Relationship between red meat allergy and sensitization to gelatin and galactose-α-1,3-galactose

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Background: We have observed patients clinically allergic to red meat and meat-derived gelatin.
Objective: We describe a prospective evaluation of the clinical significance of gelatin sensitization, the predictive value of a positive test result, and an examination of the relationship between allergic reactions to red meat and sensitization to gelatin and galactose-α-1,3-galactose (α-Gal).
Methods: Adult patients evaluated in the 1997-2011 period for suspected allergy/anaphylaxis to medication, insect venom, or food were skin tested with gelatin colloid. *In vitro* (ImmunoCAP) testing was undertaken where possible.
Results: Positive gelatin test results were observed in 40 of 1335 subjects: 30 of 40 patients with red meat allergy (12 also clinically allergic to gelatin), 2 of 2 patients with gelatin colloid–induced anaphylaxis, 4 of 172 patients with idiopathic anaphylaxis (all responded to intravenous gelatin challenge of 0.02-0.4 g), and 4 of 368 patients with drug allergy. Test results were negative in all patients with venom allergy (n = 241), nonmeat food allergy (n = 222), and miscellaneous disorders (n = 290). ImmunoCAP results were positive to α-Gal in 20 of 24 patients with meat allergy and in 20 of 22 patients with positive gelatin skin test results. The results of gelatin skin testing and anti–α-Gal IgE measurements were strongly correlated ($r = 0.46$, $P < .01$). α-Gal was detected in bovine gelatin colloids at concentrations of approximately 0.44 to 0.52 μg/g gelatin by means of inhibition RIA.
Conclusion: Most patients allergic to red meat were sensitized to gelatin, and a subset was clinically allergic to both. The detection of α-Gal in gelatin and correlation between the results of α-Gal and gelatin testing raise the possibility that α-Gal IgE might be the target of reactivity to gelatin. The pathogenic relationship between tick bites and sensitization to red meat, α-Gal, and gelatin (with or without clinical reactivity) remains uncertain. (J Allergy Clin Immunol 2012;129:1334-42.)

Key words: *Food allergy, anaphylaxis, red meat, α-galactose, gelatin, colloid*

Allergic reactions to red meat are relatively uncommon and responsible for 3% of food allergy (FA) cases in some series, as recently reviewed.[1] Beef is the most commonly reported meat allergen, with up to 20% of children with cow's milk allergy reported as having beef allergy.[2] Previous studies describe BSA and bovine IgG as the dominant beef allergens and, to a lesser extent, muscle-derived proteins, such as actin, myosin, or tropomyosin.[3] Allergic reactions to bovine- and porcine-derived gelatin are less commonly described,[4-8] but clinical reactivity to red meat and gelatin in the same patient has not previously been reported. Nonetheless, gelatin is an ingredient of some processed foods[9] and gelatin colloids[10] and is used as a stabilizing agent in some vaccines[11,12] and is thus potentially a cryptic allergen. Finally, adverse reactions to pork, lamb, rabbit, chicken, and turkey are relatively uncommon, with case reports of kangaroo, seal, and whale meat allergy reflecting different regional exposures.[13-18]

Recent research has demonstrated the importance of the IgE response to the cross-reactive carbohydrate determinant galactose-α-1,3-galactose (α-Gal) as a potential mediator of adult-onset red meat allergy[19] and a possible relationship with exposure to tick bites in Australian[20] and US[21] studies. The fortuitous observation of 1 patient allergic to red meat and topical gelatin[4] and 2 patients with initial anaphylaxis to intraoperative gelatin colloid followed by anaphylaxis to red meat on separate occasions[5] prompted a prospective 15-year evaluation of the clinical significance of gelatin sensitization and the predictive value of a positive skin test result and an examination of the relationship between allergic reactions to red meat and sensitization to gelatin and α-Gal.

