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Different Pattern of IgE Binding to Chicken Egg Yolk between Patients with Inhalant Allergy to Birds and Food-Allergic Children

Key Words

Bird
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Canary
Chicken
Egg
Parrot
Yolk

Abstract

IgE binding antigens in budgerigar, canary and parrot serum were analyzed by immunoblotting. With 4 sera from patients with a respiratory allergy to birds, a closely related pattern of IgE binding to SDS-PAGE separated sera of the 3 birds was found. In all 3 bird sera a major IgE binding structure was present with a MW of about 60 kD. The pattern of IgE binding to SDS-PAGE separated egg yolk in sera from the patients allergic to birds, was different from sera of food-allergic children. Whereas in the bird-allergic patients major IgE binding occurred to a 60 kD component in egg yolk, IgE from food-allergic children was preferentially bound to a 35-kD structure in egg yolk.

Introduction

Bird breeder's lung is a form of allergic alveolitis, caused by inhalation of bird antigens [1, 2]. In serum from the affected patients precipitins are present that are directed against antigens from avian feathers, sera and droppings. Hypersensitivity to avian antigens may also occur through an IgE-mediated allergy and cause asthma in the affected patients [3].

In 1985, we and others [4, 5] described patients with an IgE-mediated bronchial allergy to birds; whenever IgE against bird serum could be demonstrated, IgE against chicken egg yolk was also present [4, 5]. The patient, described by de Maat-Bleeker et al. [4], not only experienced respiratory symptoms to birds but also urticaria and angioedema after ingestion of eggs. Hoffman and Guenther [6] described a patient with asthma upon exposure to birds, who experienced vomiting and diarrhea after consumption of egg containing products. The simultaneous sensitization to avian feathers, sera or droppings and to chicken egg yolk was later termed 'the bird egg syndrome' [7].

Eggs from chickens are an important source of allergens for infants and young children [8]. Although the main allergens are found in egg white, egg yolk also contains IgE-binding constituents [9].

In the present study, the IgE response against chicken egg yolk in patients with an inhalant allergy to birds was compared with this IgE response in food-allergic patients by immunoblotting. Furthermore, IgE-binding antigens in canary, parrot and budgerigar serum were analyzed by the immunoblotting technique.

Materials and Methods

Patient Sera

Four sera were obtained from patients with an established IgE-mediated respiratory allergy to birds. For 2 patients sera (No. 3 and 4 in table 1), IgE binding to budgerigar and canary serum proteins and to chicken egg yolk has been documented before [5]; IgE binding to parrot serum for 2 other patient sera (No. 1 and 2 in table 1) has been described elsewhere [10]. Patient 2 experienced symptoms of angioedema on eating eggs; there was no clinical evidence of allergy to eggs in

Table 1. Specific IgE against chicken egg yolk, chicken egg white, canary, parrot and budgerigar serum in sera from bird fanciers

Patient	Total IgE IU/ml	Chicken egg yolk, %	Chicken egg white, %	Canary serum, %	Parrot serum, %	Budgerigar serum, %	Age years
1	500	2.1	3.0	14	21	15	47
2	340	46	23	ND	32	ND	19
3	640	20	25	27	ND	35	38
4	430	12	10	16	ND	28	21
C1	790						
C2	870						

Percentage = Binding of added ^{125}I -labeled anti-IgE; ND = not done; C1, C2 = control sera.

patients 1, 3 and 4. Five sera with IgE against chicken egg white were obtained from patients with symptoms of food allergy (eczema and/or urticaria, diarrhea, recurrent vomiting). Control sera were selected from samples, submitted for routine IgE antibody analysis that were negative in RASTs for egg white, cow's milk, fish, wheat, peanut and soy. All sera stored at -20°C .

Allergens

Budgerigar, canary and parrot serum were obtained from Laboratorium Diephuis, Houten, The Netherlands. Defatted chicken egg yolk allergen was prepared as described previously [5].

Total IgE was determined by a competitive radioimmunoassay (IgE RIA, Pharmacia, Woerden, The Netherlands). Specific IgE against budgerigar, canary and parrot serum and against chicken egg yolk were determined as described previously [5, 10], using agarose beads as allergen support [11]. IgE against chicken egg white was estimated by using allergen discs or CAPs according to the manufacturer's (Pharmacia) instructions.

