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The relationship between red meat allergy and sensitization to gelatin and galactose-alpha-1,3-galactose

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Abstract

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 and an examination of the relationship between allergic reactions to red meat and sensitization to gelatin and alpha-Gal.

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Conflict of interest statement

Dr Mullins has received unrestricted investigator-initiated grants from Commonwealth Serum Laboratories Australia and Alphapharm Australia (the past and current Australian distributors of EpiPen), Abbott Nutrition Australia and the Ilhan Food Allergy Foundation within the last 5 years. Dr Commins is a volunteer committee member for the American Academy of Allergy, Asthma & Immunology (AAAAI) and the American College of Allergy, Asthma & Immunology and is an author for Up-to-Date. Dr. Platts-Mills has a patent on the use of streptavidin solid phase to evaluate IgE antibodies to recombinant molecules.

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Methods—Adult patients evaluated 1997-2011 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 tests were observed in 40/1335 individuals; 30/40 patients with red meat allergy (12 also clinically allergic to gelatin); 2/2 with gelatin colloid anaphylaxis; 4/172 with idiopathic anaphylaxis (all responded to intravenous gelatin challenge of 0.02 to 0.4g); 4/368 with drug allergy. Testing was negative in all patients with venom allergy (n=241), non-meat food allergy (n=222), and miscellaneous disorders (n=290). ImmunoCap was positive to alpha-Gal in 20/24 meat allergics and in 20/22 with positive gelatin skin tests. The results of gelatin skin testing and anti-alpha-Gal IgE were strongly correlated (r=0.46; P<0.01). Alpha-Gal was detected in bovine gelatin colloids at concentrations of ~ 0.44 to 0.52ug/gm gelatin by inhibition radioimmunoassay.

Conclusion—Most patients allergic to red meat were sensitized to gelatin and a subset was clinically allergic to both. The detection of alpha-Gal in gelatin and correlation between the results of alpha-Gal and gelatin testing raises the possibility that alpha-Gal IgE may be the target of reactivity to gelatin. The pathogenic relationship between tick bites and sensitization to red meat, alpha-Gal and gelatin (with or without clinical reactivity) remains uncertain.

Keywords

food allergy; anaphylaxis; red meat; alpha-galactose; gelatin; colloid

INTRODUCTION

Allergic reactions to red meat are relatively uncommon, 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 percent of cow's milk-allergic children reported as being beef allergic (2). Previous studies describe bovine serum albumin 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), gelatin colloids (10) and as stabilizing agents 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-alpha-1,3-galactose (alpha-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 USA (21) studies. The fortuitous observation of one patient allergic to red meat and topical gelatin (4) and two patients with initial anaphylaxis to intraoperative gelatin colloid followed by anaphylaxis to red meat on separate occasions (5) prompted a prospective 14-year evaluation of the clinical significance of gelatin sensitization, the predictive value of a positive skin test and an examination of the relationship between allergic reactions to red meat and sensitization to gelatin and alpha-Gal.

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PATIENTS AND METHODS

Study Population

The study was undertaken in a mixed adult/pediatric specialty allergy/immunology practice in the Australian Capital Territory in South-Eastern 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 (RJM). Clinical and demographic data were entered prospectively into a searchable database (Blue Chip Clinical Research Module, Health Communication Network, Sydney; Microsoft Access, Microsoft Corporation, Redmond, WA, USA). Data (and accuracy) were analyzed and verified retrospectively. The characteristics of all patients aged ≥18 years evaluated in the calendar years 1995 to 2011 were analysed. The Human Research and Ethics committee (Calvary Bruce/Calvary John James Private Hospitals) approved the study.

Patient evaluation

Glycerinated commercial food allergen extracts (beef and pork; Hollister Stier, Spokane, WA, U.S.A) 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% weight/volume slurry was prepared using ground meat in saline, with the supernatant used for skin prick testing (SPT) when required. 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, NSW, Australia), respectively. Gelatin in these products is extracted from bovine bones only, excluding the skull (Hartley Atkinson, AFT Pharmaceuticals; Howard Johnson, B Braun Pharmaceutics, personal communications, 2007), using a combination of acid and alkaline hydrolysis, followed by heat extraction at temperatures up to 90°C, then sterilised at temperatures >100°C. SPT testing and intradermal testing (IDT) were performed on the volar aspect of the forearm and interpreted according to standard guidelines (22). SPT was performed using metal lancets (Stallergenes, Antony, France). A positive SPT was defined as a wheal size of at least 3 mm greater than a negative control (saline) at 15 minutes. Insulin syringes with 27 gauge needles were used for IDT to introduce ~0.02ml allergen. A positive IDT was defined as a wheal ≥ 5 mm greater than 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 SPT and IDT. When SPT with beef, pork were negative, IDT was undertaken with the same commercial extracts freshly diluted 1/100 in saline as previously described (19). When SPT with gelatin colloid was negative, IDT was undertaken using undiluted colloid. The primary indication for undertaking SPT/IDT was a history of possible red meat and/or gelatin allergy. Secondary indications (for research purposes) were suspected drug, 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) who were not considered likely to have IgE-mediated FA but where testing was undertaken for purposes of patient reassurance. Following 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. IgE-mediated FA was diagnosed only if there was also a history of acute systemic allergic reaction (one or more of urticaria, vomiting, bronchospasm or vascular collapse) following known allergen exposure,

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combined with a positive SPT or IDT 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, sweating) or severe (cyanosis, hypotension, confusion, collapse, loss of consciousness, incontinence). A diagnosis of anaphylaxis was assigned if either of the first two criteria of the 2005 National Institute 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 one or more was considered to be the cause of FA.

