Original article

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;■■■:■■■-■■■.)

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

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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

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.

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).

RESULTS

Patients' characteristics

Between 1995 and 2011, 1159 adults aged 18 to 101 years (423 male subjects) assessed by the first author (R.J.M.) were given a diagnosis of FA triggered by seafood (n = 284), peanut (n = 189), tree nuts (n = 188), systemic allergic reactions to fruits/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 patients with red meat allergy identified, 18 (46%) were male and aged 18 to 78 years (median, 48 years), and 27 had anaphylaxis. Patients estimated symptom onset at 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 (the M group) and 30 to red meat and gelatin (the MG group) on allergy testing. There was no relationship between onset time and likelihood of anaphylaxis ($P = .88$) or gelatin sensitization and likelihood of anaphylaxis ($P = .13$). With the exception of 2 vegetarians (MG1 and MG26), most patients given a diagnosis of red meat allergy reported tolerance on other occasions.

Meat and gelatin cosensitization and coreactivity

Thirty-two patients were cosensitized to red meat and gelatin, including 2 patients with intraoperative gelatin colloid anaphylaxis who were red meat tolerant (GC1 and GC2) and 29 patients given a diagnosis of red meat allergy (MG1-MG29, Table I). Of this MG group, 12 reported anaphylaxis when red meat was not ingested, including 2 additional patients with intraoperative gelatin colloid–induced anaphylaxis before presentation with red meat allergy (MG22 and MG24).[5] One additional patient (MG12) with recurrent red meat–induced anaphylaxis remained well for 5 years on a meat/gelatin-free diet. Despite wearing a MedicAlert (MedicAlert Foundation, Eastwood, South Australia) bracelet to warn of her possible gelatin allergy, she was given 40 mL of intravenous Gelofusine (approximately 1.6 g of gelatin) after a myocardial infarction and had urticaria, bronchospasm, hypoxia, and hypotension (hospital records verified by R.J.M.; Table I). Nine additional patients reported systemic reactions after oral gelatin consumption on separate occasions (eg, desserts) when meat ingestion was denied.

Prospective evaluation of gelatin sensitization

Between 1997 and 2011, 1335 subjects underwent gelatin IDTs. Positive results were observed in 40 (2.8%) subjects: 30 (75%) of 40 given a diagnosis of red meat allergy (the M+MG group); 2 of 2 patients with gelatin colloid–induced anaphylaxis (cosensitized to meat and gelatin but meat tolerant clinically, the GC group), 4 (2.3%) of 172 patients with idiopathic anaphylaxis (the ID group), and 5 (0.4%) of 1121 others tested without suspected meat/gelatin allergy (Tables I and II). Sensitization to gelatin was titratable in all patient groups (Fig 1). Five years after evaluation for possible insect venom allergy, however, 1 normally vegetarian subject with a positive gelatin test result of unknown significance returned after meat-induced anaphylaxis, was sensitized to red meat and gelatin on testing, and was reclassified into the MG group (MG26).

Gelatin challenges

In the 4 patients classified as having idiopathic anaphylaxis assessed from 2001-2003 (but with positive gelatin test results), red meat was implicated historically on multiple occasions, but sensitization to red meat could not be demonstrated on SPTs; meat IDTs were not undertaken at the time until reports of its utility.[19] Because removal of dietary meat was undesirable without further evidence, all agreed to an oral challenge with gelatin and, if the result was negative, with intravenous gelatin colloid. Although all tolerated a supervised oral challenge with 10 g of gelatin, each had urticaria and bronchospasm with intravenous gelatin colloid challenge at doses of 1.2 g (ID1; Fig 2), 0.1 g (ID2), 0.024 g (ID3), and 4.1 g (ID4). Avoidance of red meat and gelatin has reduced patient-reported episodes on follow-up from 1 episode in 6 months to 1 episode in 8 years (ID1), from 10 episodes per year to none in 6 years (ID2), from 3 episodes in 2 months to none in 6 years (ID3), and from 5 episodes per year to none in 5 years (ID4). See Appendix for additional information.