Immunoblotting

SDS-PAGE was performed according to the method of Laemmli [12], with a 3% polyacrylamide stacking gel on top of the separation gel (12.5%). For analysis by electrophoresis, 5 mg defatted chicken egg yolk were shaken for 1 h with 1 ml distilled water at room temperature. After centrifugation for 10 min at 1,400 g and 4°C , the supernatant was stored in small aliquots at -20°C . 0.04 ml of this supernatant were mixed with an equal volume of sample buffer and heated for 5 min at 100°C (sample buffer: 0.125 M Tris HCl, 4% SDS, 20 mM dithiothreitol, 20% glycerol and 0.005% bromophenol blue, pH 6.8). To 0.01 ml bird serum, 0.03 ml physiological saline and 0.04 ml sample buffer were added. These mixtures were also heated for 5 min at 100°C . Per electrophoretic run, 0.04 ml of the heated mixtures were applied. Low molecular weight markers were obtained from Pharmacia. Transfer to nitrocellulose (0.45 μm , BioRad, Utrecht, the Netherlands) was done by electroblotting [13], as described previously [14]. After protein blotting, unoccupied binding sites on the nitrocellulose membrane were blocked by incubation of the nitrocellulose sheet in PBS, 0.1% Tween 20 and 0.02% NaN_3 (PBS-Tween) for 1 h at 37°C ; 0.5 cm wide lanes were incubated overnight with a mixture of 0.2 ml PBS-Tween and 0.2 ml patient serum in disposable incubation trays

(Schleicher & Schuell, 's-Hertogenbosch, The Netherlands). After three washings with PBS-Tween, a mixture of 0.1 ml ^{125}I -labeled anti-IgE (1.2 microC/ml, Pharmacia) and 0.3 ml PBS-Tween was added to each lane. After 4 h of incubation at room temperature and three washes with PBS-Tween, the lanes were air-dried and exposed for 10 days to Kodak direct exposure film, DEF 392 (Kodak, Heidelberg, Germany), as described previously [14].

Results

Table 1 shows the IgE binding to one or more bird sera after incubation of 4 sera from patients with an respiratory allergy to birds. Initially, patient sera were only tested for IgE against those birds, they were exposed to. After completion of the immunoblotting experiments, not enough serum was left to test for IgE against all bird sera

Figures 1 and 2 show the results of immunoblot experiments with the bird sera; a closely related pattern of IgE binding to SDS-PAGE separated sera of the 3 birds was found. Lanes 1 and 4 in figure 1 and lane 4 in figure 2a, b indicate that major IgE binding occurs to a component with an estimated molecular weight of about 60 kD; with canary serum and budgerigar serum (fig. 1, 2), IgE binding to components with molecular weight higher than 94 kD was also seen. With canary serum, IgE binding also occurred to a component of 25 kD. Two control sera (see table 1) gave no or very weak IgE binding to the fractionated bird sera and chicken egg yolk. In figures 1–3 the results for one of these sera are depicted for each allergen.

Figure 3a shows that IgE from patients with respiratory allergy to birds (sera 1–4) was mainly directed against a 60-kD component in egg yolk. In agreement with table 1, patient 1 showed only weak IgE binding to egg yolk. Sera from

Table 2. IgE against egg white and egg yolk in 5 patients with symptoms of food allergy

Patient	Age years	Chicken egg white PRU/ml	Chicken egg yolk PRU/ml (kU/L) ¹	Total IgE IU/ml	Budgerigar serum, %
5	9	7.5	17.6 ¹	10,300	16.8
6	1	11.0	4.0 ¹	1,150	2.6
7	3	15.9	7.6 ¹	190	ND
8	1	>20	>20	940	ND
9	2	>20	4.7	705	ND

Percentage = Binding of added ¹²⁵I-labeled anti-IgE; ND = not done; ¹ Egg yolk Immuno CAP.

patients 2, 3 and 4 also recognized IgE binding structures with molecular weights of 40 and 32 kD, respectively.

In contrast, IgE from sera of most food-allergic patients was preferentially bound to a 35-kD antigen in egg yolk (fig. 3b). The IgE binding pattern of serum 5 however (fig. 3b) resembled more the picture of patients with an inhalant allergy to birds. Table 2 shows the levels of IgE antibody to chicken egg white in the food allergic patients.

