

Occupational Inhalant Allergy to Pork followed by Food Allergy to Pork and Chicken: Sensitization to Hemoglobin and Serum Albumin

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Established Facts

- Allergic reactions to meat in an occupational environment are clinically well documented. However, few responsible allergens have been identified so far.

Novel Insights

- Cross-reactive IgE antibodies to hemoglobin were identified in a patient allergic to pork and chicken who worked in the meat processing industry.
- Testing for sensitization to serum albumin and hemoglobin should be considered in the allergy work-up of patients employed in the meat processing industry.

Key Words

Chicken meat • Hemoglobin • Occupational allergy • Pork • Serum albumin

Abstract

Background: Animal-derived proteins are implicated in primary food allergies, but also in inhalant allergies with secondary food allergy symptoms. The objective of this study was to define the allergen(s) implicated in a case of food allergy to chicken meat, which developed in a person previously sensitized to pork after occupational exposure. **Methods:** A 42-year-old female with a history of occupational inhalant allergy to pork reported rhinitis, asthma, dysphonia

and conjunctivitis 30 min after ingestion of chicken. Skin tests were positive to chicken meat. Protein extracts were prepared from chicken meat. Allergens were characterized by IgE immunoblotting, N-terminal sequencing and ELISA. **Results:** The patient showed specific IgE binding to chicken meat proteins at 12, 14, 26, 55 and 65 kDa. N-terminal amino acid sequencing identified the 12- and 14-kDa proteins as the α - and β -chain of hemoglobin. ELISA and immunoblot showed specific IgE binding to hemoglobin purified from chicken blood. IgE antibodies to chicken serum albumin were detected by ELISA. Inhibition studies with chicken and porcine hemoglobin as well as with serum albumins demonstrated cross-reactive IgE antibodies. **Conclusions:** We report a case of confirmed occupational inhalant allergy due

to pork followed by food allergy to pork and 3 years later by food allergy to chicken. Porcine and chicken hemoglobin were found to be cross-reactive allergens. Cross-reactivity between porcine and chicken serum albumin was possibly linked to a prior sensitization to cat serum albumin.

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Introduction

Epidemiological studies have determined that the prevalence of food allergy in adults is between 2 and 5% [1–3]. The most frequent food allergens are cow's milk, hen's eggs, peanuts, tree nuts, fish and shellfish, with percentages of people affected varying regionally [3]. Allergy to meat is uncommon. In a study among 57 patients with a suspected meat allergy, the prevalence of sensitization was 73% for beef, 58% for pork and 41% for chicken [4].

Serum albumins are major allergens found in mammalian meat [5]. Bovine γ -globulin [6–8], myoglobin [9] and tropomyosin [4] have been identified as beef allergens. The only avian meat allergen described so far is chicken serum albumin (Gal d 5), formerly called α -live-tin. Gal d 5 is a heat-labile inhalant and food allergen implicated in the bird-egg syndrome [10–12].

The aim of this study was to characterize the sensitizing allergen(s) in a case of food allergy to chicken meat in the absence of a positive *in vitro* IgE diagnosis.

The allergic reaction to chicken meat developed in a female patient employed in the meat processing industry after she had changed her workplace because of respiratory symptoms triggered by pork handling.

Case Report

A 39-year-old female patient employed in the pork processing industry reported immediate urticaria, rhinitis and asthma when operating a sausage stuffing machine. She was exposed to natural pork sausage skin into which crude meat was packed. She was also in contact with evaporated soaking water as the guts were continuously hydrated. The investigations performed showed that she experienced an immediate bronchoconstriction after inhalation of pig-gut soaking water. Asthma attacks and cutaneous reactions resolved on weekends and vacations and began again when she ran the sausage-stuffing machine. She also became free of symptoms when she was transferred to another workplace in the same company.

One year after these first symptoms, she had asthma attacks after ingestion of cooked pork meat. She was known to be allergic to cats. At that time, cat and pig serum albumins as well as cat and pig γ -globulins were identified as allergens [13]. Symptoms disap-

peared when the patient was transferred to another workplace in the same company.

