

ORIGINAL ARTICLE

EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Laminin γ -1 and collagen α -1 (VI) chain are galactose- α -1,3-galactose-bound allergens in beef

H. Takahashi¹, Y. Chinuki¹, A. Tanaka² & E. Morita¹

¹Department of Dermatology, Shimane University Faculty of Medicine, Shimane; ²Thermo Fisher Scientific K.K., Tokyo, Japan

To cite this article: Takahashi H, Chinuki Y, Tanaka A, Morita E. Laminin γ -1 and collagen α -1 (VI) chain are galactose- α -1,3-galactose-bound allergens in beef. *Allergy* 2013; DOI: 10.1111/all.12302.

Keywords

allergens; carbohydrate; food allergy; mammalian meat; α -Gal.

Correspondence

Hitoshi Takahashi, Department of Dermatology, Shimane University Faculty of Medicine, Enya 89-1, Izumo 693-8501, Shimane, Japan.
Tel.: +81-853-20-2210
Fax: +81-853-21-8317
E-mail: tkh4@med.shimane-u.ac.jp

Accepted for publication 14 September 2013

DOI:10.1111/all.12302

Edited by: Reto Cramer

Abstract

Background: Sensitization to the carbohydrate galactose- α -1,3-galactose (α -Gal) has been reported in patients with beef allergy. However, the proteins responsible for this allergy have not yet been identified. This study aimed to identify beef proteins that predominantly react with serum IgE in Japanese patients with beef allergy.

Methods: Sera were collected from 29 patients with beef allergy who had allergic reaction(s) such as urticaria, abdominal pain, vomiting, and anaphylactic shock after ingestion of beef and pork; the sera tested positive for IgE against beef and pork. IgE-binding proteins were detected by immunoblotting sera from the patients and identified using a combination of two-dimensional gel electrophoresis and peptide mass fingerprinting techniques. The involvement of carbohydrate in the binding of IgE to allergens was examined by periodate treatment and an inhibition assay with cetuximab by immunoblotting. Specific IgE binding to cetuximab was measured using the CAP-fluorescent enzyme immunoassay.

Results: Two IgE-binding proteins (240 kDa and 140 kDa) were detected in beef extract and identified as laminin γ -1 and the collagen α -1 (VI) chain from *Bos taurus*, respectively. Periodate treatment or the inhibition assay resulted in the loss of IgE binding to these proteins. Immunoblotting with anti- α -Gal antibody revealed the presence of α -Gal on the 240- and 140-kDa beef proteins. The amount of IgE bound to cetuximab was significantly correlated with that to beef in the patients with beef allergy.

Conclusion: The carbohydrate moiety (α -Gal) on laminin γ -1 and collagen α -1 (VI) chain are possibly common IgE-reactive proteins in the Japanese patients with beef allergy.

Meat accounts for 3% of the causative foods of immediate-type food allergies in the Japanese population (1). An allergic reaction to mammalian meat, referred to as beef allergy or red meat allergy, develops after the ingestion of mammalian meat such as beef and pork, but not chicken or fish. Investigations of allergens causing a meat allergy have mainly focused on cross-reactive allergens in cow's milk in patients with atopic dermatitis who were sensitized to beef; bovine serum albumin (BSA), bovine immunoglobulin G (IgG), and bovine actin have been identified as the cross-reactive allergens (2–8).

Chung et al. (9) reported that the carbohydrate galactose- α -1,3-galactose (α -Gal), which is present in the heavy chain of cetuximab (a chimeric monoclonal antibody against

epidermal growth factor receptor), is a major allergen causing hypersensitivity reaction after the first cetuximab infusion. In addition, they showed that the distribution of IgE antibodies to α -Gal was highly variable depending on the region in the United States (9). Based on this finding, a series of studies have clarified that patients with cetuximab hypersensitivity have a red meat allergy, and α -Gal in red meat is responsible for red meat allergy (10–16). However, red meat proteins bearing α -Gal have not yet been identified.

We screened IgE-binding proteins in red meat (beef and pork) using sera from Japanese patients with beef allergy and identified laminin γ -1 and the collagen α -1 (VI) chain as major allergens possessing an α -Gal moiety.

