick testing (1:10240 w/v and 0.0019 mg/mL, respectively). The dose was increased in twofold increments at intervals of 10 minutes and FEV₁ and FVC were measured at 5 and 10 min after inhalation of each concentration. Bronchial 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 had been given. At the end of the inhalation challenge test, spirometry was performed at 20, 30, 40 and 60 min after challenge, and again the following day. Peak expiratory flow rate (PEF) was measured before and every hour after bronchial challenge until bedtime, and again the day after. A fall in FEV₁ of 20% or more from the lowest postsaline within 60 min of challenge was considered a positive immediate reaction while a fall in PEF greater than 25% between 2 and 24 h after challenge was considered a positive late reaction if no change was observed during the control day.

Conjunctival challenge test

Conjunctival challenge test in patient 2 was performed as described by Möller *et al.* [21] with ovomucoid and lysozyme extracts. Two atopic patients were also tested as control subjects.

Eosinophil cationic protein measurement

Eosinophil cationic protein in patient's serum was determined by Pharmacia CAP System ECP FEIA that was kindly supplied by Pharmacia Diagnostics AB (Uppsala, Sweden). Eosinophil cationic protein was measured immediately before bronchial challenge with livetin extract and 24 h after specific bronchial challenge. Parameters such as blood collection, coagulation time and temperature were kept within specified limits following manufacturer's instructions. ECP was released in the blood collection tube by clotting for 60 min at room temperature (22 °C), centrifuged at 1000 *g*, and then the standard CAP ECP fluoroimmunoassay was performed. Eosinophil cationic protein is present in eosinophil granules and it is secreted upon stimulation. Determination of released eosinophil cationic protein may be used to monitor conditions involving eosinophil activation such as asthma [22].

Total and specific IgE determination

Total serum IgE was measured by Pharmacia CAP System IgE FEIA (Uppsala, Sweden) according to the manufacturer's instructions. Specific IgE against egg white, egg yolk, ovalbumin, ovomucoid, lysozyme and chicken meat

were determined by Pharmacia CAP System RAST FEIA. To detect specific IgE antibodies against livetins, the livetin extract was coupled to nitrocellulose disks as previously described [23] and the standard RAST method was followed using reagents from Pharmacia and the results expressed in Phadebas RAST units (PRU/mL) as derived from the Phadebas RAST reference system.

CAP inhibition assays

CAP inhibition studies were carried out to investigate a possible crossreactivity between egg and avian proteins. Twenty microlitres of three progressive 10-fold dilutions of the inhibitor allergens in PBS and 20 µL of PBS as negative control were preincubated with 50 µL of patient's serum for 15 min and then the assay was continued following the standard CAP technique.

The inhibition of CAP to egg yolk with serum from patient 1 was determined using the following liquid-phase allergens: livetins, chicken serum, chicken albumin, feathers, egg yolk and ovomucoid. Inhibition of CAP to egg white with serum from patient 2 was investigated with ovomucoid, lysozyme, feathers, egg yolk and egg white as liquid-phase allergens. Because of the possible contamination of the ovomucoid extract with lysozyme (as observed in SDS-PAGE), we also assessed whether lysozyme could inhibit CAP to ovomucoid.

Results were expressed as the percentage inhibition of CAP obtained with the inhibitor allergen as compared with a control assay with PBS inhibitor.

Histamine release test

Histamine release tests were carried out with using an automated fluorometric method according to Siraganian [24]. Twenty-five microlitres of four fivefold dilutions (1:5, 1:25, 1:125, 1:625 w/v) of the extracts of egg yolk, egg white, livetins, feathers mix, ovalbumin, ovomucoid, and chicken serum were added. Before allergen challenge, blood samples were tested with four dilutions of equine antihuman IgE (Diagnostic Pasteur, France). These assays were also performed in two control subjects. The percentage of histamine release was calculated as follows:

$$\frac{(\text{allergen-induced histamine release} - \text{histamine release with diluent}) \times 100}{(\text{total histamine content} - \text{histamine release with diluent})}$$

A result greater than 10% release was considered to be positive [24].

