

# Airborne allergy to sunflower seed

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## Summary

**Background:** There is increasing evidence that bird fanciers may develop airborne allergies to unusual allergens.

**Objective:** To detect the allergen source in a bird fancier with a history of asthma associated with bird cage cleaning activities and with contact with a Brazil parrot.

**Methods:** SPT with a large series of both airborne and food allergens were carried out. IgE reactivity to allergens causing wheal and flare reactions was confirmed by in-vitro investigations including ELISA/ELISA inhibition and immunoblot analysis.

**Results:** Strong skin reactivity to sunflower seed was observed. Immunoblot analysis showed IgE reactivity to low m.w. proteins, most probably 2S albumin, and ELISA inhibition studies showed the absence of cross-reactivity to mustard.

**Conclusion:** Sunflower seed dust may sensitize patients via the respiratory tract. Differently from previously reported cases of sunflower seed allergy, no cross-reactivity to 2S albumin from botanically unrelated seeds was found.

**Key words:** Asthma, Food allergy, Sunflower seed, 2S albumins

## Introduction

There is increasing evidence that bird keepers may become sensitized via the inhalant route to proteins that are also potential food allergens. Sensitization to alpha-livetin may cause the so-called "bird-egg syndrome" [1], and allergy to millet dust in a group of bird keepers was recently described [2]. A case of airborne allergy most probably caused by sensitization to sunflower seed proteins is reported in the present study.

A 57-year-old man who had been a bird fancier for more than 10 years was recently seen at this allergy center for a series of asthma attacks during the last year. All the attacks shortly followed the cleanup of his bird cages except the last one which occurred at a friend's home about 2 minutes after a Brazil parrot was put on his shoulder. In all cases asthma subsided spontaneously within 15-20 min after the patient left the environment where birds were present. Asthma attacks ceased completely for the last two months, after the man gave up breeding birds. The patient, who had never had asthma before, reported a history of slight seasonal rhino-conjunctivitis easily controlled by anti-histamines.

## Methods

### Skin tests

Skin prick tests (SPT) were carried out on the volar side of the forearm using sterile 1mm-tip lancets (Dome-Hollister/Stier). Readings were taken after 15 min. Reactions were expressed as mean wheal diameter (adding the longest diameter to the orthogonal diameter and dividing by two). A wheal diameter of 3 mm or more was considered positive [3]. Histamine 10 mg/ml and saline were used as positive and negative control, respectively. SPT with commercial extracts of both seasonal and perennial airborne allergens (Allergopharma, Reinbeck, Germany) including grass, mugwort, ragweed, pellitory, plantain, birch, hazel, olive, cypress, several moulds, house dust mite, cat dander, dog dander, and feather mix showed hypersensitivity to both grass pollen and *Candida albicans*. Since these findings could not reasonably explain the asthma episodes, factors more strictly associated to birds, such as bird proteins and bird food, were taken into consideration. Thus, SPT with commercial extracts of egg white, egg yolk, chicken

Table 1. IgE specific for sunflower seed and inhibition studies.

Serum	IgE to sunflower seed	mustard extract	sunflower seed
A	3330	0	92
B	350	ND	ND

A. Patient's serum; B: Normal serum

All values expressed as optical density (OD): negative if <500

meat, wheat, maize, peanut, walnut, hazelnut, almond, sunflower seed, and sesame seed (all by ALK-Abelló, 1:20 w/v) were performed; moreover, skin tests with other food allergens routinely tested during food allergy investigations including milk, rice, carrot, peach, banana, and potato were carried out.

### SPT results

Sunflower seed induced a strong skin reaction (mean wheal diameter 12 mm); SPT with all the remaining food extracts scored negative. In view of our previous finding of cross-reactivity between 2S albumins from sunflower seed and mustard [4] the patient underwent further SPT with a commercial mustard (Maille brand, Dijon type) by the prick-prick technique, but no

reactivity was observed. The labels of several brands of ready-to-use mixtures to feed birds sold in a local supermarket were examined, but they reported only "mixed cereals and mixed seeds". Thus, a local pet shop owner was interviewed about the composition of bird foods; the man stated that sunflower seeds represent about 20-30% of mixtures for parrots. Notably, there were two couples of parrots among the birds owned by our patient. Unfortunately, causal relationship between asthma and sensitization to sunflower seed could not be further investigated because the patient refused to undergo a controlled inhalation challenge with the brand of bird food he used for his parrots. The patient reported that he did not eat sunflower seeds or sunflower oil and was not able to recall any ingestion of sunflower seeds in the past. In view of the severity of symptoms induced by the ingestion of a very small amount of sunflower seed in a previously reported allergic patient [4], oral challenge tests were not considered to be ethical in this patient.