Additional *in vitro* testing

Where available, sera were tested for allergen-specific IgE to meat, gelatin, and α-Gal (Table III). In the M/MG groups 23 of 26 patients were sensitized to 1 or more red meat and 20 of 24 patients were sensitized to α-Gal (plus 2 borderline results), but only 1 of 25 patients were sensitized to gelatin despite a positive gelatin IDT result in 20 of these patients (Table III). There was no association between anti–α-Gal IgE and the likelihood of anaphylaxis and only a weak inverse relationship ($r = 0.37$, $P = .074$) between time of onset and the anti–α-Gal IgE level. There was a strong correlation between the anti–α-Gal IgE level and positive gelatin IDT reactivity: of 22 IgE α-Gal–positive sera from the M, MG, ID, and G groups, 20 patients had positive gelatin skin test results. Of 22 patients with positive gelatin IDT results, 20 were anti–α-Gal IgE positive. Furthermore, there was a correlation between mean gelatin IDT wheal diameter and *in vitro* IgE levels to α-Gal ($r = 0.46$, $P < .01$).

Comparison of testing methods

Where positive, the results of beef and pork SPTs and IDTs suggested sensitization to both (data not shown). Of 40 patients given a diagnosis of red meat allergy (the M+MG group), meat SPT responses were positive in 26 (65%) of 40, meat IDT results were positive in 18 (100%) of 18, gelatin IDT results were positive in 30 (75%) of 40, meat ImmunoCAP results were positive in 23 (88%) of 26, and anti–α-Gal IgE results were positive in 25 (96%) of 26.

Detection of α-Gal in gelatin and bovine products

Given the positive correlation between the results of gelatin skin testing and anti–α-Gal IgE levels, we examined whether α-Gal might be detectable in gelatin by using a sensitive RIA.[10]

TABLE I. Clinical characteristics of patients given a diagnosis of red meat or gelatin allergy and other patient groups, as assessed by means of skin testing

Patient group	Sex	Age (y)	Trigger(s) of clinical reactivity	Onset (h)	Severity	Anaphylaxis	Meat test (SPT/IDT wheal size [mm])	Gelatin test (SPT/IDT wheal size [mm])	No. of episodes (time period)
Meat									
M1	M	37	B	2	Moderate	Yes	6/ND	0/0	3 (3 wk)
M2	M	59	B	0.5	Severe	Yes	4/ND	0/0	2 (6 mo)
M3	F	32	B, L, P	2	Moderate	No	4/6	0/0	"All life" >4/y
M4	F	25	P	2	Moderate	Yes	0/4	0/0	"All life" >4/y
M5	M	18	B	2	Moderate	No	3/ND	0/0	"All life" >4/y
M6	F	56	B, P	2	Moderate	Yes	3/ND	0/0	2 (6 mo)
M7	M	50	H	3	Moderate	Yes	3/6	0/0	"All life" >4/y
M8	F	31	B, L	0.5	Moderate	No	5/ND	0/0	1 (10 y ago)
M9	M	36	B	3	Moderate	Yes	3/ND	0/0	10 (6 mo)
M10	F	23	B, P	2	Moderate	No	5/ND	0/0	2 (3 mo)
Meat + gelatin									
MG1	M	77	B	6	Moderate	Yes	3/15	0/15	2 (2 mo)
MG2	M	51	B, L, G	5	Severe	Yes	3/ND	0/15	10 (8 mo)
MG3	F	28	B, L, K	1.5	Moderate	Yes	0/14	0/12	>25 (6 y)
MG4	F	21	B	3	Moderate	Yes	0/8	0/8	2 (2 mo)
MG5	M	64	B	3	Severe	Yes	0/12	0/12	>10 (4 y)
MG6	F	18	P	6	Moderate	Yes	6/10	0/8	"All life" >4/y
MG7	M	52	B, G	6	Mild	No	3/10	0/12	3 (3 mo)
MG8	F	16	B	6	Moderate	No	0/8	0/6	1 (2 mo)
MG9	M	78	B	8	Severe	Yes	0/10	0/15	6 (3 y)
MG10	F	60	L	2	Moderate	No	4/10	0/10	1 (2 mo)
MG11	M	52	B, L, G	5	Moderate	Yes	3/ND	0/15	4 (18 mo)
MG12	F	71	B	3.5	Severe	Yes	3/ND	0/12	>20 (2 y)
MG13	F	51	B, L, P, G	0.25	Severe	Yes	3/ND	2/12	>10 (2 y)
MG14	F	28	L, K	1.5	Severe	Yes	5/ND	0/12	3 (3 mo)
MG15	F	73	B	4	Mild	No	3/ND	0/10	3 (5 y)
MG16	F	63	B, GC	2	Severe	Yes	3/ND	0/5	>50 (10 y)
MG17	F	35	B, L, P, G	0.25	Severe	Yes	3/ND	0/15	2 (8 mo)
MG18	M	44	B	9	Moderate	Yes	0/15	0/12	2 (6 mo)
MG19	F	27	B	2	Mild	No	3/ND	0/10	1 (6 mo)
MG20	M	46	B, L	6	Severe	Yes	3/ND	0/12	5 (3 y)
MG21	F	48	B, P	8	Mild	No	3/ND	0/6	>5 (5 y)
MG22	M	69	B, GC, G	6	Severe	Yes	3/ND	3/10	>10 (3 y)
MG23	M	66	B, L, P, GC	7	Severe	Yes	3/ND	0/12	>30 (4 y)
MG24	M	72	GC, G, P	0.5	Severe	Yes	3/ND	0/5	2 (6 mo)
MG25	F	41	G, P	2	Mild	No	3/ND	3/10	4 (2 y)
MG26	F	39	B	7	Mild	No	0/10	0/10	2 (6 mo)
MG27	M	74	B, L	3	Severe	Yes	0/15	0/15	18 (1 y)
MG28	F	61	B, L, G	4	Severe	Yes	0/12	0/12	8 (2 y)
MG29	M	19	B, P	4	Severe	Yes	0/12	0/12	1 (1 mo)
MG30	F	65	B, L	1	Moderate	No	2/10	0/12	6 (10 y)
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 mo)
ID2	F	22	B	2	Moderate	No	0/ND	0/7	5 (1 y)
ID3	F	35	B, P	5	Moderate	Yes	0/ND	0/6	3 (2 mo)
ID4	F	54	B, L	3	Severe	Yes	0/ND	0/8	>20 (3 y)