In vitro testing

Sera were aliquoted and stored at -5°C in the ACT until February 2011, then transported on dry ice to the University of Virginia and stored at -20°C until analysis. Total and specific IgE antibodies were measured by using either commercially available ImmunoCAP (Phadia US, Portage, Mich, USA) 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-alpha-Gal specific assay was defined as >0.35 IU/mL. IgE antibodies to alpha-Gal were measured by streptavidin CAP technique, by adding approximately 5 μg biotinylated antigen to each CAP before adding 40 μL undiluted serum. IgE antibodies to beef (f27), pork (f26), lamb (f88) and bovine gelatin (c74) were measured using commercially available assays.

Detection of alpha-Gal in gelatin and bovine products

The concentrations of alpha-Gal in bovine-derived gelatin colloids (Gelofusine & Haemaccel), whipped cream (ultra-pasteurized whipped cream), cow's milk and beef thyroglobulin (Sigma-Aldrich) were measured using a modified inhibition radioimmunoassay (RIA) (**19**). Cetuximab (ImClone Systems and Bristol-Myers Squibb, New York and Princeton, New Jersey) and fish-derived gelatin were included as positive and negative controls, respectively, as cetuximab is known to contain alpha-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 5mg of cetuximab were each incubated for two hours with a dilution of serum from a subject with known high titer IgG antibodies to alpha-Gal. A standard curve was created using serial dilutions of the linear trisaccharide Galα1-3Galβ1-4GlcNAc (V-Labs, Inc, Covington, LA) (online Figure e1). I^{125} radiolabeled Galα1-3Galβ1-4GlcNAc-BSA (V-Labs, Inc, Covington, LA) was then added and incubated at room temperature for two hours. Finally, goat anti-human IgG (Strategic Biosolutions, Newark, DE) was added as a precipitating antibody and stored overnight at 4°C, followed by washing of precipitates in PBS three times and measurement of radioactivity with a gamma counter (Perkin Elmer, Waltham, MA).

Challenge procedures

When clinically indicated, open oral challenges with food grade gelatin confectionary were performed under medical supervision, until a total of ~10gm 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 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/minute, were doubled every 5

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minutes. Once 8 ml/minute was reached, the protocol was restarted using undiluted colloid. When reactions occurred, patients were observed for an additional 4 hours after symptom resolution.

Statistical analysis

We compared quantitative measures of IgE to alpha-Gal and the presence or absence of positive gelatin skin tests with the risk of anaphylaxis using unpaired t tests. The relationships between anti-alpha-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 <0.35 or > 100 KU/L were treated as 0.35 or 100, respectively for these calculations. A two-sided *P* value <0.05 was considered statistically significance. Statistical analyses were performed with SPSS software, version 18.0 (SPSS, Inc, Chicago, Ill), and GraphPad Prism, version 4 (GraphPad Software, Inc, La Jolla, Calif).

RESULTS

Patient characteristics

Between 1995 and 2011, 1159 adults assessed by the first author (RJM) aged 18 to 101 years (423 male) were diagnosed with FA, triggered by seafood (n=284 patients), peanut (n=189), tree nuts (n=188), systemic allergic reactions to fruit/vegetables (n=134), wheat (n=77), egg (n=57), red meat (n=40), sesame seed (n=22), cow's milk (n=21) or soybean (n=8). Of 40 red meat allergics identified, 18 (46%) were male aged 18-78 years (median 48) and 27 had anaphylaxis. Patients estimated symptom onset between 15 minutes and 9 hours after ingestion (median 3 hours) with significantly delayed onset associated with nocturnal episodes after the evening meal. Ten were sensitized to red meat only (M) and 30 to red meat and gelatin (MG) on allergy testing. There was no relationship between onset time and likelihood of anaphylaxis (P=0.88) or gelatin sensitization and likelihood of anaphylaxis (P=0.13). With the exception of two vegetarians (MG1, MG26), most patients diagnosed with red meat allergy reported tolerance on other occasions.

Meat and gelatin co-sensitization and co-reactivity

32 patients were co-sensitized to red meat and gelatin, including two patients with intraoperative gelatin colloid anaphylaxis who were red meat tolerant (GC 1 and 2) and 29 patients diagnosed with red meat allergy (MG 1-29; Table 1). Of this MG group, 12 reported anaphylaxis when red meat was not ingested, including two additional patients with intraoperative gelatin colloid anaphylaxis prior to presentation with red meat allergy (MG22 and MG24; 5). One additional patient (MG12) with recurrent red meat anaphylaxis remained well for five years on a meat/gelatin-free diet. Despite wearing a MedicAlert bracelet to warn of her possible gelatin allergy, she was given 40ml of intravenous Gelosfusine (~1.6gm gelatin) following a myocardial infarction and developed. urticaria, bronchospasm, hypoxia and hypotension (hospital records verified by the first author, RJM; Table 1). Nine additional patients reported systemic reactions following oral gelatin consumption on separate occasions (e.g. desserts) where meat ingestion was denied.

