

Original article

Isolation and characterization of a heat-resistant beef allergen: myoglobin

Background: Meat allergy is rarely reported. Most of the described cases are sensitizations to bovine serum albumin.

Objective: The aim of the study was to describe a case of allergy to a new meat allergen and, after its characterization.

Methods: A 35-year-old nonatopic female with allergic episodes after ingestion of several types of meat was studied. Skin tests (prick and prick-to-prick); total and specific immunoglobulin E (IgE) determination; sodium dodecyl sulphate-polyacrylamide gel electrophoresis and specific IgE determination by immunoblotting under different conditions were performed. A 17-kDa allergen was semipurified by ethanol fractionation and its amino-terminal sequence was determined. The existence of specific IgE directed to this protein was studied by immunoblot in 80 atopic patients.

Results: The patient showed specific IgE antibodies to a 17-kDa protein. During the isolation of this allergen it was found that a 70–90% (vol/vol) ethanol concentration was able to purify the protein. The characterization revealed that it was a heat-resistant protein without disulfide bonds. N-terminal amino acid sequence (16 residues) showed identity with myoglobin. The study of specific IgE to this allergen among atopic patients showed that it was recognized by about 1% of the subjects.

Conclusions: We describe a case of meat allergy caused by myoglobin. This is the first described case of monosensitization to this protein.

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Key words: allergy; BSA; heat-labile proteins; heat-resistant proteins; immunoblotting; meat.

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Accepted for publication 14 April 2003

The most frequent food allergens in childhood are milk, eggs, peanut, soy and wheat, whereas in adults the hypersensitivity reactions are usually caused by peanut, fish, shellfish and tree nuts (1, 2). Allergic reactions to beef and other vertebrate meats are infrequently reported, and sometimes these adverse reactions have been attributed to the presence of antibiotics in meat (3).

Most of the cases of meat allergy are due to bovine serum albumin (BSA) sensitization, as demonstrated by double blind placebo-controlled food challenges. The majority of the studies have been performed in atopic children. This protein has been described as one of the most important allergens present in bovine meat, together with bovine gamma globulin (BGG) (3). Different studies have shown that the reactivity and allergenicity of BSA is modified when beef proteins are heat-processed (3–6). Because both BSA and BGG are heat-labile proteins, Werfel et al. (3) reported that patients sensitized to heat-labile proteins might tolerate well-cooked meat, while patients with specific IgE to heat-resistant proteins did not tolerate both raw and well-cooked meat.

The aims of this study were to describe a case of meat allergy caused by a heat-resistant protein and, after its

characterization, to study the detection of specific IgE to this protein in atopic population.

Material and methods

Case report

A 35-year-old nonatopic female showed episodes of oral pruritus and angioedema of the lower lip, lasting several hours. She referred these symptoms since 8 months ago. The episodes occurred after ingestion of cow, beef and lamb's meats and fish. Also, she showed pruritus and swelling in hands and forearms after the manipulation of fish and cow, beef and lamb's meats without gloves. She tolerated chicken, turkey, pork and other foods (milk, fruits, vegetables, nuts, and eggs). The physical examination and routine blood tests were normal.

Skin prick test and prick-to-prick tests

Skin prick tests were performed with commercial inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*; dog, cat and horse dander; *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* species; tree, grass and weed pollens) and food allergens (cow's milk, egg, fruits, vegetables, fish, meat, shellfish, nuts, albumin

and *Anisakis simplex*) of commercial origin. Prick-to-prick tests were done with the following raw and cooked meat types: beef, lamb, turkey, rabbit, as well as with several fish species (raw and cooked).

Total and specific IgE determination

The CAP System (Pharmacia Upjohn, Uppsala, Sweden) was used for determination of the total and specific immunoglobulin E (IgE) antibodies in the serum of the patient.

Antigenic crude extracts

Meat (beef) and fish (hake) crude extracts were prepared as follows: 10 ml of phosphate-buffered saline (PBS) were added to 10 g of raw meat or fish, and grounded. The mixtures were shaken during 30 min, centrifuged at 4500 *g* for 15 min and the supernatants were recovered.

SDS-PAGE and immunoblotting

The supernatants obtained were studied by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 16% acrylamide minigel. After electrophoresis, the proteins were transferred to nitrocellulose by diffusion during 18 h and the immunoblotting was performed as described (7).

