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Letter to the Editor

Bird-egg syndrome induced by α -livetin sensitization in a budgerigar keeper: Successful induction of tolerance by avoiding exposure to avians

Dear Editor,

Hen's egg allergy is one of the most common childhood food allergies and symptoms often improve with age¹; however, adult-onset egg allergy is much less frequent.² In cases in children, the egg white is the dominant cause of hen's egg allergy. We herein report the first case of bird-egg syndrome induced by inhalational sensitization to an avian protein, α -livetin, which was successfully tolerated by avoiding contact with the protein.

A 50-year-old woman presented with several episodes of allergic reactions within 1 h after the ingestion of raw or incompletely cooked egg or chicken. At the age of 47, she had developed facial edema 30 min after ingesting a fried egg with a runny yolk. Her facial edema disappeared within 3 h. However, her symptoms were exacerbated after the ingestion of hen's egg. At the age of 50, she developed facial swelling, especially of the eyelids and lips, and generalized urticaria immediately after the ingestion of teriyakidon, a traditional Japanese dish consisting of broiled chicken, scrambled eggs, and steamed rice. Approximately 1 h after the ingestion of oyako-don, consisting of chicken cooked medium rare, hen's egg with a runny yolk, and steamed rice, she experienced itching of the palms and irritation and itching in her oral cavity, followed by facial edema, generalized urticaria, nasal obstruction, and cough. Consequently, she realized that she tolerated well-cooked egg, such as boiled egg, but not raw or partially cooked hen's egg and chicken. Several years before the onset of these food allergies she noticed that she developed itching of the eyes and nose, sneezing, nasal discharge, and cough whenever she cleaned the birdcage of her budgerigar (*Melopsittacus undulatus*), which she had taken care of for 15 years. The patient's medical history revealed allergic rhinitis and rice bran allergy.³ She tolerated steamed rice, but not rice bran.

To identify the causative allergen involved with allergic reactions after the ingestion of hen's egg and chicken meat, skin prick tests, IgE measurements, and open challenge tests were performed. Specific IgE measurements using the ImmunoCAP system (Thermo Fisher Scientific, MA, USA) showed positivity for egg yolk (2.41 UA/mL), egg white (2.07 UA/mL) and chicken meat (0.90 UA/mL), but not for ovomucoid (Table 1). In terms of inhalational allergens, ImmunoCAP results for budgerigar feathers (6.15 UA/mL) and budgerigar droppings (1.44 UA/mL) were positive. Skin prick testing (Torii Pharmaceutical, Tokyo, Japan) showed positivity for egg white (wheal

diameter = 6.9×5.4 mm) (positive control, 10 mg/mL histamine hydrochloride, wheal diameter = 10.6×5.6 mm; negative control, saline, wheal diameter = 0×0 mm). The prick tests with cooked eggs and uncooked eggs were not performed as the patient did not give consent for the tests. Oral challenge with a spoonful of raw hen's egg white induced discomfort of the tongue. Oral challenges with the entire egg white from one raw egg induced oropharyngeal itch. In addition, oral challenge with a spoonful of raw egg yolk induced strong itching and tingling in the oral cavity, including the tongue.

To investigate the causative allergen component of her egg allergy, an ImmunoCAP ISAC test, including specific IgE measurements against 4 hen's egg components, was performed (Table 1). The results showed positivity for serum albumin, α -livetin (Gal d 5), but not ovomucoid (Gal d 1), ovalbumin (Gal d 2), or conalbumin (Gal d 3).

To confirm cross-reactivity between egg yolk allergens and budgerigar feather allergens, we performed inhibition tests in IgE-immunoblotting (Supplementary Materials). The patient serum IgE was mainly bound to an egg yolk protein band at 69 kDa, which corresponded to the molecular weight of Gal d 5 (Fig. 1). Immunoblotting with the budgerigar feather extract showed the main band at 70 kDa, which corresponded to the molecular weight of the budgerigar serum albumin.⁴ In IgE-immunoblotting, the IgE binding to the 70 kDa protein of the budgerigar feather extract was inhibited by the preincubation of the patient's serum with the egg yolk extract, indicating that there was cross-reactivity between egg yolk Gal d 5 and budgerigar feather containing albumin. However, the other IgE-binding protein band of the budgerigar feather extract at 90 kDa was inhibited by the preincubation of the patient's serum with the egg yolk extract. Therefore, the allergens apart from serum albumin might be also involved in the cross-reactivity between the egg yolk and the budgerigar feather.

The patient was diagnosed with bird-egg syndrome by Gal d 5 sensitization via the respiratory tract while taking care of a pet budgerigar. After diagnosis, she avoided raw or partially cooked hen's egg and chicken cooked medium rare. She also avoided cleaning the bird cage, and her budgerigar passed away 2 years later. Interestingly, specific IgE levels against egg yolk and white and Gal d 5 gradually have been decreasing and reached normal limits 7 years after the diagnosis (Table 1). The challenge with raw hen's egg was negative and its avoidance was stopped. Thereafter she has eaten hen's egg and chicken meat indiscriminately, without any symptoms.