Prospective evaluation of gelatin sensitization

Between 1997 and 2011, 1335 individuals underwent gelatin IDT. Positive results were observed in 40 (2.8%) individuals: 30/40 (75%) diagnosed with red meat allergy (M+MG); 2/2 patients with gelatin colloid anaphylaxis (co-sensitised to meat and gelatin but meat tolerant clinically; GC), 4/172 (2.3%) with idiopathic anaphylaxis (ID) and 5/1121 (0.4%) others tested without suspected meat/gelatin allergy (Tables 1, 2). Sensitization to gelatin was titratable in all patient groups (Figure 1). Five years after evaluation for possible insect

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venom allergy, however, one normally vegetarian subject with a positive gelatin test of unknown significance returned following meat anaphylaxis, was sensitised to red meat and gelatin on testing and reclassified in the MG group (MG26).

Gelatin challenges

In the four cases classified as idiopathic anaphylaxis assessed 2001-2003 (but with with positive gelatin tests), red meat was implicated historically on multiple occasions but sensitization to red meat could not be demonstrated on SPT; meat IDT was not undertaken at the time until reports of its utility (19). Since removal of dietary meat was undesirable without further evidence, all agreed to an oral challenge with gelatin and if negative, with IV gelatin colloid. While all tolerated a supervised oral challenge with 10gm gelatin, each developed urticaria and bronchospasm with intravenous gelatin colloid challenge at doses of 1.2gm (ID1; online Figure e2), 0.1gm (ID2), 0.024gm (ID3) and 4.1gm (ID4). Avoidance of red meat and gelatin has been reduced patient reported episodes on followup from 1 episode in 6 months to 1 episode in 8 years (ID1), from 10 episodes/year to none in 6 years (ID2), from 3 episodes in 2 months to none in 6 years (ID3) and from 5episodes/year to none in 5 years (ID4).

Additional in vitro testing

Where available, sera were tested for allergen specific IgE to meat, gelatin and alpha-Gal (Table 3). In the M/MG groups, 23/26 were sensitised to one or more red meat, 20/24 to alpha-Gal (plus 2 borderline results) but only 1/25 to gelatin despite positive gelatin IDT in 20 of these patients (Table 3). There was no association between anti-alpha-Gal IgE and likelihood of anaphylaxis and only a weak inverse relationship ($r=0.37$; $P=0.074$) between time of onset and anti-alpha-Gal IgE. There was a strong correlation between anti-alpha-Gal IgE and positive gelatin IDT reactivity: of 22 IgE alpha-Gal positive sera from the M, MG, ID and G groups, 20 had positive gelatin skin tests. Of 22 with positive gelatin IDT, 20 were anti-alpha-Gal IgE positive. Furthermore, there was a correlation between mean gelatin IDT wheal diameter and in vitro IgE levels to alpha-Gal ($r=0.46$; $P<0.01$),

Comparison of testing methods

Where positive, the results of beef and pork SPT and IDT suggested sensitization to both (data not shown). Of 40 patients diagnosed with red meat allergy (M+MG), meat SPT was positive in 26/40 (65%), meat IDT positive in 18/18 (100%), gelatin IDT positive in 30/40 (75%), meat ImmunCap positive in 23/26 (88%) and anti-alpha-Gal IgE in 25/26 (96%).

Detection of alpha-Gal in gelatin and bovine products

Given the positive correlation between the results of gelatin skin testing and anti- alpha-Gal IgE, we examined whether alpha-Gal might be detectable in gelatin, using a sensitive RIA (10). Alpha-Gal was detected in both gelatin colloids: 0.52μg ± 0.1μg of alpha-Gal/gm of Gelofusine and 0.44μg ± 0.2μg/gm of Haemaccel). Using similar techniques, the concentrations of alpha-Gal were 5.6μg of alpha-Gal per gram of beef thyroglobulin and 1.4μg / g of heavy cream. By contrast, no detectable alpha-Gal was found in cow's milk (skim, 1% or 2% milk fat). Of the 21 oligosaccharides identified on cetuximab, approx 30% have one or more alpha-1,3 linked galactosyl residues as measured by peak area on TOF-MS spectra (28) and alpha-Gal was detected at a concentration of 10.2μg / 5mg of cetuximab in the inhibition RIA. By contrast, alpha-Gal was undetectable in fish gelatin (lower limit of assay = 0.01μg).

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Relationship between red meat allergy and tick exposure

When questioned about exposure to (and adverse reactions from) tick bites, 24/40 meat-allergics described large local bite reactions and 26 lived in (or visited) tick-endemic areas. Conversely, of 10 tick allergic patients evaluated (6 with tick anaphylaxis, none with FA), 7/10 were sensitised to red meat on skin and/or in vitro testing, 3/7 tested were sensitised to gelatin on IDT and 7/9 had serum anti- alpha-Gal IgE (Table 4).

DISCUSSION

We have identified a significant relationship between adult onset red meat allergy and sensitization and clinical reactivity to gelatin, supported by the results of intraoperative exposure (MG23, 24; GC1,2), accidental exposure (MG12), observed challenge (ID1-4) or claims of reactivity to oral gelatin. Consistent with previous studies, SPT reactivity using commercial meat extracts were relatively small and sometimes negative (19, 29), and IDT and in vitro testing were more sensitive at detecting sensitization (19, 30). In most cases, symptom onset was delayed for a median of 3 hours after ingestion; most were sensitised to alpha-Gal; and there was a historical association between tick bite exposure, sensitization and allergy to red meat (20, 21). While beef was the dominant meat triggering symptoms, this may reflect non allergic factors such as cost, availability, the amount consumed at any one sitting as well as popularity: beef consumption accounts for more than the sum total of all other meat consumed in Australia (31).