Heat treatment of the meat extract

Several aliquots containing 500 μ l of meat extract were heated at 50, 60, 70, 80, 90 and 100°C for 15 min. After heating, 25 μ l of each sample were subjected to SDS-PAGE and immunoblotting as previously described.

Ethanol fractionation

Four aliquots of 1 ml of the supernatant obtained in the meat crude extract preparation were mixed with increasing amounts of absolute ethanol to obtain 50, 60, 70 and 80% vol/vol. After 30 min at RT, the mixtures were centrifuged at 2500 *g* for 15 min. The supernatants were mixed again with ethanol to increase to 90% vol/vol and left 30 min at RT. Then, the mixtures were centrifuged and the pellets obtained were reconstituted at original volumes by addition of 1 ml of PBS.

N-terminal amino acid sequence

The N-terminal sequence analysis was performed by Dr Juan Antonio López at Proteomics Laboratory (Centro Nacional de Biotecnología, CSIC) protein sequence facility on a Procise Protein Sequencing System sequenator (model 494; Applied Biosystems, Foster City, CA).

Specific IgE determination by immunoblotting in atopic patients

Sera from the 80 last patients received in our laboratory for further *in vitro* studies such as drug, food, occupational, insect, pollen and mould allergy diagnosis were studied. The immunoblotting was performed with 100 μ l of the meat crude extract mixed with the same quantity of sample buffer and loaded in a gel prepared without lanes. After electrophoresis, proteins were transferred to nitrocellulose and blocked with 3% Nonidet P-40 for 30 min. Then, the

membranes of nitrocellulose were placed on Mini-Protean II Multiscreen (BioRad, Hercules, CA, USA). The different sera were diluted 1 : 6 in incubation buffer, and 0.6 ml of the mixtures were incubated overnight in each independent chamber. The next day, the membranes were taken out of the device, and the assay was followed as previously described (7).

Results

The patient showed negative skin prick tests with inhalant and food allergens, including cow's milk. Prick-to-prick tests were positive to several fish species, and also to meat (beef and lamb), both raw and cooked. Total serum IgE was 900 kU/l. Specific IgE was 10.2 kU/l to beef, 10.8 kU/l to lamb and <0.35 kU/l to pork. Specific IgE determination for eight different fish species were positive and the highest value found was 4.64 kU/l for cod.

Specific IgE detection in the serum of the patient showed different results depending on the experimental conditions. Usually, immunoblotting in our laboratory is performed using an incubation buffer containing 5% fetal bovine serum (FBS). Under these conditions, the patient showed an IgE-recognition of a 12-kDa protein in the fish extract and a weak signal to a protein with higher MW in the meat extract (Fig. 1B). When 5% FBS was omitted in the incubation buffer, this protein was strongly detected by the serum of the patient (Fig. 1C).

When SDS-PAGE and immunoblotting with a heated meat extract were performed, almost all protein bands except a 17-kDa one, disappeared (Fig. 2). The immunoblotting revealed that the specific IgE of the patient recognized this protein in all the lanes (Fig. 2B), a fact that proved the thermic stability of the epitopes.

This allergen was semipurified by means of ethanol fractionation. As shown in Fig. 3, most of the proteins of the crude extract precipitated at 70% (vol/vol) ethanol concentration, leaving the 17-kDa protein in solution. No differences were observed when SDS-PAGE with and without 2-mercaptoethanol was performed, suggesting a molecular structure without chains linked by disulfide bridges (Fig. 4).

The analysis of the N-terminal amino acid sequence on 16 residues gave the following sequence: GLS DGEWQLVLNAWGK which corresponded to cow myoglobin.

Eighty sera from subjects with suspected atopy were studied by immunoblot using a beef crude extract. One patient recognized a band at 17 kDa and a second band about 65 kDa. Three patients recognized only one band at this 65-kDa protein (data not shown).