Three years later, the patient presented at the clinic for allergic symptoms, triggered by eating chicken leg, a food of which she had previously been tolerant. She reported rhinitis, asthma, dysphonia and conjunctivitis 30 min after ingestion of chicken meat. She reported that she had been free of occupationally related symptoms except that, 1 year earlier, she had suffered palpebral edema and at work she had had 1 episode of cough and dysphonia, which resolved in 15 min. Skin prick-to-prick tests were positive with raw and cooked chicken meat taken from the chicken leg (4 and 5 mm, respectively). Specific IgE to chicken meat (Phadia-CAP system; Phadia, Uppsala, Sweden) were negative. Skin prick tests were positive for cat and dog (Stallergènes, Antony, France; 3 mm in both cases).

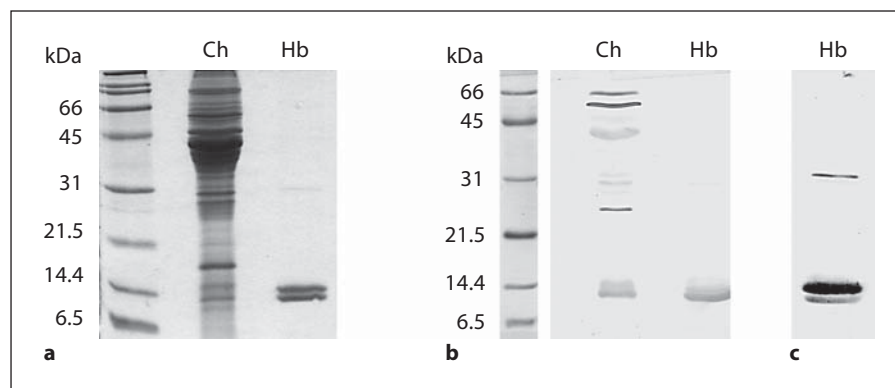
The recent history of the patient indicated that she had mild symptoms after eating duck leg. She was tolerant of rabbit and fish and had stopped eating pork or lamb. She also stopped eating blood pudding as she had experienced an asthmatic reaction immediately after ingesting it.

Methods

Raw chicken leg muscle was homogenized and extracted in single detergent lysis buffer (50 mM Tris HCl pH 8.0, 150 mM NaCl, 0.1% Triton X-100) containing a mixture of protease inhibitors (Roche, Mannheim, Germany). The protein extract was dialysed against phosphate-buffered saline (PBS) and concentrated. The patient's IgE antibodies to chicken meat were analyzed by immunoblot. Proteins were separated by 15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions and visualized by Coomassie Brilliant Blue (Gelcode® Blue; Pierce, Rockford, Ill., USA) colloidal staining. For immunoblotting, proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, Mass., USA), which were incubated overnight with the patient serum. Bound IgE antibodies were detected with anti-human IgE antibody labeled with alkaline phosphatase (Sigma, St. Louis, Mo., USA) and the blots were developed by addition of nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP; Promega, Madison, Wisc., USA). Rabbit anti-human hemoglobin antibody was from Sigma. Chicken leg muscle proteins were purified by ion exchange chromatography using a Resource S column (GE Healthcare, Amersham, UK) and a 0–500-mM NaCl gradient in 20 mM MES pH 6.3.

For N-terminal sequencing, proteins were separated by SDS-PAGE and transferred onto PVDF membranes by semi-dry transfer using CAPS-buffer (CAPS 10 mM, MeOH 20%). Bands of interest were subjected to Edman degradation on a Procise 49X HT protein sequencer (Applied Biosystems, Foster City, Calif., USA). Hemoglobin was purified from fresh chicken blood. Erythrocytes were obtained by a polysaccharide gradient (Lymphoprep™; Axis-Shield, Oslo, Norway) using a centrifugation step at 800 g for 20 min. The pellet was resuspended in hemolysis buffer (Tris-HCl 10 mM, EDTA 1 mM, MgCl₂ 5 mM, pH 7.5). Cellular debris were removed by centrifugation at 20,000 g for 20 min. The supernatant containing hemoglobin was dialysed against PBS using Amicon® Ultra 5K (Millipore). Hemoglobin was mixed with potassium hexacyanoferrate III (Merck, Darmstadt, Germany) at a molar ratio of 1:5 and incubated at 37°C to induce conversion into methemoglobin, which has a brown color.

