

*Rhinology Unit & Smell Clinic
ENT Department
Hospital Clinic - IDIBAPS
Villarroel 170
08036 Barcelona, Catalonia
Spain
Tel.: +34 932 27 9872
Fax: +34 934 51 5272
E-mail: jmullo@clinic.ub.es

Accepted for publication 19 March 2009
Allergy 2009; 64:1556–1557
© 2009 John Wiley & Sons A/S
DOI: 10.1111/j.1398-9995.2009.02092.x

References

1. Alobid I, Benítez P, Bernal M, Roca J, Alonso J, Picado C et al. Nasal polyposis and its impact on quality of life: comparison between the effects of medical and surgical treatments. *Allergy* 2005;**60**:452–458.
2. Holbrook E, Leopold D. An updated review of clinical olfaction. *Curr Opin Otolaryngol Head Neck Surg* 2006;**14**:23–28.
3. Simola M, Malmberg H. Sense of smell in allergic and nonallergic rhinitis. *Allergy* 1998;**53**:190–194.
4. Guilemany JM, García-Piñero A, Alobid I, Cardelús S, Centellas S, Bartra J et al. Persistent allergic rhinitis has a moderate impact on the sense of smell, depending on both nasal congestion and inflammation. *Laryngoscope* 2009;**119**:233–238.
5. Cardesín A, Alobid I, Benítez P, Sierra E, de Haro J, Bernal M et al. Barcelona Smell Test 24 (BAST-24): validation and smell characteristics in the healthy Spanish population. *Rhinology* 2006;**44**:83–89.

Food allergy to chicken meat with IgE reactivity to muscle α -parvalbumin

A. Kuehn*, C. Lehnert, C. Hilger, F. Hentges

Key words: allergen; alpha-parvalbumin; chicken meat; chicken parvalbumin; food allergy.

IgE-mediated allergic food reactions provoked by consumption of chicken meat have been described in patients with egg allergy and bird feather hypersensitivity (1). Chicken serum albumin

(Gal d 5), also known as α -live-tin, has been shown to be the cross-reactive allergen (2). A few cases have been published

concerning chicken meat allergy without sensitization to egg proteins. In these studies, the exact nature of the responsible allergens has not been defined (3, 4).

We report the case of a 54-year-old patient who experienced severe allergic reactions to poultry products. Swelling of tongue and oral mucosa, vomiting and hypotension occurred a few minutes after ingestion of chicken meat, turkey meat or chicken broth. The patient reported mild oral reactions when eating tuna and salmon. The patient tolerated chicken eggs.

This is the first report of chicken meat allergy with α -parvalbumin identified as food allergen.

Skin prick tests (SPT) performed with a series of commercial aeroallergens (ALK, Varennes en Argonne, France) were positive for tree and grass pollen. Skin prick tests with food allergens were positive with chicken meat, turkey meat and different fishes (tuna, salmon, cod and carp).

The total IgE level was 80 kU/l (Phadia ImmunoCAP System, Uppsala, Sweden). Specific IgE were positive for chicken meat (12 kU/l), turkey meat (6 kU/l), pork meat (1 kU/l), cod (3 kU/l), tuna (3 kU/l) and salmon (2 kU/l), but negative for egg yolk and egg white (<0.35 kU/l).

Protein extracts were prepared from leg muscle of chicken and turkey. In IgE immunoblot analysis, a distinct 14 kDa protein band was detected in poultry extracts with patient serum (Fig. 1A). In addition, several addi-