Discussion

Previous reports demonstrated that fish allergy is common in children and young adults, while meat allergy is

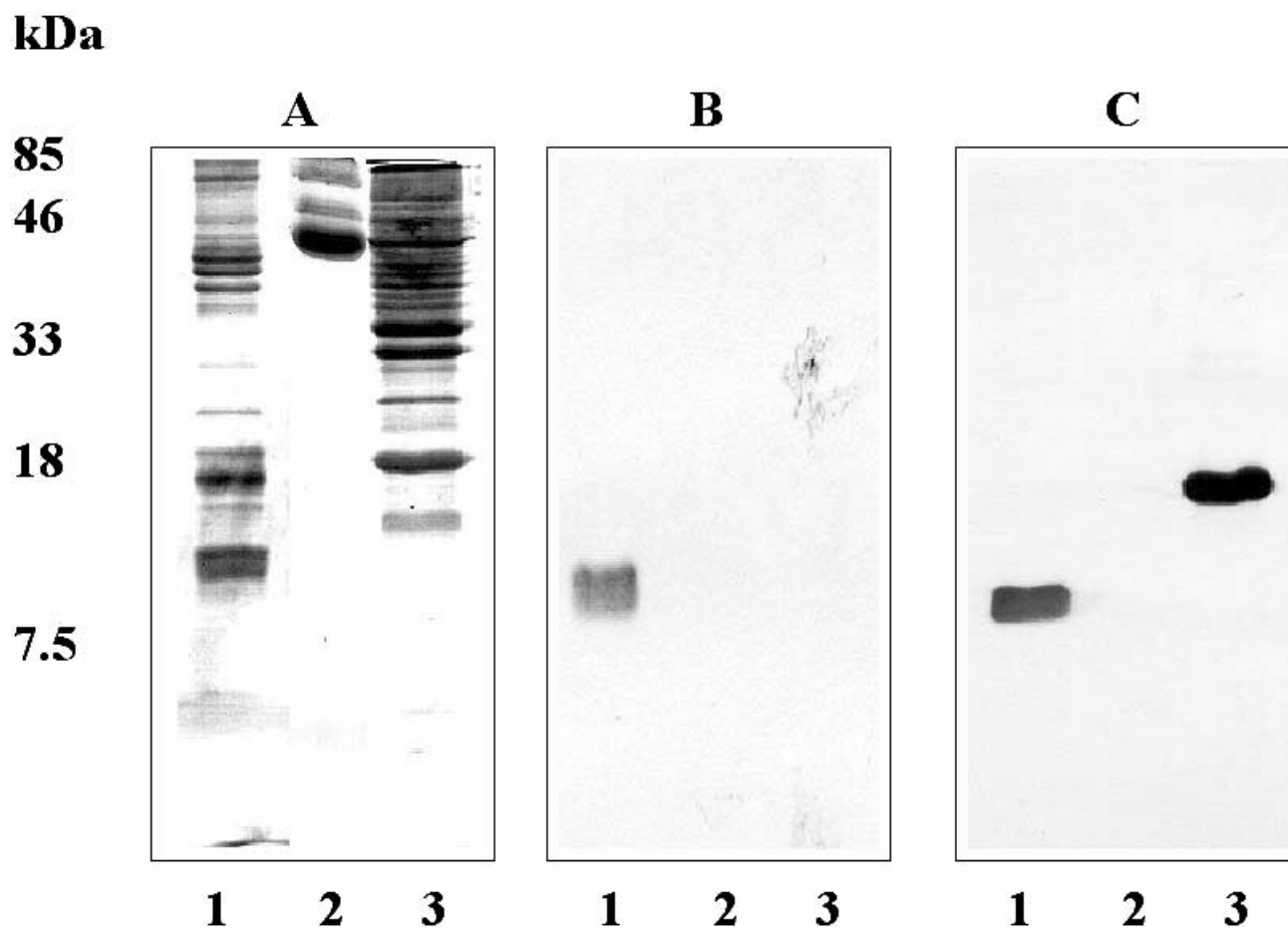


Figure 1. Effect of the fetal bovine serum (FBS). (A) Sodium dodecyl sulphate-polyacrylamide gel electrophoresis with fish extract (lane 1), FBS (2) and beef extract (3). (B) Immunoglobulin E-immunoblotting using FBS. (C) Immunoblotting without FBS.