Discussion

When IgE against serum from a specific bird is detected in serum from a patient, IgE against many other bird sera can be found [4, 5, 10]. Figures 1 and 2 show a closely related pattern of IgE binding to the 3 bird sera, suggesting a close relationship between the allergens in these sera. Not enough serum was available to test the cross-reactivity between the sera by mutual RAST inhibition. IgE from the patients with inhalant allergy to birds recognized both in the bird sera (fig. 1, 2) and in egg yolk (fig. 3) a component with an estimated molecular weight of 60 kD. Pauli et al. [15] suggested that the IgE binding structure, common to bird sera and egg yolk, is serum albumin.

The egg yolk and bird serum preparations were heated for 5 min at 100°C before electrophoresis. It is possible that heat-labile allergens lost their IgE-binding capacity by this procedure.

Four of 5 sera from patients with symptoms of food allergy showed a pattern of IgE binding to egg yolk proteins that was markedly different from this picture of the patients with an inhalant allergy. In the patients with inhalant allergy to avian antigens, IgE antibodies against chicken egg yolk

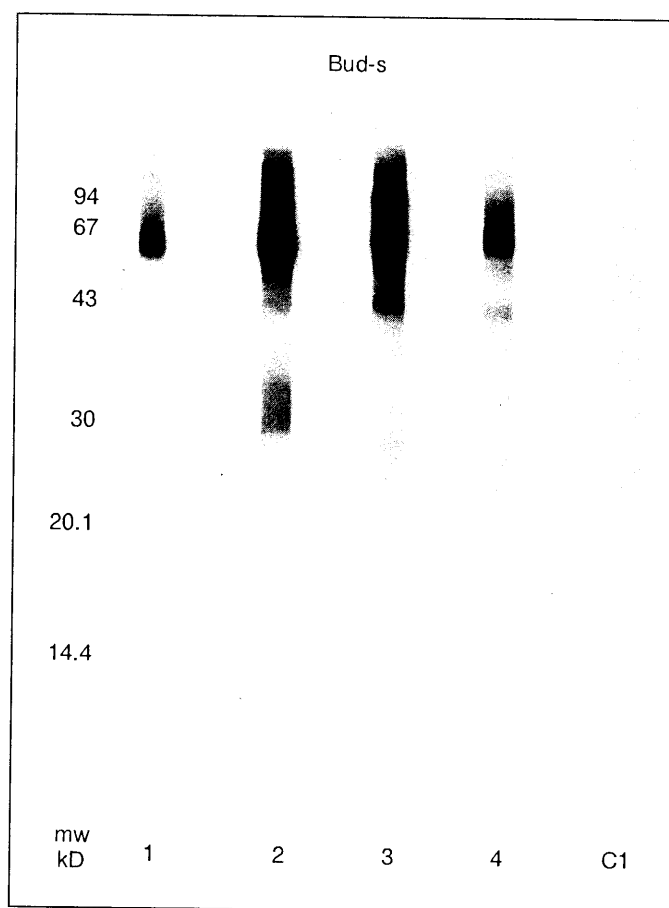


Fig. 1. IgE binding to SDS-PAGE separated components of budgerigar serum (bud-s) after incubation with 4 patient sera and a control serum (see table 1). On the left the positions of molecular weight markers (mw) are indicated.

were possibly induced by sensitization to bird serum antigens [4].

Other factors which may have contributed to the different patterns of IgE binding are the route of sensitization and the age of the patients.

The route by which allergens enter the body may determine the sensitization pattern; antigen handling in the lung may be different from antigen handling in the gastrointestinal tract. In patients with symptoms of food allergy and IgE against coriander, IgE binding to coriander could be inhibited by mugwort pollen [16], whereas cross-reactivity between coriander and mugwort pollen was absent in a patient with occupational asthma caused by inhalation of powdered coriander [17].

