Anaphylactic Reactions to Novel Foods: Case Report of a Child With Severe Crocodile Meat Allergy

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abstract

Availability of "exotic" foods is steadily increasing. In this report, we describe the first case of anaphylaxis to crocodile meat. The patient was a 13-year-old boy with severe immunoglobulin E–mediated allergy to chicken meat. When tasting crocodile meat for the first time, he developed an anaphylactic reaction. Cross-reactivity between chicken and crocodile meat was suspected to have triggered this reaction. Basophil activation and immunoglobulin E testing confirmed the boy's allergic reaction to crocodile meat proteins. Molecular analysis identified a crocodile α-parvalbumin, with extensive sequence homology to chicken α-parvalbumin, as the main cross-reactive allergen. We conclude that crocodile meat can be a potent food allergen and patients with allergy to chicken meat should be advised to avoid intake of meat from crocodile species. Both foods and people travel around the world and accessibility to exotic foods is steadily growing. As a result, novel allergic cross-reactivities are likely to become a challenge in the management of food allergy and, as our report illustrates, cross-reactivity has to be considered even between foods that might not intuitively be perceived as related.

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Dr Ballardini conceptualized and designed the study, was responsible for care of the patient, and drafted the first manuscript; Dr van Hage designed the study and was responsible for the initial immunoglobulin E (IgE) analyses, immunoblots, and cross-inhibition experiments, and critically reviewed and revised the manuscript; Drs Kuehn and Ollert planned and carried out the molecular experiments, subsequent IgE analysis, immunoblots, and cross-inhibition tests, and critically reviewed and revised the manuscript; Dr Nopp conceptualized and designed the study, performed basophil allergen threshold sensitivity analysis, and reviewed and revised the manuscript; Dr Hamsten carried out the initial IgE analyses, immunoblots, and cross-inhibition experiments and critically reviewed and revised the manuscript; Dr Vetander performed the literature review and reviewed and revised the manuscript; Dr Nilsson participated in patient care

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Food allergy is an increasing problem in the pediatric population and is estimated to affect 8% of children with a high negative impact on quality of life.[1–3] Allergy to chicken meat has been reported both in children and adults and is estimated to be rare, but reliable prevalence data are missing.[4–8] Cross reactivity between chicken and duck, turkey, and goose has been described.[4] Little is known about the causative allergens for chicken meat allergy. There are no reports of allergic reactions to crocodile meat. Although crocodiles belong to the reptile group, they are also the closest living relatives of birds.[9]

PRESENTATION

A 13-year-old boy presented to the pediatric emergency department at Sachs' Children and Youth Hospital in Stockholm, Sweden, due to an anaphylactic reaction to crocodile meat. He had been a regular outpatient at the hospital's pediatric allergy department since 5 years of age when he was diagnosed with chicken meat allergy (specific immunoglobulin E [IgE], 19 kU_A/L). He was otherwise healthy and ate all foods except poultry. At 7 years of age, an adrenaline autoinjector was prescribed after an anaphylactic reaction due to accidental consumption of turkey.

Although strict avoidance of chicken and turkey meat was recommended, he continued to experience anaphylactic reactions due to accidental consumption of foods contaminated by chicken or turkey. As a consequence, he was reluctant to eat foods outside of his home, which had a significant impact on his social life. More recently, his father, a professional chef, bought and prepared crocodile meat to create a novel meal for his son. The boy reacted at first bite with itch in the mouth and throat, facial urticaria, conjunctivitis, angioedema, chest tightness, and breathing difficulties. Intramuscular adrenaline and β-2 agonist inhalation was administered at home by the parents. At the pediatric emergency department, he presented with facial urticaria, angioedema around the eyes, bilateral redness of the sclera, and heavy breathing but no bronchoconstriction. After 4 hours of observation, he was free of symptoms and discharged.

Reviewing the literature, we found no reports of allergic reactions to crocodile. We had previously identified α-parvalbumin as a relevant chicken meat allergen[7] and because another report showed that parvalbumin is expressed in the tail muscle of *Alligator mississippiensis*,[10] we hypothesized that the reaction to crocodile was due to IgE cross-reactivity between α-parvalbumins in chicken and crocodile meat.