There are some caveats to be considered in interpreting our study. First, we specifically studied only adults with red meat allergy (due to the potential discomfort from IDT) and so our results cannot necessarily be extrapolated to children, although in vitro gelatin sensitization has been reported in children with red meat allergy (32). Second, we were only able to examine some patients repeatedly as the study developed over 14 years and as a consequence, sera for in vitro analysis were only available from a subset. While acknowledging that claims of reactions to oral gelatin (without meat) in 9 MG patients is dependent on patient reports, it is difficult to ignore the clinical significance of a positive gelatin IDT at least as a risk factor for gelatin colloid reactivity (as described above), including one case of accidental exposure where the risk was identified prospectively (MG12). Conversely, clinical reactivity to red meat was observed in 2/4 colloid-allergic patients and gelatin sensitization was predictive of red meat allergy in one “healthy control” (MG26). While the significance of gelatin sensitization in four otherwise healthy subjects with drug allergy, meat sensitization in two patients with gelatin colloid allergy and gelatin/meat sensitization in patients evaluated for tick bite allergy remains currently uncertain, this may become apparent with further observation (e.g. MG26) or may represent sensitization without clinical allergy, as recently reviewed (33). While we would have preferred to undertake more gelatin challenges in patients with a positive gelatin test, our ability to do so was constrained by patient age, co-morbidity, patient unwillingness to do so and geographical location (most lived at the coast > 200km from the inland clinical practice),

Most reports of serious allergic reactions to gelatin implicate parenteral exposure, either to gelatin colloids used as plasma expanders or to gelatin-containing vaccines. Since 1999, 129 reports of anaphylaxis (including 2 deaths) have been associated with colloid use in Australia, with colloid the only suspected trigger in 58 cases (Rob Crowdy, Australian Therapeutic Good Administration, personal communication December 2011). Both IgE-independent and -dependent mechanisms have been proposed to play a role in reactions to gelatin colloids. Evidence in favor of the former includes activation of kinin pathways and histamine release in healthy volunteers (34). That at least some reactions to gelatin colloids are IgE-mediated, however, is supported by the correlation between skin test reactivity and clinical reactions in this series as well as evidence of immunological cross-reactivity

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between gelatin and gelatin-derived colloids (8, 10). These data are further strengthened by evidence that 1) allergic reactions to gelatin-containing vaccines are also IgE-mediated; 2) IgE is directed against the alpha 2 chain of type I collagen; 3) reactions are more common in patients with prior exposure to gelatin-containing vaccines; 4) reactions are uncommon if gelatin is extensively hydrolysed; and, 5) patients generally require parenteral exposure to trigger sensitization (12, 35). Vaccine-reactive patients (7, 11, 12; sometimes also reactive to oral gelatin), however, are likely to be more sensitive to gelatin than our patients, with sensitization detectable by SPT using diluted vaccines (~0.2mg/ml gelatin vs. ~ 40mg/ml gelatin IDT in our patients) and reactivity to approximately 2mg parenteral gelatin (36), compared to 24 to 4100 mg in our challenge patients.

While our data are consistent with gelatin sensitization being a risk factor for gelatin colloid allergy, a relevant issue is whether sensitization also conveys a significant risk of clinical reactivity to oral gelatin and, by implication, the need for ongoing dietary restrictions. Eluted when meat is cooked and cooled, gelatin is present in some confectionery (e.g. marshmallows), food thickeners, dips, glazes and icing and act as a fat substitute in yoghurt, mayonnaise and ice cream (9). Gelatin can be found in sausage coatings, salami, tinned hams, pâté and meat stock, and used to clarify fruit juice and wine (9). Gelatin can thus be considered a potential occult food allergen as exposure is ubiquitous, and the method of extraction (acid and alkaline hydrolysis with heat treatment) make it more likely to survive food preparation than heat-labile meat proteins such as bovine gamma globulin and bovine serum albumin (3).

That reactions to topical or oral gelatin can occur is supported by rare case reports of allergic reactions to “hydrolysed protein” (gelatin) in shampoo, collagen implants, “catgut” sutures, collagen-derived contact lenses, as well as to gelatin present as binding agent in tablets, capsules, suppositories or confectionary (4, 6, 9, 37-40). While sensitization alone is not equivalent to being clinically allergic (41), 9 of our patients reported systemic allergic reactions (including anaphylaxis) after ingestion of gelatin containing food without red meat. While these claims (and the scarcity of published cases) may reflect poor recognition of gelatin as a possible trigger, episodes erroneously labelled as being idiopathic (due to negative routine allergy tests), the absence of co-factors, or a higher risk from parenteral exposure, one potential clue may be the oral dose required to trigger an allergic reaction. Our challenge patients failed to react to 10gm of oral gelatin, in retrospect a relatively small dose compared to the large amount intravenously required to trigger anaphylaxis in the same individuals. While ongoing studies await the results of challenges using higher doses of oral gelatin, our clinical practice in the meantime has been to advise patients with red meat allergy *and* gelatin sensitization to be cautious about ingesting substantial quantities and to wear a MedicAlert bracelet warning of potential risk from gelatin colloid exposure, a prudent approach that did not protect one patient in our series.