SDS-PAGE and immunoblotting

Gel electrophoresis of egg and avian extracts was carried

out in 12% polyacrylamide gel (chicken serum, egg yolk, feathers, livetins, and ovomucoid 20–30 µg per minigel) or in a 15% polyacrylamide gel (chicken albumin, lysozyme and ovomucoid 20 µg) in reducing conditions according to Laemli *et al* [25] using a SE250 Mighty Small mini-Vertical Units (Hoefer Scientific Instruments, San Francisco, CA, USA). Broad molecular weight markers Multimark™ multi-coloured standard (Novex, San Diego, CA, USA) were used.

After running, one set of proteins was stained with Coomassie brilliant blue, and other set of separated proteins were transferred to Immobilon-P membranes (Millipore Corp., Bedford) following the method of Towbin [26]. Unreacted membrane sites were blocked with PBS containing 3% bovine serum albumin and 0.05% Tween 20 (PBS-T BSA). The membranes were then incubated overnight with patient's serum diluted 1/5 in PBS-T BSA. After three washings with PBS-T the membranes were incubated for 60 min at room temperature with monoclonal antihuman IgE alkaline phosphatase-conjugated (Binding Site Ltd, Birmingham, UK) diluted 1/500, washed and developed with bromochloroindolyl phosphate/nitro blue tetrazolium (Bio-Rad Lab., Hercules, CA., USA).

Results

Skin tests

Skin-prick tests with common inhalants were positive in both patients to grass and *O. europaea* pollens and to cat dander. In patient 1, skin-prick tests were positive with all dilutions of livetin extract from 1:10 w/v (15 mm weal with pseudopods) to 1:10 240 w/v (3 mm) and with chicken albumin extract from 10 mg/mL (15 mm weal with pseudopods) to 0.0019 mg/mL (3 mm). Positive skin responses were also observed to egg yolk and egg white, ovalbumin, feathers, chicken serum and raw chicken meat in this patient. Patient 2 showed positive responses only to egg white, ovomucoid and lysozyme. Results of skin tests are shown in Table 1.

Skin-prick tests to these avian-derived extracts in 10 control subjects were negative.

Bronchial provocation tests

Inhalation challenge with methacholine in patient 1 revealed bronchial hyperresponsiveness with a PC₂₀ of 0.9 mg/mL. Specific bronchial provocation test with livetin extract (1:10 240 w/v) and with chicken albumin extract (0.015 mg/mL) elicited an early asthmatic response, with a maximum fall in FEV₁ of 22% and 25% from baseline, respectively, without late asthmatic response (Fig. 1). Eosinophil cationic protein concentration in patient's serum before bronchial challenge was 3 µg/L and 24 h after inhalation

challenge with livetin extract increased up to 18.1 µg/L. Baseline ECP in atopic patients (control group) was 4.9 ± 5.1 µg/L. Bronchial provocation test was negative in two control non-atopic subjects with both antigens and no significant changes in serum ECP were observed after inhalation challenge in these patients.

Conjunctival challenge test

Conjunctival provocation in patient 2 with ovomucoid extract yielded a doubtful result (mild itching and congestion) at the maximum concentration of 10 mg/mL. However, exposure of the conjunctiva to lysozyme extract elicited a strong response (intense itching and redness, tearing, followed by sneezing and nasal discharge) at a concentration of 1 mg/mL.

Total and specific IgE determination

Total serum IgE was 1693 kU/L in patient 1 and 90 kU/L in patient 2. Results of specific IgE to egg and avian proteins are shown in Table 1. RAST against livetins was positive in patient 1 and negative in patient 2 and in two control subjects.

CAP inhibition assays

The CAP to egg yolk using serum from patient 1 could be inhibited in a dose-dependent manner up to 95–98% with chicken serum, chicken albumin, livetins, feathers, and egg yolk as liquid-phase allergens (Fig. 2). On the contrary, CAP to egg yolk could not be inhibited with ovomucoid. With serum from patient 2, the CAP to egg white was inhibited up to 80% with ovomucoid and lysozyme as liquid-phase allergen, while the maximum inhibition obtained with egg white was of 43% and no significant inhibition was observed with feathers and egg yolk extracts (Fig. 3). Interestingly, the CAP to ovomucoid in this patient could be inhibited up to 88% with lysozyme extract and repeated experiments showed that this inhibition was reproducible.