(Continued)

α-Gal was detected in both gelatin colloids (0.52 ± 0.1 μg of α-Gal/g of Gelofusine and 0.44 ± 0.2 μg/g of Haemaccel). By using similar techniques, the concentrations of α-Gal were 5.6 μg of α-Gal per gram of beef thyroglobulin and 1.4 μg of α-Gal per gram of heavy cream. By contrast, no detectable α-Gal was found in cow's milk (skim or 1% or 2% milk fat). Of the 21 oligosaccharides identified on cetuximab, approximately 30% have 1 or more α-1,3–linked galactosyl residues, as measured by peak area on time-of-flight mass spectrometric spectra,[28] and α-Gal was detected at a concentration of 10.2 μg/5 mg of cetuximab

TABLE I. *(Continued)*

Patient group	Sex	Age (y)	Trigger(s) of clinical reactivity	Onset (h)	Severity	Anaphylaxis	Meat test (SPT/IDT wheal size [mm])	Gelatin test (SPT/IDT wheal size [mm])	No. of episodes (time period)
Drug allergy									
D1	F	35	NA	NA	NA	NA	ND/ND	ND/5	NA
D2	M	64	NA	NA	NA	NA	ND/ND	ND/12	NA
D3	F	37	NA	NA	NA	NA	ND/ND	ND/5	NA
D4	F	20	NA	NA	NA	NA	ND/ND	ND/7	NA

Patients given a diagnosis of allergy to red meat, gelatin, or both, as well as control groups, underwent SPTs or IDTs with meat-derived allergen and gelatin colloid. IDTs were normally only undertaken if SPT responses were negative. Results are described as mean wheal diameter in millimeters or not done *(ND)*, as indicated. For the purposes of this study, red meat was defined as beef *(B)*, pork *(P)*, lamb *(L)*, horse *(H)*, or kangaroo *(K)*. Other patients were clinically allergic to oral gelatin *(G)* or gelatin colloid *(GC)*. The time of symptom onset after meals was a patient estimate, with 1 patient (MG18) reporting symptoms after 6 hours on 1 occasion and 9 hours after another.
F, Female; *M*, male; *NA*, not applicable.