The age of the patients may also influence the sensitization pattern. Calckhoven et al. [18] showed that IgE response to house dust mite differs between children and

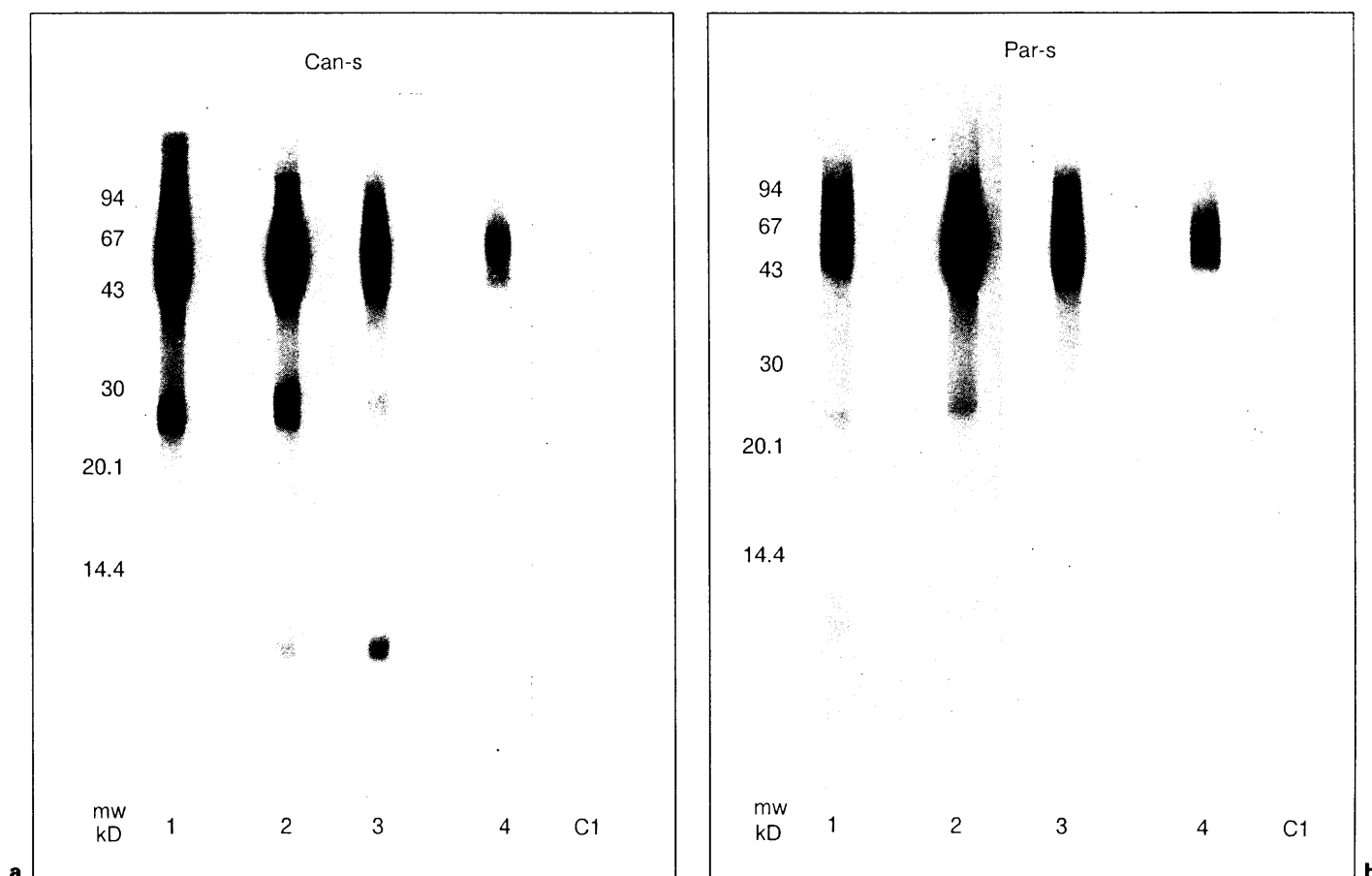


Fig. 2. IgE binding to SDS-PAGE separated components of canary serum (Can-s; **a**) and parrot serum (Par-s; **b**) after incubation with 4 patient sera and a control serum (see table 1). On the left the positions of molecular weight markers (mw) are indicated.

adults. Sera 6–9 (fig. 3) were from children less than 4 years old, whereas the bird-allergic patients were 19 years or older.