Histamine release test

This test was strongly positive in patient 1 with the extracts of livetins (44% maximum histamine release), feathers (36%), egg yolk (35%) and chicken serum (28%) and it was negative with egg white, ovalbumin and ovomucoid. This test was not conclusive in patient 2 since no histamine release was obtained with anti-IgE. No response with these extracts was observed in two control subjects.

Table 1. Results of skin-prick tests (SPT), specific IgE determinations (CAP/RAST), and specific conjunctival provocation test (CPT) and bronchial provocation test (BPT)

	Patient 1			Patient 2		
	SPT (mm weal)	CAP/RAST* (kU/L)	BPT (concentration)	SPT (mm weal)	CAP/RAST* (kU/L)	CPT (concentration)
Whole egg 6% w/v	10 × 10 ps	ND		0	ND	
Egg white 5% w/v	7 × 7	23.1		3 × 3	1.64	
Egg yolk 5% w/v	15 × 10 ps	44.7		0	< 0.35	
Ovalbumin 10 mg/mL	4 × 4	< 0.35		0	< 0.35	
Ovomucoid 10 mg/mL	0	< 0.35		5 × 5	2.11	Doubtful (10 mg/mL)
Lysozyme 10 mg/mL	0	< 0.35		10 × 8	1.81	Positive (1 mg/mL)
Livetins 10% w/v	15 × 12 ps	1.65*	Immediate response (1:10 240 w/v)	0	< 0.35*	
Feathers mix. 5% w/v	7 × 7 ps	ND		0	ND	
Chicken serum 2 × 10 ⁵ PNU/mL	9 × 9 ps	ND		0	ND	
Chicken albumin 10 mg/mL	15 × 14 ps	ND	Immediate response (0.015 mg/mL)	0	ND	
Chicken meat as is (raw)	6 × 6 ps	39.6		0	< 0.35	

ND, not done; ps, pseudopods; * RAST expressed in PRU/mL

SDS-PAGE and immunoblotting

SDS-PAGE of egg and avian proteins is shown in Fig. 4. Ovomucoid was seen as a wide diffuse band from 36 to 50 kDa. In addition to this expected band corresponding to ovomucoid, a contaminating protein at 14–16 kDa was seen as a weak band (Fig. 4B, lane 7). Immunoblots with serum from patient 1 showed IgE-binding bands of molecular weight (mol.wt.) between 60 and 80 kDa in the extracts of chicken serum, egg yolk, feathers, and livetins (Fig. 5, lanes 1–4) while no IgE reactivity to ovomucoid extract was observed (lane 5). IgE binding to a band with an apparent mol.wt. of 70 kDa was observed in egg yolk, livetins and avian extracts. Serum from patient 2 did not recognize any of these proteins (Fig. 5, lanes 6–9), but this patient showed

IgE-binding to a 14 kDa protein present in the ovomucoid extract (Fig. 5, lane 10).

Serum from patient 1 reacted with chicken albumin, that was seen as a sharp narrow band at 70 kDa (Fig. 6, lane 1), while serum from patient 2 reacted to lysozyme at 14 kDa (Fig. 6, lane 4). IgE-binding to lysozyme could be inhibited by preincubation of serum from patient 2 with lysozyme extract at 100 µg/mL (Fig. 6, lane 5).

Discussion

We report two patients with sensitization to egg-derived proteins in whom respiratory allergy symptoms developed on exposure to these antigens through the inhalative route. Moreover they developed food allergy symptoms of differ-

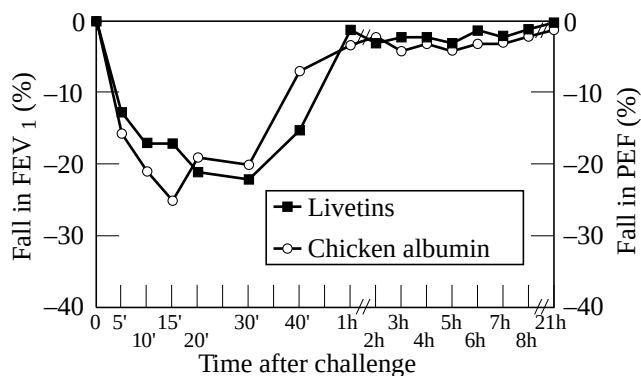


Fig. 1. Early asthmatic responses after bronchial provocation test with livetin extract (1:10 240 w/v) and chicken albumin (0.015 mg/mL). A significant increase in serum ECP was observed 24 h after challenge with livetin extract.