METHODS

Written informed consent was obtained from the patient and his parents.

Allergen Preparation

Crocodile filet was purchased from the same store that sold the crocodile meat that caused the allergic reaction. It was analyzed by the certified laboratory of the Swedish National Food Agency (Livsmedelsverket; Uppsala, Sweden) for content of DNA and protein from chicken and turkey. In addition, the crocodile filet was used to prepare a protein extract.[11] Briefly, the muscle tissue was grinded in liquid nitrogen followed by extraction in lysis buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, and 1% Triton X-100). Extract supernatants were dialyzed against phosphate-buffered saline (pH 7.2) before basophil activation assay and specific IgE–binding analysis. For the basophil activation assay and measurements of allergen-specific IgE, muscle tissue was homogenized followed by extraction in phosphate-buffered saline (pH 7.4).

Measurement of Allergen-Specific IgE

IgE antibodies against chicken (f83), turkey (f284), and crocodile meat proteins (5 μg of biotinylated crocodile extract coupled to Streptavidin ImmunoCAP) and total IgE were measured by ImmunoCAP (Phadia AB/Thermo Fisher Scientific, Uppsala, Sweden).

Basophil Activation Assay

Basophil allergen threshold sensitivity (CD-sens) was performed as previously described.[12,13] Briefly, cells were stimulated with increasing concentrations of crocodile or chicken extracts (0.5–500 ng protein/mL) or negative controls (peanut, birch, casein) and then analyzed by flow cytometry.

Allergen Analysis by IgE Immunoblot and Enzyme-Linked Immunosorbent Assay

Chicken and crocodile meat extracts were separated by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis).[11] Patient serum was diluted 5× in 3% bovine serum albumin (Sigma-Alderich, Bornem, Belgium) for immunoblot and 5–10× for IgE enzyme-linked immunosorbent assay (ELISA).[11] For IgE cross-inhibition, patient serum was preincubated with chicken or crocodile extract at a concentration of 1 mg inhibitor/mL for immunoblot and 200 μg inhibitor/mL for ELISA. Parvalbumins were detected by using an antiparvalbumin IgG antibody mix (Swant, Marly, Switzerland; Abcam, Cambridge, MA).

Biomolecular Allergen Characterization

Chicken and crocodile parvalbumins were isolated by ion exchange and gel filtration chromatography as previously reported.[7,14] Protein purity of chicken and crocodile parvalbumins was confirmed by silver-stained, two-dimensional SDS-PAGE.[11] Crocodile parvalbumin isoforms were additionally separated by isoelectric focusing (Offgel, Agilent, Diegem, Belgium) before an intact mass measurement by mass spectrometry (MS) analysis. Parvalbumins were trypsinized for MS peptide mass fingerprint analysis,[11] which revealed the complete sequence of 1 crocodile parvalbumin isoform. Unidentified peptides of the other isoform were sequenced de novo on a second MALDI TOF/TOF instrument (AB Sciex 5800, Framingham, MA), identifying 92% of the protein sequence. Protein structures were calculated using Modeller 9v2 software,[15,16] and chicken parvalbumin (P43305; 2KYF) was used to establish the surface model.

RESULTS

No contamination of the crocodile meat by chicken or turkey residues was demonstrated. The boy's serum immunoglobulin E (sIgE) levels were 40 kU_A/L to chicken, 14 kU_A/L to turkey, and 6 kU_A/L to crocodile meat extract, whereas total serum-IgE was 190 kU/L. CD-sens was clearly positive to both crocodile and chicken meat extracts (Fig 1A). In immunoblot analysis, the patient's IgE bound