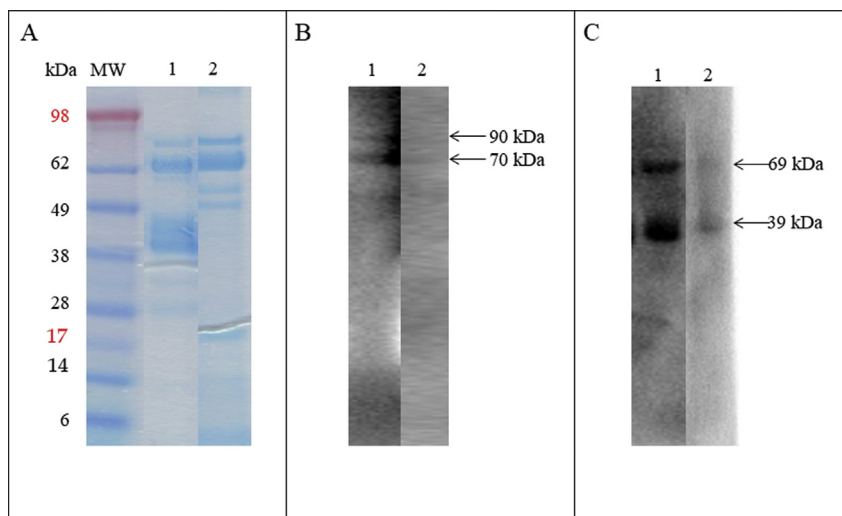
Bird-egg syndrome is mainly seen in adults who become sensitized to airborne avian allergens through exposure to feathers,

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Table 1
The results of ImmunoCAP and ISAC for components regarding avians.

Allergen	At the time of diagnosis	1 years later	Seven years later
ImmunoCAP (U _A /mL, class)			
Food allergen			
Egg white	2.07 (2)	0.89 (2)	<0.35 (0)
Egg yolk	2.41 (2)	0.71 (2)	<0.35 (0)
Chicken meat	0.90 (2)	ND	<0.35 (0)
Ovomucoid	<0.35 (0)	<0.35 (0)	<0.35 (0)
Inhalant allergen			
Budgerigar feather	6.15 (4)	ND	0.654 (1)
Budgerigar drops	1.44 (2)	ND	<0.35
ISAC (ISU, class)			
Hen's egg component			
Ovomucoid (Gal d 1)	0.0 (0)	0.0 (0)	0.0 (0)
Ovalbumin (Gal d 2)	0.0 (0)	0.0 (0)	0.0 (0)
Conalbumin (Gal d 3)	0.1 (0)	0.0 (0)	0.0 (0)
Serum albumin, α -livetin (Gal d 5)	5.0 (2)	0.9 (1)	0.2 (0)

**Fig. 1.** (A) Protein separation by SDS-PAGE and Coomassie staining. Lane 1, the egg yolk extract and lane 2, the budgerigar feather extract. (B) Inhibition experiments using IgE-immunoblotting with the budgerigar feather extract were carried out by the preincubation with 0 and 0.1 mg/mL egg yolk extract (lanes 1 and 2). (C) Inhibition experiments using IgE-immunoblotting with the egg yolk extract were carried out by the preincubation with 0 and 0.1 mg/mL egg yolk extract (lanes 1 and 2). MW, molecular weight markers.

droppings and sera of pet birds (typically budgerigars, less often canary birds and parrots).^{4–6} The causative allergens in bird-egg syndrome have been identified as serum albumins. Serum albumin from egg yolk (α -livetin, Gal d 5) is thought to induce allergic reactions after ingesting hen's eggs.^{4,5} Serum albumins can be found in various tissues, including muscle tissue, and in high amounts in egg yolk. Serum albumins have conserved amino acid sequences and a protein structure with a molecular weight of approximately 70 kDa, resulting in cross-reactivity among different avian serum albumins.^{6,7}

Patients with bird-egg syndrome usually develop allergic symptoms after the ingestion of raw or soft-boiled egg yolk, including oral and gastro-intestinal symptoms, as well as mild-to-moderately severe systemic reactions (urticaria, angioedema, asthma).^{4,5,7–9} Well-cooked egg yolk is likely to be tolerated, as observed in the present patient. However, after the ingestion of meat cooked medium rare, systemic reactions may occasionally occur because of the limited heat resistance of serum albumins.^{8,9}

Studies in children and adults who are double-positive to egg yolk and bird feathers revealed chicken meat allergy in 22% and 12% of the subjects, respectively.^{8,10} Our patient developed mild localized reactions (oral itching) after the ingestion of soft-boiled chicken meat although she tolerated well-cooked chicken meat.

Her chicken meat-related episodes were less frequent than her hen's egg-related ones. In addition, rice bran allergy of the present patient might have been enhanced by cross-reactivity to millet in the bird feed. During the time she stopped cleaning the bird cage, she also avoided eating and contact with edible rice bran. Thereafter, her rice bran allergy also got better.