ent severity after ingestion of egg-containing foodstuffs. Immunological studies showed that the symptoms reported were due to IgE-mediated sensitization to individual allergens present in egg yolk/avian extracts or egg white, that could explain the clinical pictures described.

Maat-Bleeker *et al* [2], first reported the association of hypersensitivity to ingested egg yolk and rhinitis and asthma caused by exposure to a parrot in an older woman. On the basis of clinical and serological investigations they suggested that food allergy to egg yolk was induced by respiratory sensitization to avian serum proteins. Thereafter, a patient with occupational exposure to birds who developed symptomatic respiratory allergy to bird proteins and exquisite food allergy to egg yolk was reported [3]. Serological studies in the latter patient demonstrated that hypersensitivity to egg yolk was a result of avian serum sensitization.

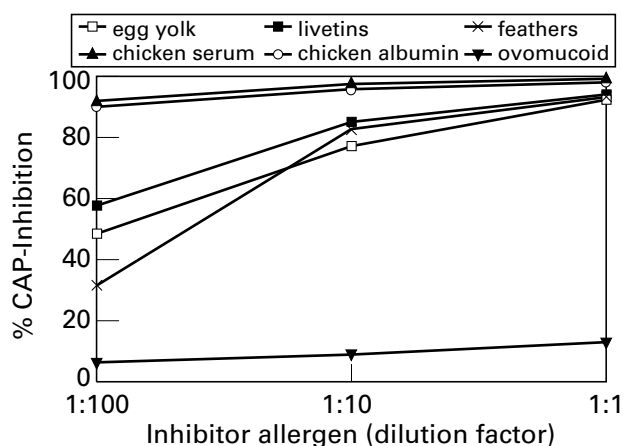


Fig. 2. Inhibition of IgE binding (CAP) to egg yolk with serum from patient 1 using livetins, feathers, egg yolk, chicken serum, chicken albumin and ovomucoid as liquid-phase inhibitor allergens.

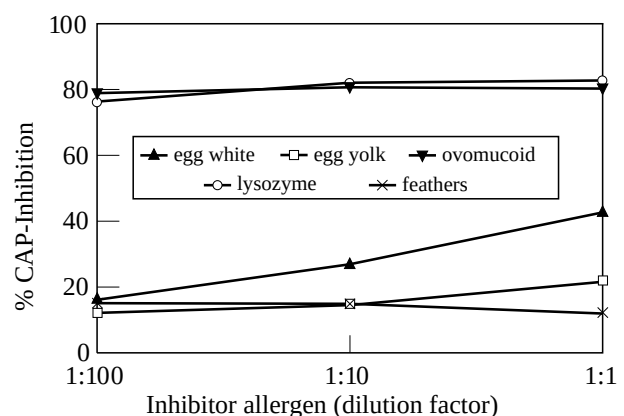


Fig. 3. Inhibition of IgE binding (CAP) to egg white with serum from patient 2 using egg white, egg yolk, ovomucoid, feathers and lysozyme as liquid-phase inhibitor allergens.

Mandallaz *et al.* [4] demonstrated by RAST-inhibition studies that livetins, the water-soluble fractions of egg yolk proteins, were the major cross-reacting antigen found in bird feathers and egg yolk and they coined the 'bird-egg syndrome' to designate this IgE-mediated association of inhalant and food allergy. Extensive crossreactivity among serum antigens from different avian species has also been demonstrated [4,27]. Burley and Vadehra [28] described five fractions in soluble egg yolk proteins by chromatography: α -livetins (70 kDa), β -livetins (42 kDa), δ -livetins (150 kDa), γ -livetins (200 kDa), and apovitelin II (20 kDa). Willians [29] identified α -livetins as chicken serum albumin, a protein with a mol.wt. of 60–70 kDa.