to chicken (14 kDa, 19/22 kDa, 30 kDa) and crocodile meat proteins (18–50 kDa) (Fig 1B). IgE-binding to chicken allergens was not inhibited by preincubation with crocodile extract, whereas IgE-binding was inhibited by preincubation with chicken extract (Fig 1B). Because chicken is never consumed raw, an extract from heated chicken meat was also included in the immunoblot analysis. The patient's sIgE recognized a single 14-kDa chicken protein only, which was confirmed to be parvalbumin by using an antiparvalbumin antibody. Homologous crocodile parvalbumins were detected as 2 bands at 6 and 14 kDa (potentially 2 isoforms, denoted "6-kDa" and "14-kDa parvalbumin"). The patient's sIgE levels were 108 kU_A/L to purified chicken α-parvalbumin and 25 kU_A/L to crocodile 14-kDa parvalbumin. The selective IgE reactivity to crocodile parvalbumins was confirmed by IgE immunoblot, where the patient's IgE antibodies only detected the 14-kDa but not the 6-kDa isoform (Fig 1B). After preincubation of serum with the respective homolog, IgE reactivity to chicken α-parvalbumin and 14-kDa crocodile parvalbumin was efficiently inhibited (94% and 100%, respectively). Amino acid sequencing revealed the complete sequence of the 14-kDa (108/108 amino acids) and most of the 6-kDa crocodile parvalbumin (100/108 amino acids). The protein identity of the 14-kDa crocodile parvalbumin compared with the chicken homolog was 94%, and a molecular model comparison visualized extensive surface identity of both allergens (Fig 2).

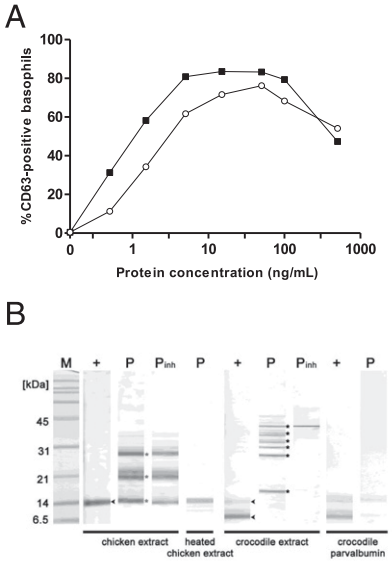

FIGURE 1

Basophil reactivity and immunoblot with chicken and crocodile meat extracts. A, Basophil reactivity (CD-sens) against crocodile (○) and chicken (■) extracts. CD-sens was also performed against peanut, birch, and casein as negative controls and basophils from a nonallergic blood donor were tested against crocodile and chicken extracts with negative results (not shown in figure). B, Detection of IgE-reactive chicken and crocodile proteins. Patient serum (P) was analyzed for IgE reactivity to raw/heated meat extracts and purified crocodile parvalbumin by immunoblot. IgE cross-inhibition (P_{inh}) was tested by serum preincubation with chicken and crocodile extracts before immunoblot. +, antiparvalbumin antibody (arrows); ★, IgE-reactive proteins; M, molecular weight marker.

DISCUSSION

To the best of our knowledge, this is the first report of a food-allergic reaction to crocodile meat. We demonstrate that this anaphylactic reaction, confirmed by the positive basophil challenge (CD-sens) with crocodile meat, was due to cross-reactivity of a highly homologous α-parvalbumin found in both chicken and crocodile meat. Our hypothesis of clinical cross-reactivity via highly conserved B cell epitopes on these parvalbumins could be confirmed by IgE cross-inhibition assays using the purified chicken and crocodile allergens. Furthermore, we showed extensive sequence identity representing putative linear epitopes and high structural homology representing putative conformational epitopes. Although chicken was assumed to be the primary sensitization source, the patient had experienced anaphylactic reactions to turkey as well. These reactions can be explained by our previous finding that chicken and turkey α-parvalbumin are 100% identical,[7] which reflects their close phylogenetic relationship. In another recent publication, we reported on 36 patients (including 17 children <14 years old) with chicken meat allergy and showed that 61% had specific IgE against the allergen Gal d 8, chicken parvalbumin.[17] Therefore, most patients with chicken meat allergy might also be at risk for allergic reactions to crocodile meat. We believe that chicken meat allergic individuals should be informed about the potential risk of cross-reactivity and advised to avoid meat from crocodile species.