In contrast to classic early-childhood egg allergy, egg allergy in bird-egg syndrome is thought to be typically adult-onset, characterized by isolated or dominant sensitization to egg yolk, and mostly persistent.^{6,9} Interestingly, tolerance to our patient's allergies to raw egg and chicken meat was successfully achieved after avoiding contact with her budgerigar, suggesting that bird-egg syndrome might be reversible by avoiding contact with avians. Therefore, in patients with adult-onset egg allergy, bird allergy due to α -livetin should be considered for allergic examinations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2018.11.004>.

Conflict of interest

The authors have no conflict of interest to declare.

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Supplementary Materials

Methods

Hen egg yolk extract

Hen egg yolk extract was prepared as follows: raw hen egg yolk was defatted as described by Toorenenbergen AW et al., with some modifications.¹ Briefly, 14g egg yolk were mixed with 150ml acetone for 3 h at room temperature. After filtering out the acetone, the remaining material was mixed with 150ml acetone for 3 h; after filtering out the acetone, the remaining material was air-dried at room temperature. 1g egg yolk protein was extracted with 100g of 10 mM phosphate buffer saline (PBS) (pH 7.4) for 3 h at room temperature. After centrifugation (3,500g $\times$ g for 30 min at 4°C), the supernatant was collected and frozen at -80°C.

Budgerigar feather extract

Budgerigar feather protein was extracted as described by Tauer-Reich I. et al., with some modifications.² Budgerigar feather allergen (30mg) was extracted by 100g of 10 mM PBS (pH 7.4) containing 0.05% NaN₃ (30ml) for 3 h at room temperature. After centrifugation (3,500g $\times$ g for 30 min at 4°C), the supernatant was concentrated using an Ultracel 3K Amicon Ultra filters (Millipore, Bedford, MA, USA) and frozen at -80°C.

Estimation of the protein concentration

Protein concentrations were quantified using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) according to the information provided by the manufacturer.

SDS-PAGE

The egg yolk extract (7.5 µg per lane) or the budgerigar feather extract (10 µg per lane) was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (8%, w/v) under reducing conditions using the electrophoresis system Bolt™ (Thermo Fisher Scientific, MA, USA) according to the method of Laemmli, following the instructions supplied by the manufacturer.³ For SDS-PAGE under reducing conditions, the samples were mixed with 0.1 M Tris (pH 6.8) containing 4% (w/v) SDS, 20% (w/v) glycerol, 10% (w/v) 2-mercaptoethanol and 0.02% (w/v) bromophenol blue. The samples were denatured by heating at 70°C for 10 min. To ensure correct protein separation and visualization, proteins on the gel were stained with Coomassie Brilliant Blue R-350 (Wako Pure Chemical Industries, Osaka, Japan) and silver staining II kit (Wako) following the instructions provided by the manufacturer.

Immunoblot analysis

Proteins separated by the SDS-PAGE under reducing conditions were transferred onto an Immobilon-P polyvinylidene difluoride (PVDF) membrane (Merck Millipore, Bedford, MA, USA) by wet transfer blotting methods. The membrane was incubated in 10 mM PBS (pH 7.5) containing 0.1% Tween 20 and 2% ECL Prime Blocking Reagent (GE Healthcare, Little Chalfont, UK) for blocking. The membrane was incubated for 1 hour at room temperature with the patient serum and the control pooled serum (diluted 1/20) indicated in PBS/0.05% Tween 20. Bound primary antibodies were detected by horseradish peroxidase-conjugated goat anti-human IgE (GeneTex, LA, USA) and an ECL advance western blotting detection kit (GE Healthcare), following the manufacturer's instructions. The obtained chemiluminescence signals were detected on X-ray films (Hyperfilm MP, GE Healthcare).

Inhibition experiments

The patient serum was diluted 1:20 in 10 mM PBS (pH 7.5) containing 0.1% Tween 20 and preincubated with 0.1 mg/mL egg yolk extract at 4°C overnight. Thereafter, PVDF strips coated with the budgerigar feather extract or the egg yolk extract, were incubated

with the preincubated serum at 4°C overnight. IgE binding bands were detected as described previously.

Results

SDS-PAGE and IgE-immunoblotting

SDS-PAGE of the egg yolk extract under reducing conditions showed multiple protein bands with molecular weights ranging from approximately 28 to 90 kDa (Figure).

SDS-PAGE of the budgerigar feather extract under reducing conditions showed multiple protein bands with molecular weights ranging from approximately 5 to 90 kDa.

The patient serum IgE was mainly bound to an egg yolk protein band at 69 kDa, which corresponded to the molecular weight of Gal d 5 (Figure). Immunoblotting with the budgerigar feather extract showed the main band at 70 kDa, which corresponded to the molecular weight of the budgerigar serum albumin. The control pooled serum did not react to the egg yolk protein band at 69 kDa and the budgerigar feather at 70 kDa.

Inhibition experiments

In IgE-immunoblotting, IgE-binding to the budgerigar feather extract at approximately 70 kDa and 90 kDa was inhibited by preincubation of the patient serum with 0.1 mg/ml

egg yolk extract. In addition, IgE-binding to the egg yolk extract at approximately 39 kDa and 69 kDa was inhibited by preincubation of the patient serum with 0.1 mg/ml egg yolk extract.

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