TABLE II. Gelatin sensitization

Clinical group	Positive test results	No. tested	Percent positive
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
Nonmeat 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	
Totals	40*	1335	2.9

One thousand three hundred twenty-five patients underwent IDTs with gelatin colloid between 1997 and 2011.
ACE, Angiotensin-converting enzyme.
*One patient returned with red meat–induced anaphylaxis 5 years after investigation for insect venom allergy and was reclassified into the red meat allergy group.

in the inhibition RIA. By contrast, α-Gal was undetectable in fish gelatin (lower limit of assay, 0.01 μg).

Relationship between red meat allergy and tick exposure

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

DISCUSSION

We have identified a significant relationship between adult-onset red meat allergy and sensitization and clinical reactivity to gelatin, which is supported by the results of intraoperative exposure (MG23 and MG24 and GC1 and GC2), accidental exposure (MG12), observed challenge (ID1-ID4), or claims of reactivity to oral gelatin. Consistent with previous studies, SPT responses to commercial meat extracts were relatively small and sometimes negative,[19,29] and IDT and *in vitro* test results 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 sensitized to α-Gal, and there was a historical association between tick bite exposure, sensitization, and allergy to red meat.[20,21] Although beef was the dominant meat triggering symptoms, this might reflect nonallergic factors, such as cost, availability, amount consumed at any one sitting, and 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 (because of the potential discomfort from IDTs), and therefore 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 15 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 patients of the MG group are dependent on patient reports, it is difficult to ignore the clinical significance of a positive gelatin IDT result at least as a risk factor for gelatin colloid reactivity (as described above), including 1 case of accidental exposure in which the risk was identified prospectively (MG12). Conversely, clinical reactivity to red meat was observed in 2 of 4 patients with colloid allergy, and gelatin sensitization was predictive of red meat allergy in 1 "healthy control subject" (MG26). Although the significance of gelatin sensitization in 4 otherwise healthy subjects with drug allergy, meat sensitization in 2 patients with gelatin colloid allergy, and gelatin/meat sensitization in patients evaluated for tick bite allergy remains currently uncertain, this might become apparent with further observation (eg, MG26) or might represent sensitization without clinical allergy, as recently reviewed.[33] Although we would have preferred to undertake more gelatin challenges in patients with a positive gelatin test result, our ability to do so was constrained by the patient's age, comorbidity, patient unwillingness to do so, and geographic location (most lived at the coast >200 km 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 IgE-dependent mechanisms have been

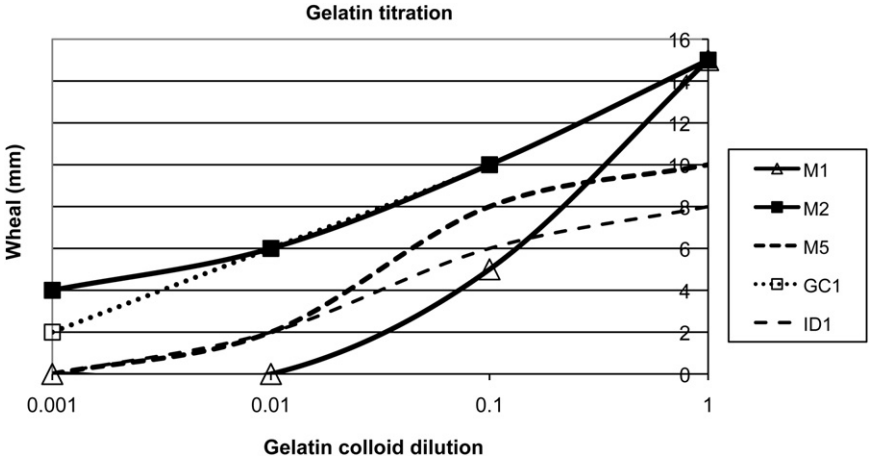

FIG 1. Titration of intradermal gelatin colloid skin testing. Intradermal testing with gelatin colloid was titrated in 17 patients, with positive test results detectable at dilutions of undiluted colloid only (3 patients) and 1:10 (6 patients), 1:100 (8 patients), and 1:1000 (0 patients) dilution. Representative examples are shown. *GC*, Gelatin colloid; *ID*, idiopathic; *M*, meat.