De Blay *et al.* [30] reported a patient who showed IgE-binding to a 66 kDa band in egg yolk and hen serum and feathers, and it was recognized as α -livetins. Recently, Szépfalusi *et al.* [6] have demonstrated, with the use of the immunoblot technique, the presence of common

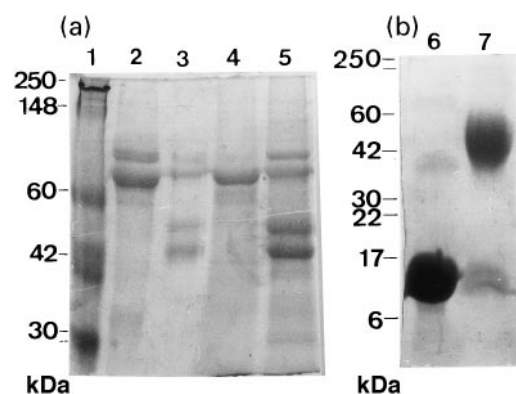


Fig. 4. SDS-PAGE of egg and avian proteins: chicken serum (lane 2), egg yolk (lane 3), feathers (lane 4), livetins (lane 5), lysozyme (lane 6), ovomucoid (lane 7). Molecular weight markers (lane 1).

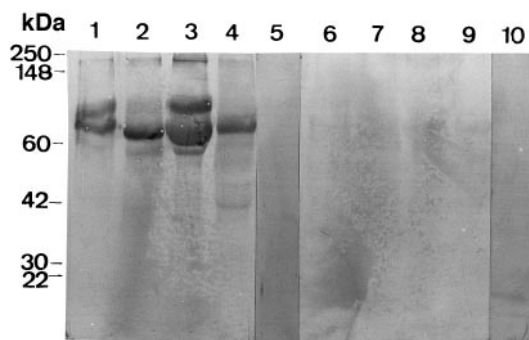


Fig. 5. IgE immunoblots using serum from patient 1 (lanes 1–5) and from patient 2 (lanes 6–10) with chicken serum (lanes 1, 6), egg yolk (lanes 2, 7), feathers (lanes 3, 8), livetins (lanes 4, 9) and ovomucoid (lanes 5, 10).

epitopes of budgerigar and hen feather and egg yolk α -livetins. They pointed out that chicken serum albumin (α -livetins) was the crossreactive protein responsible for the bird–egg syndrome. However, the implication of livetin or chicken serum albumin in this syndrome has not been demonstrated by specific bronchial challenge until now.

Our results of immunoblot and inhibition assays in patient 1 are in keeping with the previous reports and support that chicken albumin (α -livetins) is the crossreactive antigen present in egg yolk and chicken serum and feathers. We further demonstrated by means of specific bronchial challenge test that chicken albumin may act as an inhalant allergen, after being processed by the respiratory tract antigen-presenting cells, and then may induce asthmatic responses. The significant rise in eosinophil cationic protein in patient's serum after specific inhalation challenge with livetins provides evidence of eosinophil activation and release of their granule products.

Patients with bird–egg syndrome are mainly female adults, in opposition to egg white allergy that affects mainly children

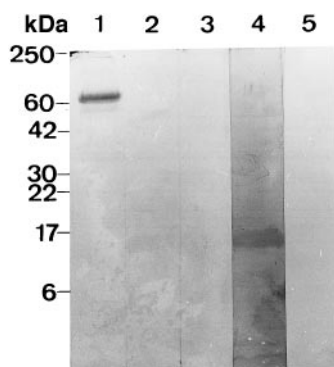


Fig. 6. IgE immunoblots using serum from patient 1 (lanes 1–2) and from patient 2 (lanes 3–5) with chicken albumin (lanes 1,3) and lysozyme (lanes 2,4). Lane 5: inhibition of IgE binding to lysozyme after preincubation of patient's serum with lysozyme.

without any sex predisposition [6]. Patients with this syndrome have a predominance of respiratory and digestive symptoms, and a longer duration of egg allergy compared with egg-allergic patients without bird sensitization [31]. The route of sensitization in bird–egg syndrome seems to be primarily respiratory, with ensuing food allergy to egg by cross-sensitization. However, a previous sensitization to egg proteins could also predispose some patients to respiratory symptoms from birds [32].