Serum No. 5, selected because of an elevated level of IgE against egg white, showed a pattern of IgE binding that resembled more the patients with a respiratory allergy to birds than the food allergic group. After completion of the immunoblot experiments, sera 5 and 6 were tested for IgE against one of the bird sera: budgerigar serum. Indeed, an elevated level of IgE against budgerigar serum was found in serum 5; in serum 6 no IgE against budgerigar serum could be demonstrated (table 2). The written case report of patient 5 (table 2) revealed that she was a highly atopic child with severe atopic dermatitis at 1 year of age. When the serum that was used for immunoblotting, was obtained, the patient was 9 years old. In this serum a class 4 RAST for rabbit was found, class 3 RASTs for house dust mite, grass pollen, cat,

dog, and chicken egg white, class 2 RASTs for hazelnut, birch and wheat; class 1 RASTs for cow's milk and guinea pig. The total IgE level was 10,300 IU/ml. Contact with a canary at home and four chickens did not markedly aggravate her symptoms of allergy. Thus, the history of patient 5 suggests that she may have developed a respiratory allergy to bird allergens. Because she was sensitive to many allergens and strongly hyperreactive, clinical sensitivity to birds may have gone unnoticed. Bausela et al. [19] recently described a patient, in which egg protein sensitization preceded bird-related asthma.

In summary, this paper shows differences in the IgE response to egg yolk proteins between adults with a respiratory allergy to avian antigens and infants and children with symptoms of food allergy.

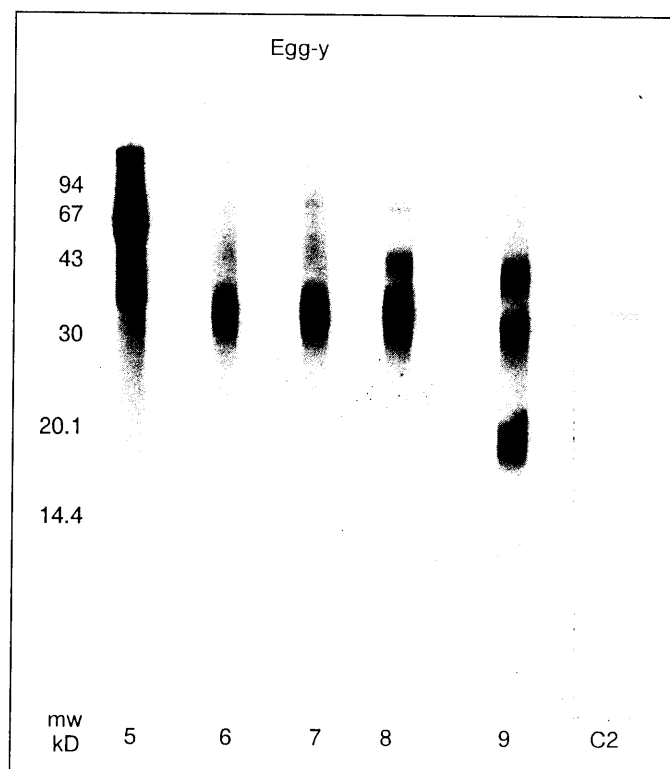
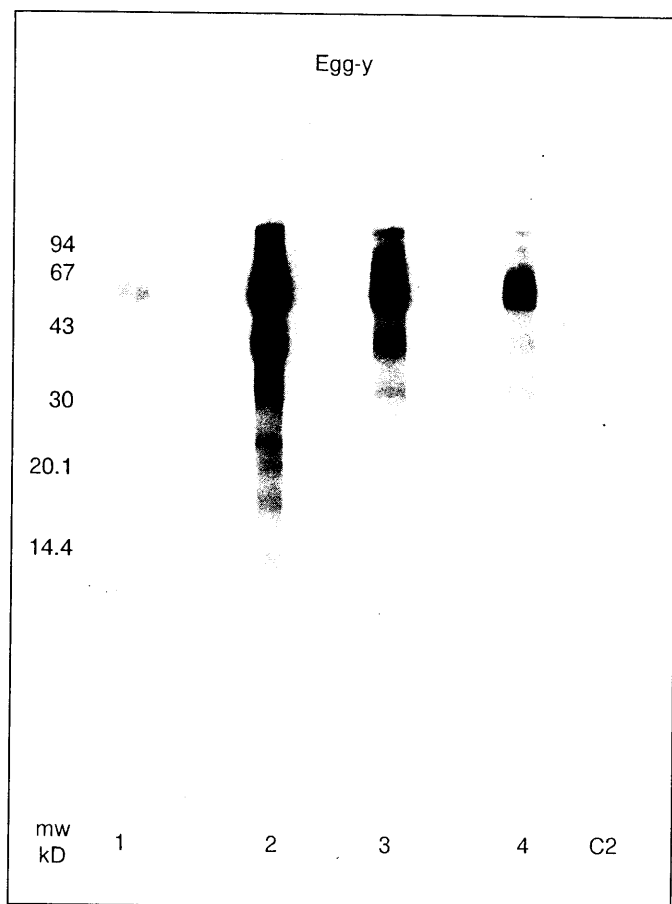