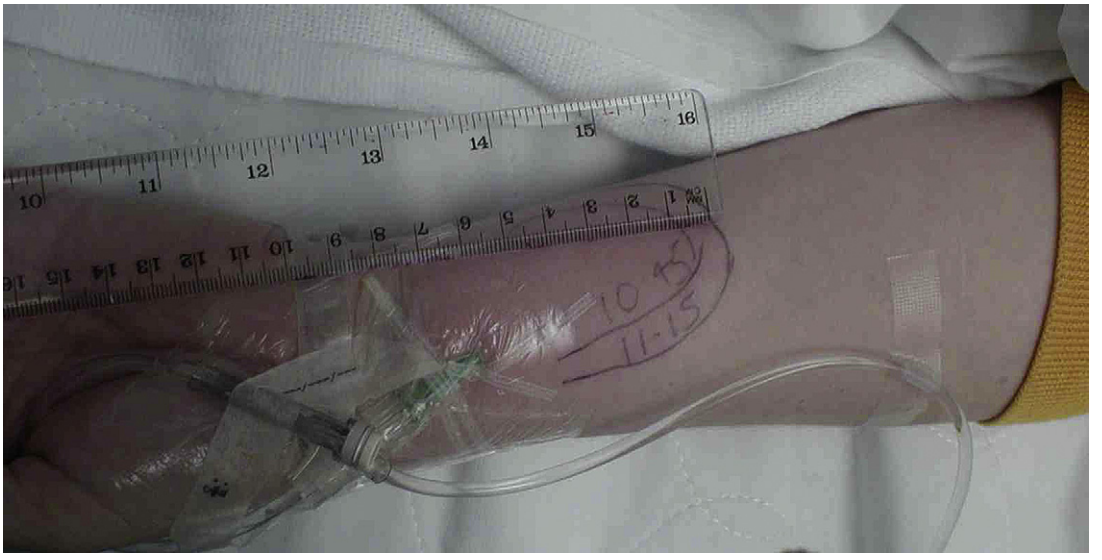

FIG 2. Patient ID1 gelatin colloid challenge. After the equivalent of 0.1 mL of undiluted gelatin colloid (Haemaccel; approximately 3.5 mg of gelatin), a 5 × 8–cm itchy wheal developed around the cannula site at 10:45 AM, with progression over the next 30 minutes. With 31 mL (approximately 1.1 g of gelatin), she had axillary and inguinal urticaria, tachycardia, itchy throat, cough, and bronchospasm, and peak flow deteriorated from 540 to 470 L/min, at which point the challenge was terminated Importantly, the cannula remained patent throughout the procedure.

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 immunologic cross-reactivity 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 α2 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 hydrolyzed, and (5) patients generally require parenteral exposure to trigger sensitization.[12,35] Vaccine-reactive patients (sometimes also reactive to oral gelatin),[7,11,12] however, are likely to be more sensitive to gelatin than our patients, with sensitization detectable by means of SPTs with diluted vaccines (approximately 0.2 mg/mL gelatin vs approximately 40 mg/mL gelatin IDTs in our patients) and reactivity to approximately 2 mg of parenteral gelatin[36] compared with 24 to 4100 mg in our challenge patients.

Although 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 confectioneries (eg, marshmallows), food thickeners, dips, glazes, and icing and acts as a fat substitute in yogurt, mayonnaise, and ice cream.[9] Gelatin can be found in sausage coatings, salami, tinned hams, pâté, and meat stock and is used to

TABLE III. *In vitro* testing in patients given a diagnosis of allergy to red meat or gelatin

Patient group	Pork (f26)	Beef (f27)	Lamb (f88)	α-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					
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 levels (in kilounits per liter) to meat, α-Gal, and gelatin were measured by using ImmunoCAP in patients given a diagnosis of allergy to and sensitized to meat only, to meat and gelatin, or to gelatin colloid and in 1 patient classified with idiopathic anaphylaxis *(ID)*. An allergen-reactive IgE level of greater than 0.35 kU/L was considered to be positive.
ND, Not done.