Consistent with previous studies (19, 30), in vitro testing appeared to be more sensitive at detecting sensitization to meat derived allergen than skin testing, with wheal sizes using commercial meat extracts being relatively small or negative and requiring IDT to detect sensitization (Table 1). Explanations for this have been discussed previously (19), including the possibility that folding of proteins within extracts might make alpha-Gal less available for mast cell cross-linking, that antibodies to uncharged carbohydrate molecules like alpha-Gal might be of low affinity (42), or perhaps of specific relevance, evidence that alpha-Gal concentration is lower in commercial meat extracts than in crude extracts of real meat (19), perhaps accounting for lower sensitivity.

In this context, it is perhaps not surprising that IDT was more sensitive at detecting gelatin sensitization yet paradoxically in vitro testing was negative in almost all samples. This

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cannot be explained by serum sample degradation due to prolonged storage, since IgE to meat and alpha-Gal was detected in parallel assays. Potential explanations include assay insensitivity due to the preparation of gelatin required for immunoassay grade stability or if alpha-Gal is the allergenic target, insufficient concentration on the ImmunoCap to detect sensitization. Alternatively, gelatin-reactive IgE may be of low affinity or low concentration (as previously suggested for anti-cross-reactive carbohydrate determinants IgE antibody; 42). Regardless of the explanation, the relative insensitivity of current commercial in vitro assays for gelatin IgE is underlined by negative results even in patients with demonstrated anaphylaxis to gelatin colloid challenge.

Our data thus supports the use of gelatin colloids where possible (not available in the USA at this time) as useful reagents for skin testing to confirm suspected gelatin allergy, and as less sensitive (but still useful) reagents to detect suspected red meat allergy, with a positive test having potential clinical relevance for avoidance strategies. While the option of using food-grade gelatin for testing may be considered in patients with suspected meat and/or gelatin allergy, the potential for processing of food-grade gelatin to yield extracts of varying molecular weights may limit its use as a testing reagent and might also explain inconsistent clinical responses to oral exposure and inconsistent in vitro assays to gelatin, as previously reported (11). While comparison of results using gelatin colloid and crude gelatin extracts for SPT/IDT merits future study, our plan to undertake IDT in >1000 patients led us to not consider using an unstandarized and non-sterile reagent for our *prospective* study in a large number of individuals in whom meat/gelatin allergy was unlikely to be present.

It is likely that allergic responses to red meat are heterogeneous, with some responses directed towards heat labile meat proteins (3), others directed towards alpha-Gal and others to gelatin. Consistent with previous studies (43, 44), the majority of our patients experienced only occasional overt reactions despite regular meat consumption. Potential explanations remain speculative, but may include the absence of co-factors (e.g. exercise), the amount ingested (other studies suggest that >75gm is generally required to trigger symptoms; 44), the way in which meat is prepared (influencing the quantity and number of allergens eluted or presence of additional heat-labile allergens) or perhaps fat content. Moreover, observations that sIgE anti- alpha-gal may decrease naturally over time without additional tick bites (Platts-Mills & Commins, unpublished observations) may account in part for varying degrees of tolerance over time. The likelihood of consumption of larger amounts of meat in the evening raises the possibility that circadian changes in gut motility (45) may influence allergen absorption. Furthermore, nocturnal onset while asleep may prevent recognition of milder reactions and delay recognition of more severe episodes, perhaps accounting for some considerable delays observed (e.g. patient MG18).

We were also able to confirm previous Australian and USA reports of an association between adult onset red meat allergy, alpha-Gal sensitization and a history of tick bite reactions (19-21), which may explain the geographical location of most of our patients. This is significant because we also found asymptomatic sensitization to gelatin, meat and alpha-Gal in a small number of patients with tick bite reactions in a different country with different tick species. The detection of alpha-Gal in gelatin preparations and the correlation between sensitization to red meat, gelatin and alpha-Gal in most cases raises one intriguing possibility: that as has been proposed for reactions to cetuximab (46), clinical reactivity to gelatin might in some cases be mediated by anti- alpha-Gal IgE. If so, this might in part help explain the poor correlation between the results of in vitro and IDT testing to gelatin in our adult population compared to younger and more sensitive patients reacting to lower doses of gelatin in vaccines (7, 11, 12) with IDT testing acting as an indirect marker of IgE reactivity to alpha-Gal, and with positive in vitro testing results in the vaccines studies perhaps reflecting either IgE to other gelatin moieties or patients with very high titers of anti-gelatin

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IgE. Of interest, if tick bite exposure is a risk factor for meat/gelatin sensitization (and precedents for geographical variation in anaphylaxis have been described; 26), then one might reasonably expect that adverse reactions to gelatin colloid might also follow a similar pattern. Unfortunately, the level of detail available in Australian adverse drug reports precludes such analysis (Nick Simpson, Australian Therapeutic Goods Administration, personal communication January 2012).

In conclusion, we found that most patients allergic to red meat are sensitized to gelatin, and that a subset will report reactions to IV (and sometimes oral gelatin) as well. Gelatin sensitization poses a risk of clinical reactivity to both red meat and gelatin, albeit not in all patients. Patients presenting with clinical reactions to either trigger thus merit evaluation for sensitization to both triggers and warned appropriately if results are positive. Taking into account the relationship between the results of gelatin and alpha-Gal testing in our patients, and the detection of alpha-Gal in gelatin, a positive test to either may also represent a risk factor for both meat, and gelatin allergy. As the syndrome of delayed anaphylaxis to mammalian meat highlights, correct diagnosis is hampered by delayed onset, inconsistent ability to tolerate the food on some occasions, the inability of patients to always correctly identify their dietary triggers and geographical limitations in the availability of diagnostic tests for anti-alpha-Gal and anti-gelatin IgE. Future challenges include determining whether a salivary component common to multiple tick species from different continents may be the sensitizing agent (21) and the factors contributing to delayed onset of symptoms to red meat compared to other foods.