### In-vitro studies

Sunflower seed hypersensitivity was further investigated in-vitro both by ELISA and immunoblot analysis. Sunflower seeds were homogenized and extracted (5%) in 0.1M phosphate-buffered saline, pH 7.4 (PBS) shaking over-night at 4°C. ELISA confirmed patient's IgE reactivity to sunflower seed proteins (Table 1). In inhibition experiments patient's serum was pre-absorbed with sunflower seed extract (1 mg protein/ml) or with mustard extract (1 mg protein/ml) for 120 min before ELISA with sunflower seed was carried out. Pre-absorption of patient's serum with mustard extract did not cause any loss of IgE reactivity to sunflower seed, while pre-absorption with sunflower seed extract caused a total loss of IgE reactivity (Table 1). Electrophoresis of sunflower seed (25 µg/lane) was carried out in a 10% polyacrilamide precast Nupage Bis-Tris gel according to manufacturer's instructions (Invitrogen, Milan, Italy) at 180 mA for 1 h. The resolved proteins were stained with 0.1% Coomassie Brilliant Blue or transferred onto a nitrocellulose membrane (Protran BA 85, Schleicher and Schuell, Milan, Italy) according to Towbin [5]. The membrane was saturated in TBS buffer containing 5% skimmed dry milk before incubation with patient's

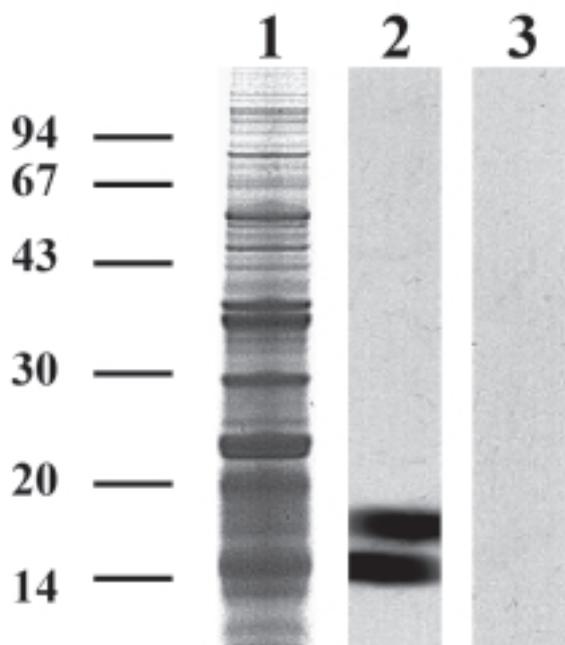


Figure 1. SDS-PAGE of sunflower seed extract and immunoblot analysis. Lane 1: SDS-PAGE; Lanes 2 and 3: IgE reactivity of patient's serum and of a normal serum to sunflower seed extract.

serum diluted 1:1.5 in saturating buffer. Bound specific IgE were detected by peroxidase-conjugated anti-human IgE serum using an ECL Western blotting kit (Amersham, Milan, Italy) as substrate. Immunoblot analysis showed IgE reactivity to sunflower seed proteins in the molecular weight range between 14-17 kDa (Figure 1), corresponding to 2S albumins.

## Discussion

This patient with a history of asthma attacks induced uniquely by exposure to caged birds showed strong hypersensitivity to sunflower seed. Although a controlled inhalation challenge with sunflower seed dust was not carried out, the possibility that asthma attacks experienced by the patient while cleaning the cages were induced by allergens other than sunflower seed seems very unlikely. Both negative SPT with egg yolk and chicken meat and the fact that the patient regularly ate eggs and poultry without any consequence ruled out the "bird-egg syndrome" caused by sensitization to bird serum albumin (alpha-livetin) contained in bird droppings. Our patient did not eat sunflower seed or sunflower oil nor did he recall the ingestion of sunflower seeds in the past; thus it is likely that sensitization occurred via the respiratory tract. Our field investigations showed that seed mixtures to feed parrots are rich in sunflower seeds. This suggests that bird food mixtures may contain sunflower seed dust particles small enough to be inhaled and to induce respiratory allergy. In effect, occupational airborne allergy to sunflower seed [6] as well as sunflower seed allergy in patients keeping caged birds fed on these seeds [4,7] have been reported, suggesting that sensitization via the inhalation route is not rare; most previously reported patients experienced anaphylaxis the first time sunflower seeds were ingested. Our patient reacted to 2S albumin, the major allergen in sunflower seed [8-10]. The immunoblotting profile showed IgE reactivity both to the immature and mature (cleaved) forms of the protein with a molecular mass of 16-17 kDa and 12 kDa, respectively [8]. In our previous study [4] immunologic cross-reactivity between 2S albumins in sunflower seed and mustard was found. This patient did not cross-react to other seeds containing 2S albumins (walnut, hazelnut, mustard, sesame, etc) suggesting that cross-reactivity to 2S albumins from botanically unrelated seeds is not the rule. Individuals

handling bird food mixtures should be aware that sunflower seed dust represents a substance able to induce sensitization via the inhalant route and to cause both respiratory and potentially severe food allergies.

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