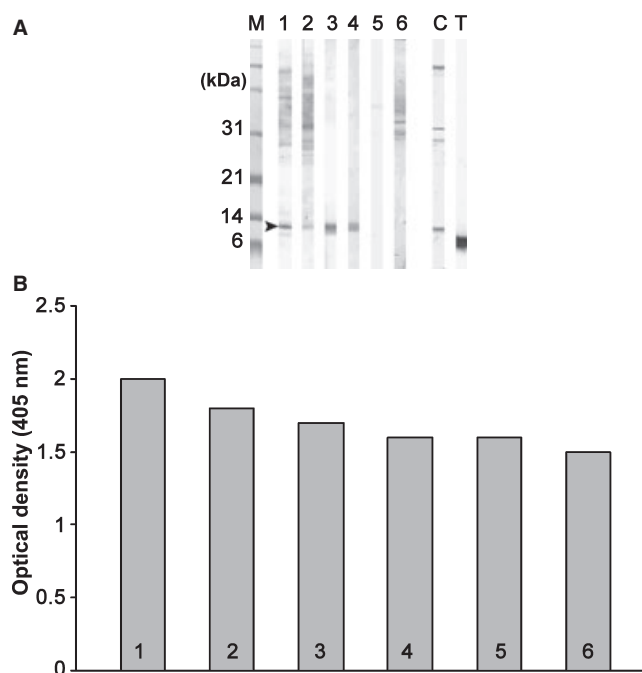


Figure 1. IgE analysis of chicken allergens. A. Allergens are detected at 14 kDa and >30 kDa by the patient's IgE antibodies in chicken (1) and turkey (2) leg meat. IgE bind specifically to purified native (3) and recombinant (4) chicken α -parvalbumin, but not to tuna (5) β -parvalbumin. IgE reactivity to chicken leg extract is inhibited by preincubation with 20 $\times$ excess of recombinant chicken parvalbumin (6). (C) Chicken parvalbumin is detected in total extracts using a commercial antibody (Abcam, Cambridge, UK). (T) Identity of purified tuna parvalbumin is confirmed by an anti- β -parvalbumin antibody (Swant, Bellinzona, Switzerland). B. IgE quantification by enzyme-linked immunosorbent assay. Similar IgE titers are obtained for native chicken (1), recombinant chicken (2) as well as for frog (3), cattle (4), horse (5) and pig (6) recombinant α -parvalbumins.

tional IgE-binding bands were observed in the molecular range from 30 to 60 kDa.

Native parvalbumins were prepared from chicken leg, tuna, salmon, cod and carp muscle by ion-exchange and size-exclusion chromatography. Chicken (FM994924), turkey, cattle (FM178223), pig (FM994923) and horse (FM994925) α -parvalbumins were cloned using rapid amplification of cDNA ends (RACE) PCR technology and expressed in *Escherichia coli* M15. Recombinant α -parvalbumin from frog (Q8JIU2) was produced as described before (5). Protein sequence analysis of α -parvalbumins showed that chicken parvalbumin was 100% identical to turkey, 83% to cattle/horse, 82% to frog, 80% to pig, 79% to human (X63070) and 54% to cod β -parvalbumin.

Purified chicken and fish parvalbumins were analyzed in immunoblot for IgE reactivity using patient's serum. Chicken α -parvalbumin was strongly positive, fish β -parvalbumin was negative (Fig. 1A). IgE recognition of the 14 kDa band was abolished by preincubation of the serum with recombinant chicken α -parvalbumin.

IgE antibodies to α -parvalbumin from chicken, frog, pig, cattle and horse were quantified by *enzyme-linked immunosorbent assay* (ELISA) (Fig. 1B). Similar values were obtained for all parvalbumins analyzed, except for fish parvalbumins which were negative (data not shown). The SPT was clearly positive with purified, native chicken parvalbumin at a concentration of 10 μ g/ml while the SPT was negative with native tuna β -parvalbumin.

In conclusion, we report a case of poultry meat allergy where we identified chicken α -parvalbumin as one of the implicated allergens. The patient was sensitized to α -parvalbumin from chicken with cross-reactivity to highly similar homologs from pig, horse and cattle. Remarkably, no IgE binding was found to the major fish allergen β -parvalbumin. The allergenic potential of α -parvalbumin is assumed to be very low in general, possibly because of their high identity to the human homolog (6). Even though expression levels of mammalian muscle

α -parvalbumins are known to be minor, they could be potent food allergens because of the high thermal stability and digestion resistance of this protein family, which gives impact to the present report. The clinical relevance of α -parvalbumins as meat allergens might be reconsidered in food allergy diagnosis.