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ABBREVIATIONS

ACT	Australian Capital Territory
alpha-Gal	galactose-alpha-1,3-galactose
FA	Food allergy
G	Gelatin
GC	Gelatin colloid
ID	Idiopathic
IDT	Intradermal testing
M	Meat
MG	Meat Gelatin
RIA	Radioimmunoassay
SPT	Skin prick testing

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Key messages

- Most patients allergic to red meat are sensitized to gelatin and a subset will be clinically allergic to both.
- The detection of alpha-Gal in gelatin and correlation between the results of alpha-Gal and gelatin testing raises the possibility that alpha-Gal IgE may be the target of reactivity to gelatin.
- The relationship between tick bite reactions in meat-allergic subjects and meat sensitization in patients with tick bite allergy is suggestive of a possible role for tick bites in meat allergy pathogenesis.

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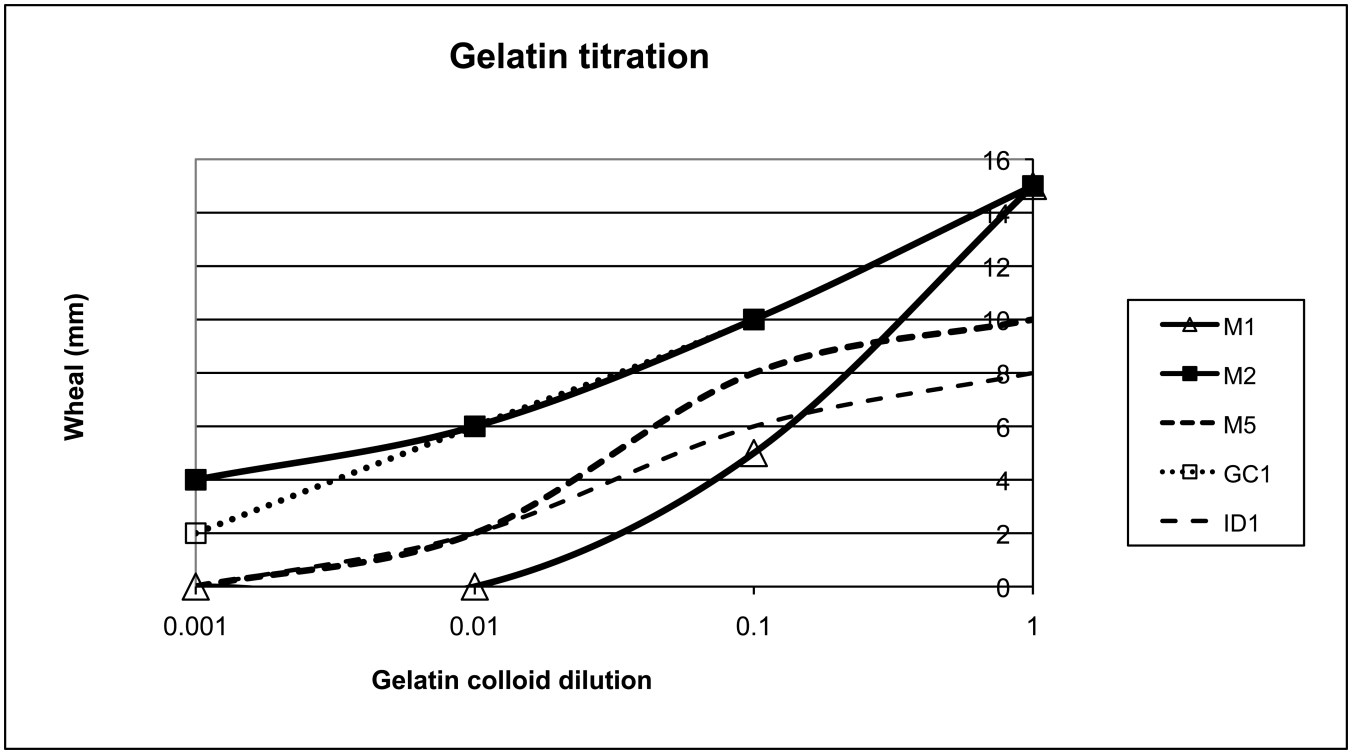

Figure 1. Titration of intradermal gelatin colloid skin testing

Intradermal testing with gelatin colloid was titrated in 17 cases, with positive tests detectable at dilutions of undiluted colloid only (3 patients), 1/10 (6 patients), 1/100 (8 patients) and 1/1000 (0 patients). Representative examples are shown.