Allergy to hen's egg white usually affects atopic children, who tend to outgrow this particular food allergy. The major allergens reside in egg white fraction, i.e. mainly ovomucoid (Gal d 1), ovalbumin (Gal d 2) and conalbumin (Gal d 3) [33]. Lysozyme (Gal d 4) has also been found to be a potent allergen for some patients and prevalence of anti-lysozyme IgE in an egg-allergic population was found to be 35% [34]. Occupational asthma due to egg white aerosols has also been reported and the main egg-derived aeroallergens are ovalbumin, ovomucoid, conalbumin and lysozyme [10–15]. Bernstein *et al.* [35] reported a patient employed in a company which manufactured egg white-derived lysozyme who developed lysozyme-induced asthma.

The commercial egg protein preparations have been shown to contain large amounts of contaminating proteins varying from 1% to 5%. It has been previously shown that 'purified' ovomucoid (Sigma T-2011) contains contaminating proteins that are able to bind specific IgE and one of these proteins has been identified as lysozyme [36]. In patient 2, using methods that do not separate the proteins such as specific IgE determination by CAP, we suspected allergy to ovomucoid. However, SDS-PAGE and immunoblotting was useful to reveal the problem of contaminating lysozyme in the ovomucoid extract and lack of IgE binding to ovomucoid itself. On the basis of skin tests, specific IgE and immunoblot results we identified lysozyme as the main allergen causing egg sensitization in this patient. In fact, CAP to ovomucoid could be nearly completely inhibited by lysozyme extract and conjunctival challenge test with lysozyme was strongly positive. Since individual egg proteins (i.e. lysozyme) are present as hidden allergens or additives in several foods and drugs [34] the need to use highly purified preparations of these proteins for the correct diagnosis of allergic reactions and avoidance of allergen should be emphasized.

References

- 1 Pauli G, de Blay F, Bessot JC, Dietmann A. The association between respiratory allergies and food hypersensitivities. *Allergy Clin Immunol News* 1992; 4:43–7.
- 2 Maat-Bleeker F, Van Dijk AG, Berrens L. Allergy to egg yolk possibly induced by sensitization to bird serum antigens. *Ann Allergy* 1985; 54:245–8.