clarify fruit juice and wine.[9] Gelatin can thus be considered a potential occult food allergen because exposure is ubiquitous, and the method of extraction (acid and alkaline hydrolysis with heat treatment) makes it more likely to survive food preparation than heat-labile meat proteins, such as bovine gamma globulin and BSA.[3]

That reactions to topical or oral gelatin can occur is supported by rare case reports of allergic reactions to "hydrolyzed protein" (gelatin) in shampoo, collagen implants, "catgut" sutures, and collagen-derived contact lenses, as well as to gelatin present as binding agent in tablets, capsules, suppositories, or confectionaries.[4,6,9,37-40] Although 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. Although these claims (and the scarcity of published cases) might reflect poor recognition of gelatin as a possible trigger, episodes erroneously labeled as idiopathic (because of negative routine allergy test results), the absence of cofactors, or a higher risk from parenteral exposure, one potential clue might be the oral dose required to trigger an allergic reaction. Our challenge patients did not react to 10 g of oral gelatin, which is in retrospect a relatively small dose compared with the large amount intravenously required to trigger anaphylaxis in the same subjects. Although ongoing studies await the results of challenges with 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 1 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 IDTs to detect sensitization (Table I). Explanations for this have been discussed previously,[19] including the possibility that folding of proteins within extracts might make α-Gal less available for mast cell cross-linking, that antibodies to uncharged carbohydrate molecules like α-Gal might be of low affinity,[42] or, perhaps of specific relevance, evidence that α-Gal concentrations are 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 IDTs were more sensitive at detecting gelatin sensitization yet paradoxically that *in vitro* test results were negative in almost all samples. This cannot be explained by serum sample degradation caused by prolonged storage because IgE to meat and α-Gal was detected in parallel assays. Potential explanations include assay insensitivity caused by the preparation of gelatin required for immunoassay grade stability or, if α-Gal is the allergenic target, insufficient concentration on the ImmunoCAP to detect sensitization. Alternatively, gelatin-reactive IgE might 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 support the use of gelatin colloids where possible (not available in the United States 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 result having potential clinical relevance for avoidance strategies. Although the option of using food-grade gelatin for testing can be considered in patients with suspected meat allergy, gelatin allergy, or both, the potential for processing of food-grade gelatin to yield extracts of varying molecular weights might 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] Although comparison of results with gelatin colloid and crude gelatin extracts for SPTs/IDTs merits future study, our plan to undertake IDTs in more than 1000 patients led us to not consider using an unstandardized and nonsterile reagent for our prospective study in a large number of subjects 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 toward heat-labile meat proteins,[3] others directed toward α-Gal, and others directed toward gelatin. Consistent with previous studies,[43,44] the majority of our patients experienced only occasional overt reactions, despite regular meat

TABLE IV. Characteristics of patients with tick allergy

Patient	Sex	Age (y)	Severity of tick reactions	Meat test (SPT/IDT wheal size [mm])	Gelatin test (SPT/IDT wheal size [mm])	Pork (f26)	Beef (f27)	Lamb (f88)	α-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 levels (in kilounits per liter) to meat, gelatin, and α-Gal were measured in patients reporting allergic reactions to tick bites but without known food allergy. The results of SPTs or IDTs are shown (mean wheal size in millimeter) are shown, with results of 3 mm or greater than the negative control defined as positive. IgE levels to meat, α-Gal, and gelatin were measured by using ImmunoCAP, with levels of greater than 0.35 kU/L considered positive.
F, Female; *M*, male; *ND*, not done.

consumption. Potential explanations remain speculative but might include the absence of cofactors (eg, exercise), the amount ingested (other studies suggest that >75 g 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–α-Gal concentrations might decrease naturally over time without additional tick bites (Platts-Mills and Commins, unpublished observations) might 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] might influence allergen absorption. Furthermore, nocturnal onset while asleep might prevent recognition of milder reactions and delay recognition of more severe episodes, perhaps accounting for some considerable delays observed (eg, patient MG18).