Fig. 1. Electrophoretic analysis of chicken meat extract and purified hemoglobin. Chicken leg meat extract (Ch) and purified chicken hemoglobin (Hb) were separated by 15% SDS-PAGE and stained with Coomassie blue (a) or immunoblotted onto PVDF membranes for IgE detection with patient serum (b) or detection with a rabbit anti-hemoglobin antibody (c).



Specific IgE antibodies were quantified by ELISA. Purified chicken hemoglobin and methemoglobin, porcine and bovine hemoglobin (Sigma), serum albumin from cat (CSA), pig and chicken (all from Sigma) were coated at a concentration of 5 µg/ml in PBS to microtiter plates. 100 µl of diluted patient serum was added to the microtiter plate for 2 h. Binding of specific IgE was measured by incubation for 1 h with biotin-labeled anti-human IgE antibodies (1/2,000; Southern Biotechnology, Birmingham, Ala., USA), followed by an incubation for 30 min with alkaline phosphatase-labeled streptavidine (1/1,000; Pharmingen). p-NPP was added as a substrate and plates were left in the dark before optical density was read at 405 nm. The serum of a patient with a known titer of specific IgE to CSA determined by the Phadia CAP-System was serially diluted to generate a standard curve on which all determinations of specific IgE to hemoglobin and albumin were calculated. In ELISA inhibition experiments, the patient serum was preincubated for 2 h with 100 µg of inhibitor before addition to the microtiter plate. The percentage of IgE binding was calculated from the mean OD values, measured with or without addition of inhibitor.

Results

Skin prick tests were positive to cat and dog dander (3 mm in both cases). Prick-to-prick tests were positive to raw and cooked chicken leg meat (4 and 5 mm), raw and cooked duck meat (3 and 6 mm), cat and porcine γ-globulins (both 2 mm), porcine serum albumin (3 mm) and porcine gut (3 mm). Total IgE was 366 kU/l, specific IgE to pork was 14.1 kU/l, porcine serum albumin 11.5 kU/l, chicken meat <0.35 kU/l and chicken serum proteins 0.78 kU/l.

A protein extract was made from chicken leg muscle and the protein pattern was analyzed by SDS-PAGE (fig. 1a, lane Ch). IgE immunoblotting with the patient's serum showed strong bands at ~12, 26, 55 and 65 kDa and some weak bands at 14, 30 and 42 kDa (fig. 1b, lane Ch).