Fig. 3. IgE binding to SDS-PAGE separated components of chicken egg yolk after incubation with 9 patients sera and a control serum (see tables 1 and 2). On the left the positions of molecular weight markers are indicated.

References

- Hargreave FE, Pepys J, Longbottom JL, Wraith DG: Bird breeder's (fancier's) lung. *Lancet* 1966;1:445-449.
- Reed CE, Sosman AJ, Baree RE: Pigeon breeder's lung. *JAMA* 1965;193:261-265.
- Faux JA, Wide C, Hargreave FE, Longbottom JL, Pepys J: Immunological aspects of respiratory allergy in budgerigar fanciers. *Clin Allergy* 1971;1:149-158.
- de Maat-Bleeker F, van Dijk AG, Berrens L: Allergy to egg yolk possibly induced by sensitization to bird serum antigens. *Ann Allergy* 1985;54:245-248.
- van Toorenenbergen AW, Gerth van Wijk R, van Dooremalen G, Dieges PH: Immunoglobulin E antibodies against budgerigar and canary feathers. *Int Arch allergy Appl Immunol* 1985;77:433-437.
- Hoffmann DR, Guenther DM: Occupational allergy to avian proteins presenting as allergy to ingestion of egg yolk. *J Allergy Clin Immunol* 1988;81:484-488.
- Mandallaz MM, de Weck AL, Dahinden CA: Bird-egg syndrome. *Int Arch Allergy Appl Immunol* 1988;87:143-150.
- Sampson HA: Food hypersensitivity and atopic dermatitis. *Allergy Proc* 1991;12:327-331.
- Anet J, Back JF, Baker RS, Barnett D, Burley RW, Howden MEH: Allergens in the white and yolk of hen's egg. *Int Arch Allergy Appl Immunol* 1985;77:364-361.
- Gerth van Wijk R, van Toorenenbergen AW, Dieges PH: Nasal allergy to avian antigens. *Clin Allergy* 1987;17:515-521.
- Adkinson NF: Measurement of total serum immunoglobulin E and allergen-specific immunoglobulin E antibody: in Rose NR, Friedman H, Fahey JL (eds): *Manual of clinical Laboratory Immunology*, ed 3. Washington, American Society for Microbiology, 1986, pp 665-674.
- Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-685.
- Bengtsson A, Karlsson A, Rolfsen W, Einarson R: Detection of allergens in mould and mite preparations by a nitrocellulose electroblotting technique. *Int Arch Allergy Appl Immunol* 1986;80:383-390.
- van Toorenenbergen AW, Huijskes-Heins MIE, Dieges PH, Leijnse B: Occupational allergy to pancreatic powder: Characterization of IgE-binding antigens by immunoblotting. *J Allergy Clin Immunol* 1991;87:650-654.
- Pauli G, de Blay F, Bessot JC, Dietemann A: The association between respiratory allergies and food hypersensitivities. *ACI News* 1992;4:43-45.
- van Toorenenbergen AW, Dieges PH: Demonstration of spice-specific IgE in patients with suspected food allergies. *J Allergy Clin Immunol* 1987;79:108-113.
- van Toorenenbergen AW, Dieges PH: Immunoglobulin E antibodies against coriander and other spices. *J Allergy Clin Immunol* 1985;76:477-481.
- Calkhoven PG, Aalbers A, Schilte PPM, Yntema JL, Griffioen RW, van Nierop JC, Oranje AP, Aalberse RC: Newly generated IgE antibodies to *Dermatophagoides pteronyssinus* in children are directed against components distinct from Der p1 or Der p11. *J Allergy Clin Immunol* 1991;88:265-271.
- Bausela BA, Esteban MM, Alzamora FM, Marcos CP, Sasas JAO: Egg protein sensitization in patients with bird feather allergy. *Allergy* 1991;46:614-618.