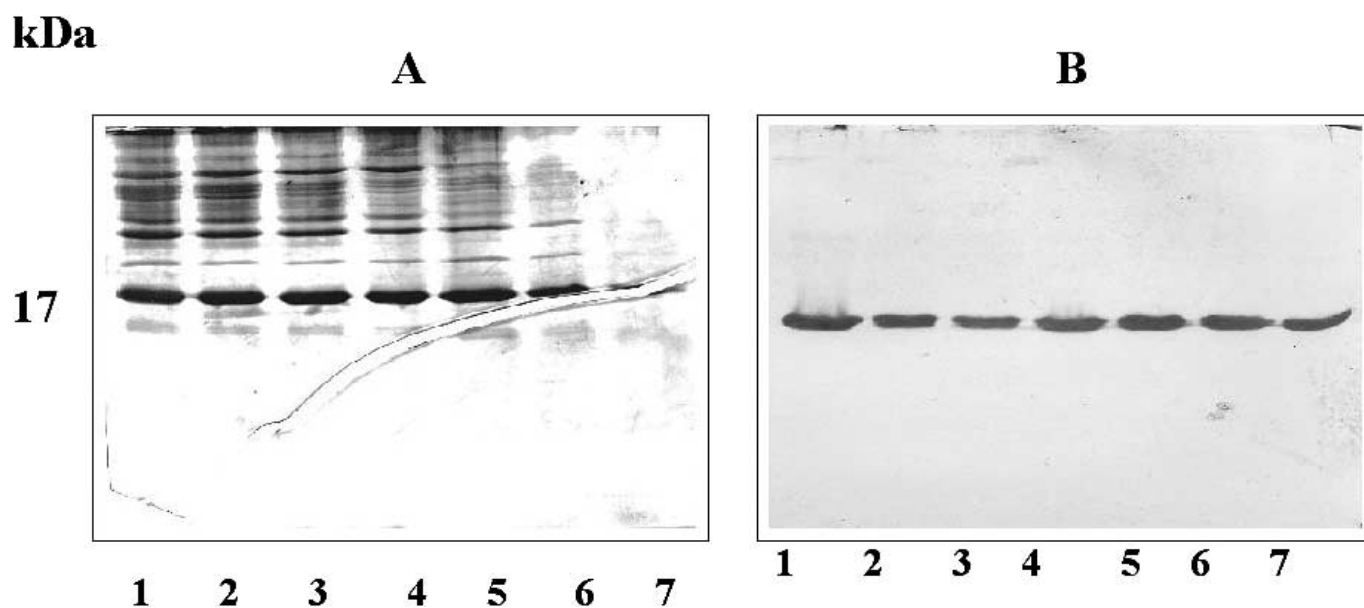


Figure 2. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoglobulin E-immunoblotting with raw beef meat after treatment at different temperatures for 15 min. (A) SDS-PAGE: room temperature (1), 50°C (2), 60°C (3), 70°C (4), 80°C (5), 90°C (6), 100°C (7). (B) Immunoblotting.