We were also able to confirm previous Australian and US reports of an association between adult-onset red meat allergy, α-Gal sensitization, and a history of tick bite reactions,[19-21] which might explain the geographic location of most of our patients. This is significant because we also found asymptomatic sensitization to gelatin, meat, and α-Gal in a small number of patients with tick bite reactions in a different country with different tick species. The detection of α-Gal in gelatin preparations and the correlation between sensitization to red meat, gelatin, and α-Gal in most cases raises an intriguing possibility: as has been proposed for reactions to cetuximab,[46] clinical reactivity to gelatin might in some cases be mediated by anti–α-Gal IgE. If so, this might in part help explain the poor correlation between the results of *in vitro* tests and IDTs to gelatin in our adult population compared with younger and more sensitive patients reacting to lower doses of gelatin in vaccines,[7,11,12] with IDTs acting as an indirect marker of IgE reactivity to α-Gal and with positive *in vitro* test results in the vaccine studies perhaps reflecting either IgE to other gelatin moieties or patients with very high titers of anti-gelatin IgE. Of interest, if tick bite exposure is a risk factor for meat/gelatin sensitization (and precedents for geographic 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 intravenous (and sometimes oral) gelatin as well. Gelatin sensitization poses a risk of clinical reactivity to both red meat and gelatin, although not in all patients. Patients presenting with clinical reactions to either trigger thus merit evaluation for sensitization to both triggers and should be warned appropriately if results are positive. Taking into account the relationship between the results of gelatin and α-Gal testing in our patients and the detection of α-Gal in gelatin, a positive test result to either might 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, inability of patients to always correctly identify their dietary triggers, and geographic limitations in the availability of diagnostic tests for anti–α-Gal and anti-gelatin IgE. Future challenges include determining whether a salivary component common to multiple tick species from different continents might be the sensitizing agent[21] and the factors contributing to delayed onset of symptoms to red meat compared with other foods.

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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 α-Gal in gelatin and correlation between the results of α-Gal and gelatin testing raises the possibility that α-Gal IgE might be the target of reactivity to gelatin.**
- **The relationship between tick bite reactions in patients with meat allergy 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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APPENDIX

Patient ID4 (with positive gelatin IDT, positive IV gelatin colloid challenge but negative meat SPT) was reviewed February 2012. She continued to have regular tick bites but no further episodes of anaphylaxis had occurred with red meat and gelatin avoidance. Meat and gelatin SPT was negative (1 mm wheals), but gelatin and meat IDT was positive (12 mm wheals). In *vitro* testing was positive to beef (7.44 kU/L), lamb (2.29 kU/L), pork (4.61 kU/L) and α-Gal (14.6 kU/L).

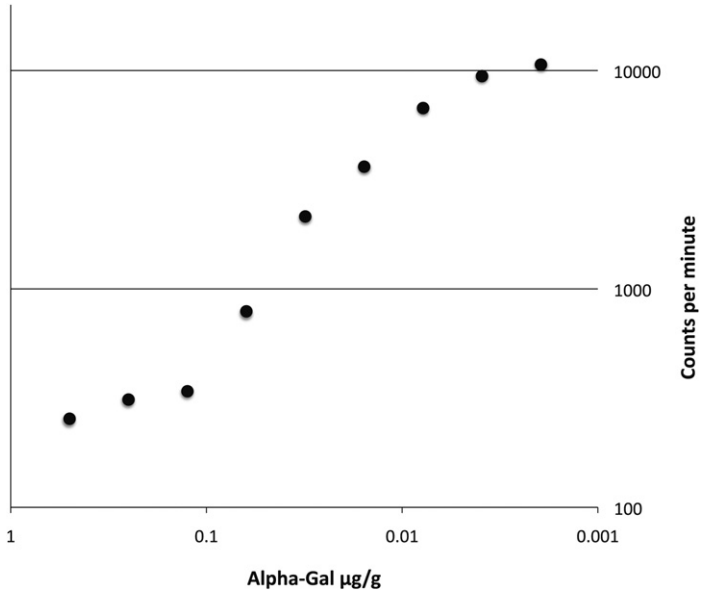

FIG E1. Standard curve of iodine 125–labeled α-gal inhibition RIA. The concentrations of α-Gal were measured by using inhibition RIA. A standard curve was created with serial dilutions of the linear trisaccharide Galα1-3Galβ1-4GlcNAc. Counts per minute of iodine 125–labeled α-Gal *(y-axis)* are compared with the concentration of α-Gal (in micrograms per gram of test substance) on the *x-axis*.