*Laboratory of Immunogenetics and Allergology
CRP-Santé
84, Val Fleuri
L-1526 Luxembourg
Luxembourg
Tel.: +352 26 97 03 35
Fax: +352 26 97 03 90
E-mail: annette.kuehn@crp-sante.lu

Accepted for publication 26 March 2009
Allergy 2009; 64:1557–1558
© 2009 John Wiley & Sons A/S
DOI: 10.1111/j.1398-9995.2009.02094.x

References

1. Bausela AB, Estekban MM, Alzamora FM, Marcos CP, Casas JAO. Egg protein sensitization in patients with bird feather allergy. *Allergy* 1991;**46**:614–618.
2. Quirce S, Maranon F, Umpierrez A, de las Heras M, Fernandez-Caldas E, Sastre J. Chicken serum albumin (Gal d 5*) is a partially heat-labile inhalant and food allergen implicated in the bird-egg syndrome. *Allergy* 2001;**56**:754–762.
3. Liccardi G, Szepfalusi Z, Noschese P, Nentwich I, D'Amato M, D'Amato G. Allergy to chicken meat without sensitization to egg proteins: a case report. *J Allergy Clin Immunol* 1997;**100**:577–579.
4. Cahen YD, Fritsch R, Wütherich B. Food allergy with monovalent sensitivity to poultry meat. *Clin Exp Allergy* 1998;**28**:1026–1030.
5. Hilger C, Grigioni F, Thill L, Mertens L, Hentges F. Severe IgE-mediated anaphylaxis following consumption of fried frog legs: definition of alpha-parvalbumin as the allergen in cause. *Allergy* 2002;**57**:1053–1058.
6. Jenkins JA, Breiteneder H, Clare Mills EN. Evolutionary distance from human homologs reflects allergenicity of animal food proteins. *J Allergy Clin Immunol* 2007;**120**:1399–1405.

Allergy for cheese: evidence for an IgE-mediated reaction from the natural dye annatto

D. G. Ebo, S. Ingelbrecht, C. H. Bridts, W. J. Stevens*

Key words: anaphylaxis; annatto; basophil; cheese.

A 58-year-old atopic man was referred to our outpatients' clinic because of four episodes in 3 years of severe anaphylaxis with urticaria, angio-oedema and finally loss of consciousness within a few minutes after consumption of a sandwich or toast with Gouda cheese. De-

tailed history, quantification of specific IgE (sIgE) and skin tests confirmed allergy for house dust mite and cat epithelium. There was neither evidence of a classical IgE-mediated food allergy (in particular, wheat and dairy products) nor of pollen or latex allergy that could have explained a secondary food allergy. Serum tryptase level was normal at the clinical visit. Careful revision of labels from different brands of Gouda cheese revealed that the reaction could potentially be elicited by the natural dye annatto (E160b).

Skin prick tests with an extract from Gouda cheese (Holland Delhaize, Belgium) and annatto (Ceska Annatto WS E160b) both tested in duplo and at a 10^{-3} dilution were positive with a wheal and flare reaction of 4/5 and 4/6 mm respectively.

Flow cytometric analysis (FACS-Calibur, BD, Immunocytometry Systems, San Jose, CA, USA) of activated basophils was performed using Alexa Fluor 448-coupled anti-IgE (Sigma-Aldrich, Chemic GmbH, Steinheim, Germany) and phycoerythrin-conjugated anti-CD63 (Pharmingen, BD Biosciences, Erembodegem, Belgium). The test included a negative control (stimulation buffer without any allergen), a positive control

A man with anaphylaxis from the natural dye annatto is presented. Diagnosis was established by history, skin tests and basophil activation test.