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Table 1

Clinical characteristics of patients diagnosed with red meat or gelatin allergy and other patient groups, as assessed by skin testing

Patient group	Gender	Age (years)	Trigger(s) of clinical reactivity	Onset (hrs)	Severity	Anaphylaxis	Meat Test (SPT/IDT wheal size in mm)	Gelatin Test (SPT/ IDT wheal size in mm)	No. episodes (time period)
Meat									
M1	M	37	B	2	moderate	Yes	6/nd	0/0	3 (3 weeks)
M2	M	59	B	0.5	severe	Yes	4/nd	0/0	2 (6 months)
M3	F	32	B, L, P	2	moderate	No	4/6	0/0	“all life” > 4/yr
M4	F	25	P	2	moderate	Yes	0/4	0/0	“all life” >4/yr
M5	M	18	B	2	moderate	No	3/nd	0/0	“all life” >4/yr
M6	F	56	B, P	2	moderate	Yes	3/nd	0/0	2 (6 months)
M7	M	50	H	3	moderate	Yes	3/6	0/0	“all life” >4/yr
M8	F	31	B, L	0.5	moderate	No	5/nd	0/0	1 (10 yrs ago)
M9	M	36	B	3	moderate	Yes	3/nd	0/0	10 (6 months)
M10	F	23	B, P	2	moderate	No	5/nd	0/0	2 (3 months)
Meat + Gelatin									
MG1	M	77	B	6	moderate	Yes	3/15	0/15	2 (2 months)
MG2	M	51	B, L, G	5	severe	Yes	3/nd	0/15	10 (8 months)
MG3	F	28	B, L, K	1.5	moderate	Yes	0/14	0/12	>25 (6 yrs)
MG4	F	21	B	3	moderate	Yes	0/8	0/8	2 (2 months)
MG5	M	64	B	3	severe	Yes	0/12	0/12	>10 (4 yrs)
MG6	F	18	P	6	moderate	Yes	6/10	0/8	“all life” >4/yr
MG7	M	52	B, G	6	mild	No	3/10	0/12	3 (3 months))
MG8	F	16	B	6	moderate	No	0/8	0/6	1 (2 months)
MG9	M	78	B	8	severe	Yes	0/10	0/15	6 (3 yrs)
MG10	F	60	L	2	moderate	No	4/10	0/10	1 (2 months)
MG11	M	52	B, L, G	5	moderate	Yes	3/nd	0/15	4 (18 months)
MG12	F	71	B	3.5	severe	Yes	3/nd	0/12	> 20 (2 yrs)
MG13	F	51	B, L, P, G	0.25	severe	Yes	3/nd	2/12	> 10 (2 yrs)
MG14	F	28	L, K	1.5	severe	Yes	5/nd	0/12	3 (3 months)
MG15	F	73	B	4	mild	No	3/nd	0/10	3 (5 yrs)

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Patient group	Gender	Age (years)	Trigger(s) of clinical reactivity	Onset (hrs)	Severity	Anaphylaxis	Meat Test (SPT/IDT wheal size in mm)	Gelatin Test (SPT/ IDT wheal size in mm)	No. episodes (time period)
MG16	F	63	B,GC,	2	severe	Yes	3/nd	0/5	> 50 (10 yrs)
MG17	F	35	B, L, P, G	0.25	severe	Yes	3/nd	0/15	2 (8 months)
MG18	M	44	B	9*	moderate	Yes	0/15	0/12	2 (6 months)
MG19	F	27	B	2	mild	No	3/nd	0/10	1 (6 months)
MG20	M	46	B, L	6	severe	Yes	3/nd	0/12	5 (3 yrs)
MG21	F	48	B, P	8	mild	No	3/nd	0/6	>5 (5 yrs)
MG22	M	69	B, GC, G	6	severe	Yes	3/nd	3/10	>10 (3 yrs)
MG23	M	66	B, L, P, GC	7	severe	Yes	3/nd	0/12	>30 (4 yrs)
MG24	M	72	GC, G, P	0.5	severe	Yes	3/nd	0/5	2 (6 months)
MG25	F	41	G, P	2	mild	No	3/nd	3/10	4 (2 yrs)
MG26	F	39	B	7	mild	No	0/10	0/10	2 (6 months)
MG27	M	74	B, L	3	severe	Yes	0/15	0/15	18 (1 yr)
MG28	F	61	B, L, G	4	severe	Yes	0/12	0/12	8 (2 yrs)
MG29	M	19	B, P	4	severe	Yes	0/12	0/12	1 (1 month)
MG30	F	65	B, L	1	moderate	No	2/10	0/12	6 (10 yrs)
Gelatin Colloid									
GC1	M	77	GC	0.5	severe	Yes	0/4	0/15	1
GC2	M	63	GC	0.5	moderate	Yes	0/10	0/8	1
Idiopathic									
ID1	F	33	P	0.25	moderate	Yes	0/nd	0/5	1 (6 months)
ID2	F	22	B	2	moderate	No	0/nd	0/7	5 (1 yr)
ID3	F	35	B, P	5	moderate	Yes	0/nd	0/6	3 (2 months)
ID4	F	54	B, L	3	severe	Yes	0/nd	0/8	> 20 (3 yrs)
Drug Allergy									
D1	F	35	n/a	n/a	n/a	n/a	nd/nd	nd/5	n/a
D2	M	64	n/a	n/a	n/a	n/a	nd/nd	nd/12	n/a
D3	F	37	n/a	n/a	n/a	n/a	nd/nd	nd/5	n/a
D4	F	20	n/a	n/a	n/a	n/a	nd/nd	nd/7	n/a

Patients diagnosed with allergy to red meat and/or gelatin as well as control groups underwent skin prick testing (SPT) or intradermal testing (IDT) using meat-derived allergen and gelatin colloid. IDT was normally only undertaken if SPT was negative. Results are described as mean wheal diameter in mm or not done (nd) as indicated. For the purposes of this study, red meat was defined as beef (B), pork (P),

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lamb (L) horse (H) or kangaroo (K). Other patients were clinically allergic to oral gelatin (G) or gelatin colloid (GC). The time of symptoms onset after meals was a patient estimate, with one patient (MG18) reporting symptoms after 6 hours on one occasion and 9 hours after another.