METHODS

Study population

The study was undertaken in a mixed adult/pediatric specialty allergy/immunology practice in the Australian Capital Territory in southeastern Australia. The practice services the local inland metropolitan population and surrounding regional (including coastal) areas. Referrals were received from general medical practitioners, accident and emergency departments, and pediatricians. Patients were assessed by the first author (R.J.M.). Clinical and demographic data were entered prospectively into a searchable database (Blue Chip Clinical Research Module, Health Communication Network, Sydney, Australia; Microsoft Access, Microsoft Corporation, Redmond, Wash). Data (and accuracy) were analyzed and verified retrospectively. The characteristics of all patients aged greater than 18 years evaluated in the calendar years 1995 to 2011 were analyzed. The Human Research and Ethics committee (Calvary Bruce/Calvary John James Private Hospitals) approved the study.

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Part of this work has been presented in abstract form in the last 5 years at meetings of the American Academy of Allergy, Asthma & Immunology and of the Australasian Society for Clinical Immunology and Allergy.

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Abbreviations used
α-Gal: Galactose-α-1,3-galactose
FA: Food allergy
IDT: Intradermal test
SPT: Skin prick test

Patient evaluation

Glycerinated commercial food allergen extracts (beef and pork; Hollister-Stier, Spokane, Wash) and histamine 10 mg/mL positive control (Hollister-Stier) were purchased from Link Pharmaceuticals Australia (Sydney). In the absence of commercial extracts (in Australia) for lamb, kangaroo, or horse meat allergy testing, a fresh 10% wt/vol slurry was prepared by using ground meat in saline, with the supernatant used for skin prick tests (SPTs) when required. The bovine gelatin–derived colloids Haemaccel (35 mg/mL gelatin) and Gelofusine (40 mg/mL gelatin) were purchased from Aventis Pharma (Sydney, Australia) and B. Braun (Castle Hill, New South Wales, Australia), respectively. Gelatin in these products is extracted from bovine bones only, excluding the skull (Hartley Atkinson, AFT Pharmaceuticals, and Howard Johnson, B Braun Pharmaceutics, personal communications, 2007) by using a combination of acid and alkaline hydrolysis, followed by heat extraction at temperatures of up to 90°C and then sterilized at temperatures of greater than 100°C. SPTs and intradermal tests (IDTs) were performed on the volar aspect of the forearm and interpreted according to standard guidelines.[22] SPTs were performed with metal lancets (Stallergenes, Antony, France). A positive SPT response was defined as a wheal size of at least 3 mm greater than that elicited by a negative control (saline) at 15 minutes. Insulin syringes with 27-gauge needles were used for IDTs to introduce approximately 0.02 mL of allergen. A positive IDT result was defined as a wheal more than 5 mm larger than that elicited by the negative control (saline) at 15 minutes accompanied by itching and surrounding flare. SPT and IDT results were recorded as the mean wheal diameter. Undiluted Haemaccel and Gelofusine were used for SPTs and IDTs. When results of SPTs with beef and pork were negative, IDTs were undertaken with the same commercial extracts freshly diluted 1:100 in saline, as previously described.[19] When results of SPTs with gelatin colloid were negative, IDTs were undertaken with undiluted colloid. The primary indication for undertaking SPTs/IDTs was a history of possible red meat allergy, gelatin allergy, or both. Secondary indications (for research purposes) were suspected drug or insect venom allergy or FA/anaphylaxis, where most adults with anaphylaxis (>90%) assessed between 1997-2011 were tested as well.

Other patients tested were those with chronic urticaria/angioedema, as well as other less common conditions described in the Results section, who were not considered likely to have IgE-mediated FA but where testing was undertaken for the purposes of patient reassurance. After descriptions of a possible relationship between tick bites and adult-onset red meat allergy,[20,21] tick bite–reactive patients were also tested.

Diagnostic criteria

Sensitization was defined as the presence of a positive SPT or IDT response. IgE-mediated FA was diagnosed only if there was also a history of an acute systemic allergic reaction (≥1 of urticaria, vomiting, bronchospasm, or vascular collapse) after known allergen exposure combined with a positive SPT or IDT result to the relevant allergen. The severity of systemic allergic reactions was classified as described by Brown[23]: mild (skin and subcutaneous tissue involvement only), moderate (features suggestive of respiratory, cardiovascular, or gastrointestinal involvement: dyspnea, wheeze, chest or throat tightness, nausea, vomiting, abdominal pain, dizziness, and sweating), or severe (cyanosis, hypotension, confusion, collapse, loss of consciousness, and incontinence). A diagnosis of anaphylaxis was assigned if either of the first 2 criteria of the 2005 National Institutes of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network Symposium definition were fulfilled.[24] For the purposes of this study, red meat was defined as beef, lamb, pork, horse, or kangaroo, and red meat allergy was diagnosed when 1 or more was considered to be the cause of FA.