Both foods and people travel around the world and accessibility to "exotic" foods is steadily growing. Food safety is an important aspect to be addressed before the introduction of these new products to the market.[18] Assessment of potential allergenicity of novel foods is highly challenging because it is not well understood what makes a food protein a potent allergen. However, as this case illustrates, additional research concerning allergic cross-reactivities is needed so that clinicians will be able to give accurate advice to food-allergic patients intending to broaden their diet.

CONCLUSIONS

Crocodile meat can be a potent food allergen. Because patients with allergy to chicken meat are at risk for reacting to crocodile meat, clinical testing with crocodile meat may need to be included in the diagnostic workup of these individuals. Novel allergic cross-reactivities are likely to become a challenge in the management of food allergy and, as our report illustrates, cross-reactivity has to be considered

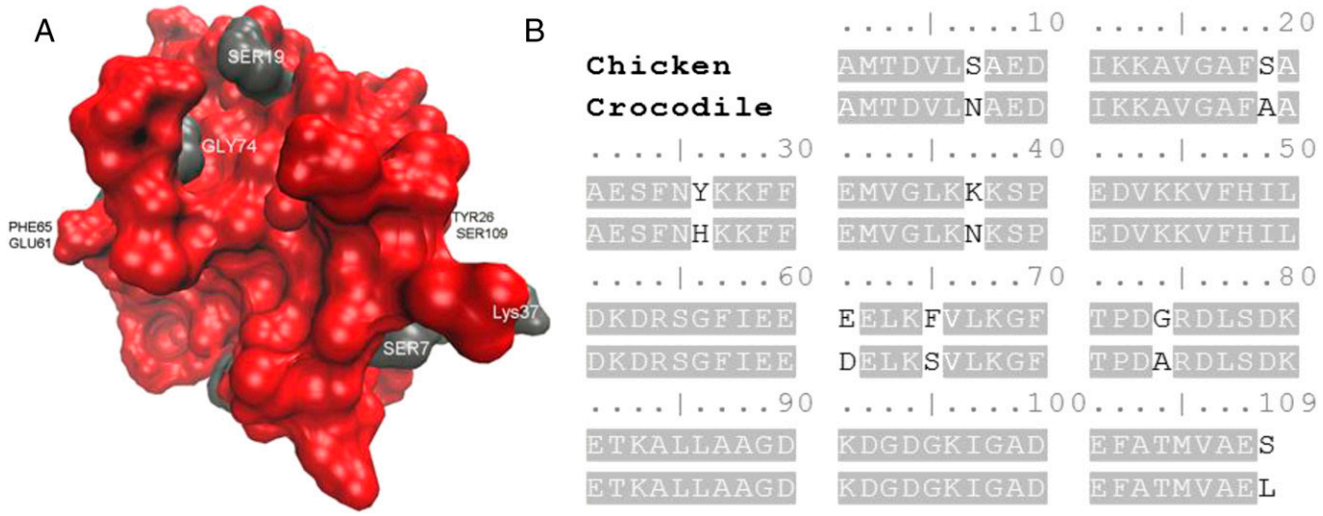

FIGURE 2

Comparison of chicken and crocodile α-parvalbumin. A, Three-dimensional model of chicken parvalbumin, protein surface coloring by identity to crocodile parvalbumin. Red, identical residues; gray, variable residues. B, Amino acid sequence alignment of chicken and crocodile parvalbumins as allergens involved in the clinical cross-reaction to both meats.

even between foods that might not intuitively be perceived as related.

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ABBREVIATIONS

CD-sens: basophil allergen threshold sensitivity
ELISA: enzyme-linked immunosorbent assay
IgE: immunoglobulin E
MS: mass spectrometry
sIgE: serum immunoglobulin E

and reviewed and revised the manuscript; Drs Melén and Flohr provided intellectual input and reviewed and revised the manuscript; and all authors approved the final manuscript as submitted.

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