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Table 2

Gelatin sensitisation

1335 patients underwent intradermal testing with gelatin colloid between 1997 and 2011.

Clinical group	+ve tests	No. tested	% +ve
Red meat	30	40	75
Gelatin colloid	2	2	100
Venom allergy	1*	242*	0.4
Idiopathic anaphylaxis	4	172	2.3
Drug allergy	4	368	1.1
Non-meat food allergy	0	222	0
Miscellaneous	0	290	0
Idiopathic angioedema	0	81	
Eosinophilic esophagitis	0	79	
Chronic urticaria	0	77	
Irritable bowel syndrome	0	24	
Anxiety disorder	0	12	
ACE inhibitor angioedema	0	8	
Laryngospasm	0	6	
Animal allergy	0	2	
Semen allergy	0	1	
Total	40*	1335	2.9

*One patient returned with red meat anaphylaxis 5 years after investigation for insect venom allergy and was reclassified in the red meat allergy group.

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Table 3

In vitro testing in patients diagnosed with allergy to red meat or gelatin

Patient group	Pork (f26)	Beef (f27)	Lamb (f88)	Alpha-gal	Gelatin (c74)
Meat					
M1	7.08	13.7	5.68	2.08	<0.35
M2	0.07	0.07	0.04	0.35	<0.35
M3	0.02	0.06	0.1	0.35	<0.35
M6	0.42	0.73	0.21	0.97	<0.35
Meat + Gelatin					
MG1	16.9	35.1	15.3	93	<0.35
MG2	17.1	29.2	10	64.2	<0.35
MG3	6.43	8.25	4.4	12.4	<0.35
MG4	0.81	1.18	0.59	2.63	<0.35
MG5	18.3	37.1	<0.10	66.9	<0.35
MG6	1.28	0.38	1.18	<0.35	<0.35
MG7	17.7	34.8	12.7	100	<0.35
MG8	1.36	1.62	1.41	3.56	<0.35
MG9	2.54	3.7	<0.35	7.9	<0.35
MG10	0.82	1.74	<0.35	12.6	nd
MG11	0.75	0.88	1.15	3.91	<0.35
MG12	0.79	1.39	0.66	2.23	<0.35
MG13	5.39	5.64	5.91	6.09	<0.35
MG14	15.7	17.9	16.6	20.7	0.42
MG15	0.37	0.41	0.31	1.22	<0.35
MG16	0.58	1.04	0.6	2.59	<0.35
MG17	0.04	0.17	0.18	0.35	<0.35
MG26	2.64	4.73	5.68	9.91	<0.35
MG27	11.4	13.7	7.68	45.2	<0.35
MG28	4.62	6.16	3.17	11.5	<0.35
MG29	13.6	14.6	10.3	29.5	<0.35
MG30	17.4	27.1	11.8	>100	<0.35
Idiopathic					

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Patient group	Pork (f26)	Beef (f27)	Lamb (f88)	Alpha-gal	Gelatin (c74)
ID3	0.04	0.05	0.02	0.35	<0.35
Gelatin colloid					
G1	<0.35	0.42	<0.35	1.54	<0.35

IgE to meat, alpha-Gal and gelatin was measured by ImmunoCap in patients diagnosed as being allergic and sensitised to meat only (M), to meat and gelatin (MG), to gelatin colloid (GC) and in one patient classified with idiopathic anaphylaxis (ID). An allergen-reactive IgE of > 0.35 KU/L was considered to be positive. nd = not done.

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Table 4

Characteristics of tick allergic subjects

Patient	Gender	Age (years)	Severity of tick reactions	Meat test (SPT/IDT wheal size in mm)	Gelatin test (SPT/IDT wheal size in mm)	Pork (f26)	Beef (f27)	Lamb (f88)	Alpha-gal	Gelatin (c74)
T1	F	65	Local	0/12	0/12	25.7	46.6	nd	100	<0.35
T2	F	66	Local	0/8	0/0	<0.35	0.61	<0.35	2.28	<0.35
T3	M	22	Local	0/7	0/7	3.17	3.82	1.82	6.57	<0.35
T4	M	67	Anaphylaxis	5/8	0/0	0.74	1.42	<0.35	2.95	<0.35
T5	F	82	Anaphylaxis	0/0	0/0	<0.35	<0.35	<0.35	<0.35	<0.35
T6	F	68	Anaphylaxis	0/nd	nd/nd	<0.35	<0.35	<0.35	0.72	<0.35
T7	M	63	Anaphylaxis	0/nd	nd/nd	<0.35	<0.35	<0.35	0.35	<0.35
T8	M	70	Anaphylaxis	nd/nd	0/0	<0.35	0.75	<0.35	5.74	<0.35
T9	F	67	Anaphylaxis	nd/nd	nd/nd	0.36	0.76	0.36	2.49	<0.35
T10	F	51	Local	0/10	0/6	0.61	1.01	0.57	nd	<0.35

IgE to meat, gelatin and alpha-Gal was measured in patients reporting allergic reactions to tick bites but without known food allergy. The results of skin prick tests (SPT) or intradermal tests (IDT are shown (mean wheal size in mm) are shown, with results ≥3mm than the negative control defined as positive. IgE to meat, alpha-Gal and gelatin was measured by ImmunoCap with levels > 0.35 KU/L considered to be positive. nd = not done.