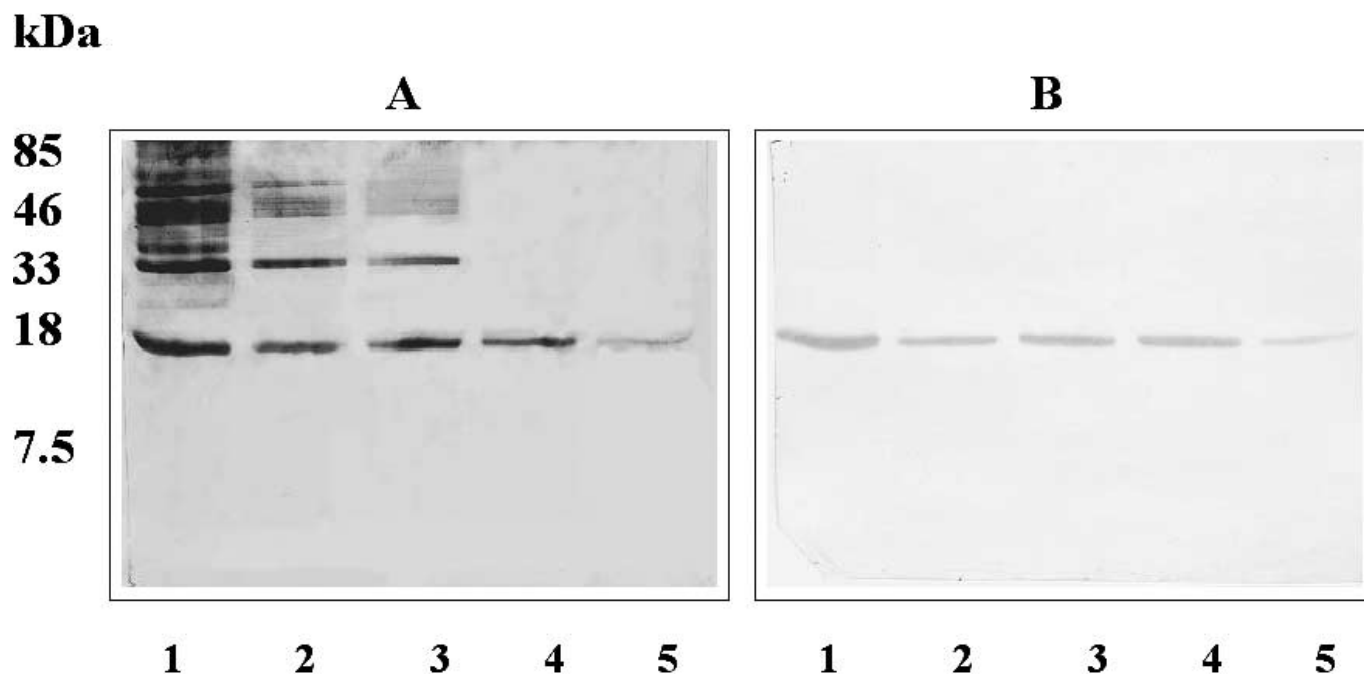


Figure 3. Ethanol fractionation of the crude beef extract. (A) Sodium dodecyl sulphate-polyacrylamide gel electrophoresis: crude extract (1), pellet obtained in a 50–90% vol/vol ethanol (2), 60–90% (3), 70–90% (4), 80–90% (5). (B) Immunoblotting.

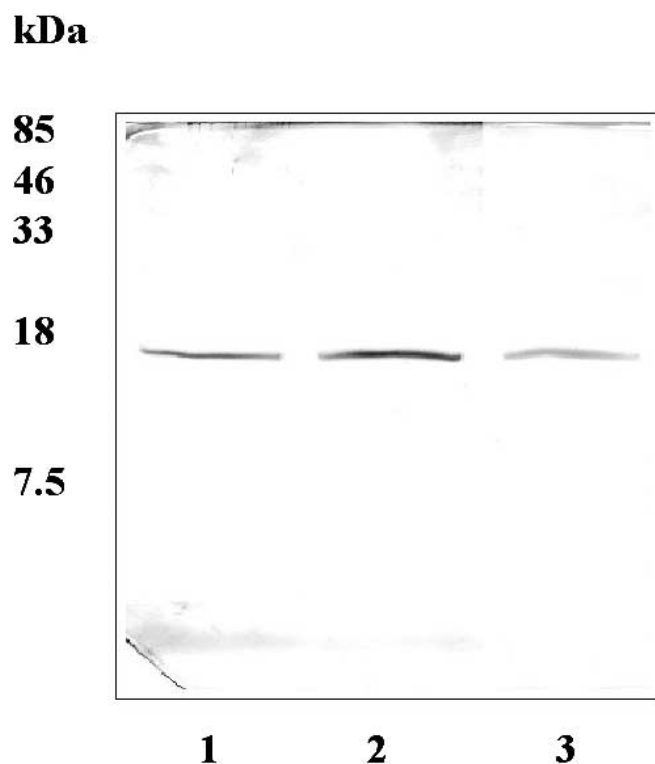


Figure 4. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of the semipurified allergen under reducing conditions in the sample buffer: (1) 2-mercaptoethanol at room temperature; (2) 2-mercaptoethanol at 100°C for 5 min; (3) nonreducing conditions.

rarely described (3, 4, 8–10). In our case, the patient presented symptoms both with fish and meat. When specific-IgE immunoblotting was performed, a protein with a molecular weight close to 12 kDa in the fish extract was detected. This band had the same molecular weight as main cod allergen (Gad c1) widely described in the literature (8, 9).

Most of the studies show that allergy to mammalian meat is associated with BSA (3, 5, 11). Its molecular weight ranges from 65 to 69 kDa and belongs to a highly conserved evolution protein group (12) that has been reported as a cause of cross-reaction between mammalian meat and danders (4, 12, 13). Different studies have demonstrated that the allergenicity of this protein is decreased by heat treatment (3, 5). For that reason, patients sensitized to this protein present symptoms only after ingestion of under-cooked meat, tolerating well-cooked meat.