- 3 Hoffman DR, Guenther DM. Occupational allergy to avian proteins presenting as allergy to ingestion egg yolk. *J Allergy Clin Immunol* 1988; 81:484–8.
- 4 Mandallaz MM, de Weck AL, Dahinden CA. Bird–egg syndrome. Cross-reactivity between bird antigens and egg-yolk livetins in IgE-mediated hypersensitivity. *Int Arch Allergy Appl Immunol* 1988; 87:143–50.
- 5 Añibarro B, Martín-Esteban M, Martínez F, Pascual C, Ojeda JA. Egg protein sensitization in patients with bird feather allergy. *Allergy* 1991; 46:614–8.
- 6 Szépfalusi Z, Ebner C, Pandjaitan R, Orlicek F et al. Egg yolk α -livetins (chicken serum albumin) is a cross-reactive allergen in the bird–egg syndrome. *J Allergy Clin Immunol* 1994; 93:932–42.
- 7 Korn DS, Florman AL, Gribetz I. Recurrent pneumonitis with hypersensitivity pneumonitis to hen litter. *JAMA* 1968; 205:114.
- 8 Berrens L, Verschuren M, Van Dijk AG, Guikers CL. Antibodies against hen's egg proteins in pigeon breeder's disease. *Clin Allergy* 1986; 16:355–63.
- 9 Hargreave FE, Pepys J. Allergic respiratory reactions in bird fanciers provoked by allergen inhalation provocation tests. *J Allergy Clin Immunol* 1972; 50:157.
- 10 Smith AB, Bernstein DI, Aw TC et al. Occupational asthma from inhaled egg protein. *Am J Ind Med* 1987; 12:205–18.
- 11 Berrens L, Edwards JH. Antibodies to bird serum proteins in confectionery workers exposed to egg spray. *Clin Allergy* 1987; 17:405–8.
- 12 Blanco JG, Juste S, Garcés M, Rodríguez P. Occupational asthma in the confectionery industry caused by sensitivity to egg. *Allergy* 1992; 47:190–1.
- 13 Valero A, Lluch M, Amat P, Serra E, Malet A. Occupational allergy in confectionery workers. *Allergy* 1996; 51:588–9.
- 14 Bernstein DI, Smith AB, Moller DR et al. Clinical and immunologic studies among egg-processing workers with occupational asthma. *J Allergy Clin Immunol* 1987; 80:791–7.
- 15 Smith AB, Bernstein DI, London MA et al. Evaluation of occupational asthma from airborne egg protein exposure in multiple settings. *Chest* 1990; 98:398–404.
- 16 Lutsky I, Teichtahl H, Bar-Sela S. Occupational asthma due to poultry mites. *J Allergy Clin Immunol* 1984; 73:56–60.
- 17 Paggiaro PL, Loi AM, Toma G. Bronchial asthma and dermatitis due to spiramycin in a chick breeder. *Clin Allergy* 1979; 9:571–4.
- 18 Perfetti L, Cartier A, Malo JL. Occupational asthma in poultry-slaughterhouse workers. *Allergy* 1997; 52:594–5.
- 19 Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: A method and clinical survey. *Clin Allergy* 1977; 7:235–43.
- 20 Chan-Yeung M, Kinsella M, Ostrow DN. Specific broncho-provocation testing. *Clin Rev Allergy* 1990; 8:147–57.
- 21 Möller C, Björkstén B, Nilsson G, Dreborg S. The precision of conjunctival provocation test. *Allergy* 1984; 39:37–41.
- 22 Venge P. Serum measurement of eosinophil cationic protein in bronchial asthma. *Clin Exp Allergy* 1993; 23 (Suppl. 2):3–7.
- 23 de la Hoz B, Fernández-Rivas M, Quirce S et al. Swiss chard hypersensitivity: clinical and immunologic study. *Ann Allergy* 1991; 67:487–92.
- 24 Siraganian RP. Histamine release and assay methods for the study of human allergy. In: Rose NR, Friedman H, eds *Manual of clinical immunology*. American Society for Microbiology. Washington D.C. 1976: 603–15.
- 25 Laemli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227:680–5.
- 26 Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci USA* 1979; 76:4350.
- 27 Baxendale W, Courtenay JS, Phillips AW, Zola H. Some properties of pigeon and other avian serum proteins. *Int J Biochem* 1971; 2:419.
- 28 Burley RW, Vadehra DV. Chromatographic separation of the soluble proteins of hen's egg yolk: an analytical and preparative study. *Anal Biochem* 1979; 94:53–9.
- 29 Williams J. Serum proteins and the livetins in hen's-egg yolk. *Biochem J* 1962; 83:346–55.
- 30 De Blay F, Hoyet C, Candolfi E, Thierry R, Pauli G. Identification of α -livetins as a cross reacting allergen in a bird–egg syndrome. *Allergy Proc* 1994; 15:77–8.
- 31 Añibarro B, García-Ara MC, Martín-Esteban M et al. Peculiarities of egg allergy in children with bird protein sensitization. *Ann Allergy Asthma Immunol* 1997; 78:213–6.
- 32 Mandallaz M, Wyss M, Huwyler T, Wüthrich B. Bird-egg and egg–bird syndrome. *Allergologie* 1991; 7:275–8.
- 33 Hoffman DR. Immunochemical identification of the allergens in egg white. *J Allergy Clin Immunol* 1983; 71:481–6.
- 34 Frémont S, Kanny G, Nicolas JP, Moneret-Vautrin DA. Prevalence of lysozyme sensitization in an egg-allergic population. *Allergy* 1997; 52:224–8.
- 35 Bernstein JA, Kraut A, Bernstein DI et al. Occupational asthma induced by inhaled egg lysozyme. *Chest* 1993; 103:532–5.
- 36 Ebbehøj K, Dahl AM, Frøkiaer HN et al. Purification of egg-white allergens. *Allergy* 1995; 50:133–41.