In vitro testing

Sera were placed in aliquots and stored at −5°C in the Australian Capital Territory and then transported on dry ice to the University of Virginia and stored at −20°C until analysis. Total and specific IgE antibody levels were measured by using either commercially available ImmunoCAP (Phadia US, Portage, Mich) or a modification of the assay with streptavidin on the solid phase, as previously described.[19,25] The assays were performed with the ImmunoCAP 250 instrument, and the results were expressed as international units per milliliter, with the international unit both for specific and total IgE being approximately 2.4 ng. A positive anti–α-Gal–specific assay result was defined as greater than 0.35 IU/mL. IgE antibodies to α-Gal were measured with the streptavidin-CAP technique by adding approximately 5 μg of biotinylated antigen to each CAP before adding 40 μL of undiluted serum. IgE antibodies to beef (f27), pork (f26), lamb (f88), and bovine gelatin (c74) were measured by using commercially available assays.

Detection of α-Gal in gelatin and bovine products

The concentrations of α-Gal in bovine-derived gelatin colloids (Gelofusine and Haemaccel), whipped cream (ultrapasteurized whipped cream), cow's milk, and beef thyroglobulin (Sigma-Aldrich, St Louis, Mo) were measured by using a modified inhibition RIA.[19] Cetuximab (ImClone Systems and Bristol-Myers Squibb, New York and Princeton, NJ) and fish-derived gelatin were included as positive and negative controls, respectively, because cetuximab is known to contain α-Gal[26] and fish gelatin is not known to cross-react with mammalian gelatin.[27] One-gram samples of gelatin colloid, whipped cream, cow's milk, beef thyroglobulin, or fish gelatin and 5 mg of cetuximab were each incubated for 2 hours with a dilution of serum from a subject with known high-titer IgG antibodies to α-Gal. A standard curve was created by using serial dilutions of the linear trisaccharide Galα1-3Galβ1-4GlcNAc (V-Labs, Covington, La; see Fig E1 in this article's Online Repository at www.jacionline.org). Iodine 125–radiolabeled Galα1-3Galβ1-4GlcNAc-BSA (V-Labs) was then added and incubated at room temperature for 2 hours. Finally, goat anti-human IgG (Strategic Biosolutions, Newark, Del) was added as a precipitating antibody and stored overnight at 4°C, followed by washing of precipitates in PBS 3 times and measurement of radioactivity with a gamma counter (PerkinElmer, Waltham, Mass).

Challenge procedures

When clinically indicated, open oral challenges with food-grade gelatin confectionaries were performed under medical supervision until a total of approximately 10 g of oral gelatin was consumed, followed by a 3-hour wait after the last dose was consumed. Intravenous challenges were performed in an intensive care unit by using either Haemaccel or Gelofusine (35 or 40 mg/mL gelatin, respectively), according to product availability in the challenge hospital. Infusions of a 1:10 dilution of colloid in normal saline, initially 1 mL/min, were doubled every 5 minutes. Once 8 mL/min was reached, the protocol was restarted with undiluted colloid. When reactions occurred, patients were observed for an additional 4 hours after symptom resolution.

Statistical analysis

We compared quantitative measures of IgE with α-Gal and the presence or absence of positive gelatin skin test results with the risk of anaphylaxis using unpaired *t* tests. The relationships between anti–α-Gal IgE levels and speed of symptom onset, as well as gelatin IDT wheal size, were examined by calculating Pearson correlation coefficients. Allergen-specific levels of less than 0.35 kU/L or greater than 100 kU/L were treated as 0.35 or 100 kU/L, respectively, for these calculations. A 2-sided *P* value of less than .05 was considered statistically significant. Statistical analyses were performed with SPSS software, version 18.0 (SPSS, Inc, Chicago, Ill), and GraphPad Prism software, version 4 (GraphPad Software, Inc, La Jolla, Calif).