In a recent study, Werfel et al. (3), described a small group of patients who were intolerant to either under-cooked or well-cooked meat. The sera recognized several protein bands, one of them was a protein with a molecular weight of 17.8 kDa. This protein could correspond to myoglobin, as detected by us. The IgE-immunoblotting with meat crude extract at different temperatures showed that this 17 kDa protein is heat-resistant and this fact could explain why prick to prick tests with rare and well-cooked meat were positive in our case. This feature suggests that the patients allergic to this protein should not tolerate under-cooked or even cooked meat. We believe that this fact has a great clinical relevance and it

could be important in order to decide the kind of diet for these sensitized patients.

Myoglobins of mammalian meats have a great degree of homology in their amino acid sequences. The patient tolerated pork meat, whose sequence has 11 differences in relation to human myoglobin, while bovine myoglobin has 23 variations. At the same time, 19 differences between pork and bovine myoglobin are found scattered along the molecule. Therefore, myoglobin allergy may be based on subtle changes of amino acids.

In summary, BSA is not the cause of all cases of meat allergy. Other cause may be a heat-resistant protein of

17 kDa that could be implicated in those patients that do not tolerate well-cooked meat. In this paper we present the first case of meat allergy by monosensitization to a heat-resistant 17 kDa protein.

Acknowledgments

This work was supported by a grant from Fundación de la Sociedad Española de Alergología e Inmunología Clínica.

References

1. SAMPSON HA. Food allergy. Part 1: Immunopathogenesis and clinical disorders. *J Allergy Clin Immunol* 1999;**103**: 717–725.
2. SAMPSON HA. Food allergy. Part 2: Diagnosis and management. *J Allergy Clin Immunol* 1999;**103**:981–988.
3. WERFEL SJ, COOKE SK, SAMPSON HA. Clinical reactivity to beef in children allergic to cow's milk. *J Allergy Clin Immunol* 1997;**99**:293–300.
4. AYUSO R, LEHRER SB, LÓPEZ M, REESE G, IBÁÑEZ MD, MARTÍN-ESTEBAN M, et al. Identification of bovine IgG as a major cross-reactive vertebrate meat allergen. *Allergy* 2000;**55**:348–354.
5. FIOCCHI A, RESTANI P, RIVA E, MIRRI GP, SANTINI I, BERNARDO L et al. Heat treatment modifies the allergenicity of beef and bovine serum albumin. *Allergy* 1998;**53**:798–802.
6. RESTANI P, FIOCCHI A, BERETTA B, VELONA T, GIOVANNINI M, GALLI C. Effects of structure modifications on IgE binding properties of serum albumins. *Int Arch Allergy Immunol* 1998;**117**:113–119.
7. MONEO I, CABALLERO ML, GÓMEZ F, ORTEGA E, ALONSO MJ. Isolation and characterization of a major allergen from the fish parasite *Anisakis simplex*. *J Allergy Clin Immunol* 2000;**106**:177–182.
8. BUGAJSKA-SCHRETTTER A, ELFMAN L, FUCHS T, KAPIOTIS S, RUMPOLD H, VALENTA R, et al. Parvalbumin, a cross-reactive fish allergen, contains IgE-binding epitopes sensitive to periodate treatment and Ca²⁺ depletion. *J Allergy Clin Immunol* 1998;**101**:67–74.
9. BERNHISEL-BROADBENT J, STRAUSE D, SAMPSON HA. Fish hypersensitivity. II: Clinical relevance of altered fish allergenicity caused by various preparation methods. *J Allergy Clin Immunol* 1992;**90**:622–629.
10. KANNY G, HAUTECLOCQUE C, MONERET-VAUTRIN DA. Food anaphylaxis to bovine serum albumin. *J Allergy Clin Immunol* 1998;**101**:137–139.
11. VERDAGUER J, COROMINAS M, BAS J, VALLS A, MESTRE M, ROMEU A et al. IgE antibodies against bovine serum albumin in a case of eosinophilic gastroenteritis. *Allergy* 1993;**48**:542–546.
12. PANDJAITAN B, SWOBODA I, BRANDEJSKY-PICHLER F, RUMPOLD H, VALENTA R, SPITZAUER S. *Escherichia coli* expression and purification of recombinant dog albumin, a cross-reactive animal allergen. *J Allergy Clin Immunol* 2000;**105**: 279–285.
13. SPITZAUER S, PANDJAITAN B, SÖREGI G, MÜHL S, EBNER C, KRAFT D et al. IgE cross-reacts against albumins in patients allergic to animals. *J Allergy Clin Immunol* 1995;**96**:951–959.