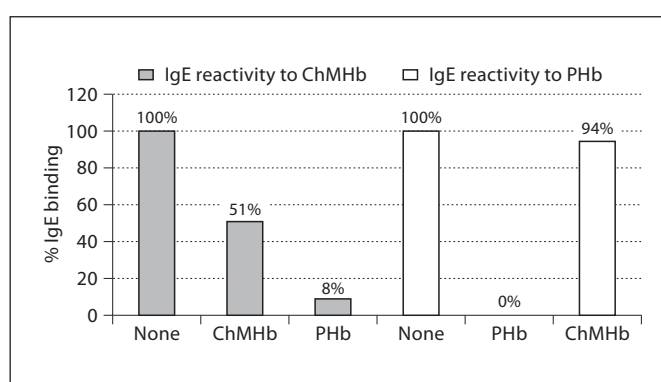


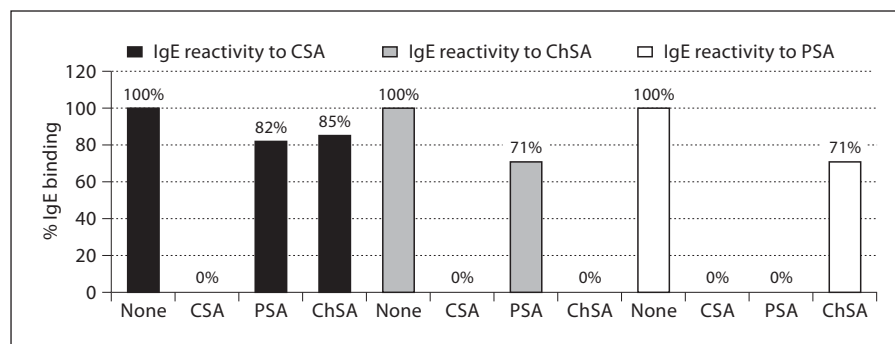
Fig. 2. Inhibition of IgE binding to hemoglobin. Patient serum was tested by enzyme-linked immunosorbent assay for IgE binding to chicken and porcine hemoglobin. The y-axis displays the percentage of IgE binding after preincubation with inhibitor compared to the binding without inhibitor. Inhibitors added are indicated below the x-axis. ChMHb = Chicken methemoglobin; PHb = porcine hemoglobin.

N-terminal sequence analysis identified the 12 and 14 kDa bands as, respectively, the α- and β-chain of chicken hemoglobin, and the 30-kDa band as the dimer of α- and β-hemoglobin.

In order to confirm the identity of the 12-kDa protein, hemoglobin was purified from chicken blood (fig. 1a, lane Hb). The IgE antibodies of the patient recognized the α chain of chicken hemoglobin and, to a lesser extent, the β chain (fig. 1b, lane Hb). A commercial anti-human hemoglobin antibody recognized preferentially the β chain of the purified molecule, as well as the dimeric form at 30 kDa (fig. 1c, lane Hb).

Specific IgE antibodies to different hemoglobins and chicken serum albumin were determined by ELISA. Respective IgE titers were 1.4 kU/l for chicken methemoglobin

Fig. 3. Inhibition of IgE binding to serum albumin. Patient serum was tested by enzyme-linked immunosorbent assay for IgE binding to cat (CSA), porcine (PSA) and chicken (ChSA) serum albumin. The y-axis displays the percentage of IgE binding after preincubation with inhibitor compared to the binding without inhibitor. Inhibitors added are indicated below the x-axis.



bin, 1.2 kU/l for chicken hemoglobin, 3.0 kU/l for porcine hemoglobin and 1.5 kU/l for bovine hemoglobin. Specific IgEs to chicken serum albumin were 1.3 kU/l.

Proteins of chicken leg muscle extract were separated by ion exchange chromatography, and the N-terminal sequence of the IgE-reactive 55-kDa protein was determined. The sequence PLSADPHFKLLEWH aligned 100% on chicken glucose-6-phosphate isomerase. This protein has 82% aa identity with human glucose-6-phosphate isomerase. The identity of the 26-kDa protein could not be defined by N-terminal sequencing.

The patient's history suggested a possible cross-reactivity between pork and chicken. ELISA inhibition studies showed that IgE reactivity to chicken hemoglobin was nearly completely inhibited by porcine hemoglobin. However, IgE antibodies to porcine hemoglobin were only weakly inhibited by chicken hemoglobin (fig. 2).

As mammalian albumins are known to be cross-reacting allergens, the IgE binding pattern to cat, porcine and chicken serum albumin was evaluated by IgE inhibition in ELISA. Addition of CSA totally abolished IgE binding to porcine and chicken serum albumin (fig. 3).

Discussion

A pork processing industry worker first presented respiratory symptoms when manipulating pork gut at work. She had also respiratory symptoms when eating pork [13]. Later she developed allergic reactions when eating chicken. The immunological investigation driven by positive skin tests to chicken meat showed that IgE antibodies recognized protein bands in chicken leg muscle extract migrating at 12, 14, 26, 55 and 65 kDa. The 12- and 14-kDa bands were identified as chicken hemoglobin alpha and beta chain. The 55-kDa protein was assumed to be glucose-6-phosphate isomerase, based on the N-terminal se-

quence, and the 65-kDa protein probably is chicken serum albumin.

Amino acid sequence identities between chicken and human α - and β -hemoglobin chains are very high (70%). Between pig and human, they are even higher (85%). This degree of identity is of the same order of magnitude as that between mammalian serum albumins (porcine and human 76%, cat and human 82%). It has been proposed that molecules with a sequence identity of more than 62% rarely elicit allergic reactions [14]. Serum albumins, however, have been shown to be highly cross-reactive molecules in respiratory and food allergy [5, 15, 16]. Our results show that vertebrate hemoglobin can generate IgE antibodies under certain exposure conditions despite their high sequence homology to human hemoglobin. In our patient, IgE antibodies to porcine hemoglobin were cross-reacting with chicken hemoglobin. Homologies between vertebrate hemoglobins are high, and cross-reactivity is thus to be expected.

As the chronology of the patient's sensitization was from pork to chicken, cross-inhibition experiments were performed to confirm the direction of sensitization. IgE reactivity to chicken hemoglobin was inhibited by porcine hemoglobin. In our patient, porcine hemoglobin can be assumed to be primarily a respiratory allergen as she had been exposed to pig gut and crude pork meat in her first occupational job. The patient then became allergic to chicken meat, as she showed respiratory symptoms after ingestion of chicken leg. She tolerates chicken white meat which contains very little hemoglobin. However, as the patient has IgE antibodies to chicken hemoglobin and serum albumin which are both present in meat and blood, it is difficult to define the clinical relevance of each of these allergens.

As the patient had allergic rhinitis in the presence of cats, CSA was also included in the inhibition assay. CSA was able to inhibit totally IgE binding to porcine but, sur-

prisingly, also to chicken serum albumin. These results suggest that the primary sensitization was to CSA and that the sensitizations to porcine and chicken serum albumin are cross-sensitizations. CSA and chicken serum albumin share only 46% amino acid identities and cross-reacting IgE antibodies are less frequently reported than cross-reacting IgE between mammalian serum albumins [5, 15].

An increasing number of studies have documented allergic reactions to food following inhalation, especially in occupational environments [17]. Respiratory allergy to animal food allergens is well known for fish and shellfish as well as for egg proteins, which become aerosolized in the food-processing industries [18–21]. Symptoms typically include respiratory manifestations such as rhinoconjunctivitis, coughing, wheezing, dyspnea and asthma. Rhinitis symptoms caused by high-molecular-weight agents such as proteins are generally more intense than those triggered by low-molecular-weight agents [22].

Several studies report immediate type skin reactions to animal proteins [23]. Occupational contact dermatitis has been described in slaughterhouse workers and butchers. One study reported a 22% occurrence rate of contact dermatitis in 144 slaughterhouse workers [24]. A scratch

patch test with meat was positive for 12 out of 31 workers. For some patients an IgE-mediated allergy was suggested by positive prick tests or specific IgE to meat [25–27]. A butcher experiencing asthma, dizziness and angioedema after consumption of beef was reported [28]. Skin prick tests and a positive challenge to cooked beef confirmed the immediate type allergy. Concomitant pork meat contact dermatitis and occupational asthma was also reported in a patient working in a pork meat processing plant [29]. Recently, González-Muñoz [30] reported a case of occupational contact dermatitis to chicken meat in a butcher. Interestingly, specific IgE to chicken meat were negative as in our case, but the prick test as well as the basophil activation assay were positive.

In conclusion, we have documented a case of sequential allergy to pork and then chicken in a patient employed in the pork-processing industry. Serum albumin as well as a new allergen, hemoglobin, could be identified as IgE cross-reactive proteins. Testing for sensitization to serum albumin and hemoglobin seems promising for patients with immediate type allergy to meat but possibly also for patients with contact dermatitis who work in the meat-processing industry.

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