Materials and methods

Patients and sera

Sera were obtained from 29 patients with beef allergy; sera were stored at -20°C until analysis. Beef allergy was diagnosed by the presence of clinical episode(s) of allergic reaction such as urticaria, abdominal pain, vomiting, and anaphylactic shock within 8 h after ingesting mammalian meat, and positive serum-specific IgE tests of beef and pork. Table 1 lists the clinical features of the patients. Serum-specific IgE values were measured by a CAP-fluorescent enzyme immunoassay (CAP-FEIA; ImmunoCAP[®]; Thermo Fisher Scientific, Uppsala, Sweden). This study was approved by the Ethics Committee of the Shimane University Faculty of Medicine (approval No. 469).

Preparation of meat proteins

Meat (beef, pork, and chicken) was homogenized in a 10 $\times$ volume of ice-cold phosphate-buffered saline (PBS) containing a protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland). The homogenate was centrifuged at 20 000 *g* for 15 min at 4°C , and the supernatant was collected as water-soluble proteins. The pellet was solubilized in 2% sodium dodecyl sulfate (SDS) aqueous solution with constant mixing at 95°C for 5 min and centrifuged at 20 000 *g* for 15 min at 4°C , and the supernatant was collected as water-insoluble proteins. Protein concentrations were determined using a protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Immunoblot analysis

For IgE immunoblotting, meat proteins were separated by 7.5% SDS–polyacrylamide gel electrophoresis (PAGE) (25 $\mu\text{g}/\text{lane}$) under reducing conditions and subsequently transferred electrophoretically to a polyvinylidene difluoride (PVDF) membrane (Immobilon-P; Millipore, Billerica, MA, USA). The PVDF membrane was incubated with a blocking solution (20 mM Tris–HCl, pH 8.0, 0.15 M NaCl, 0.1% Tween-20, and 0.6% polyvinylpyrrolidone) for 1 h and further incubated with patient sera diluted to 10% with blocking solution for 16 h at 28°C . After washing the PVDF membranes with TBST (20 mM Tris–HCl, pH 8.0, 0.15 M NaCl, 0.1% Tween-20) three times, bound IgE was visualized on RX-U Fuji medical X-ray film (FUJIFILM Co., Tokyo, Japan) using horseradish peroxidase (HRP)-conjugated goat anti-human IgE antibody (Kirkegaard & Perry Laboratories, Inc., KPL, Gaithersburg, MD, USA) and the ECL prime kit (GE Healthcare UK Ltd, Buckinghamshire, UK). Total protein in the gel was stained with Coomassie Brilliant Blue (CBB).

For α -Gal detection, mouse anti- α -Gal monoclonal antibody (M86 clone; Enzo Life Sciences, Inc., Farmingdale, NY, USA) and HRP-conjugated anti-mouse IgM (μ) antibody (KPL) were used. The chemiluminescent signal was detected as described above.

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)

To obtain the allergen-rich fraction, water-soluble beef proteins were precipitated with ammonium sulfate at concentrations of 0–20%, 20–40%, 40–60%, and 60–80% at 4°C . Each precipitate was dissolved in PBS, and the suspension was dialyzed twice in PBS at 4°C for 16 h. IgE immunoblot analysis with patient sera revealed that the precipitate obtained with 20–40% ammonium sulfate contained IgE-reactive proteins with relative molecular masses of 240 kDa and 140 kDa.

The allergen-rich fraction was cleaned up with a Ready-Prep 2-D Cleanup kit (Bio-Rad) for isofocusing electrophoresis, and the resultant precipitate was dissolved in a buffer containing 60 mM Tris–HCl (pH 8.8), 6 M urea, 1 M thio-urea, 3% CHAPS, and 1% TritonX-100. Residual particles were removed by ultracentrifugation at 200 000 *g* for 20 min at 4°C . Protein concentration was determined using an RC DC protein assay kit (Bio-Rad).

2D-PAGE was performed according to the manufacturer's instructions. First-dimension protein separation (40 μg) was carried out in agarGEL (pH 3–8, ϕ 2.5 $\times$ 75 mm; ATTO corp., Tokyo, Japan) at 300 V for 3.5 h. The agarGel was subsequently used for second-dimension SDS-PAGE on a 7.5% acrylamide gel under reducing conditions. For IgE immunoblot analysis, proteins separated by 2D-PAGE were electrophoretically transferred to PVDF membranes and further incubated with 10% patient sera. Bound IgE was detected as described above.

Identification of beef allergens

Protein spots were manually excised from the CBB-stained gel, subjected to reductive alkylation by iodoacetamide, and digested with trypsin gold (Promega, Madison, WI, USA) using ProteaseMAX[™] Surfactant (Promega) following the manufacturer's instructions. Peptides were crystallized with α -cyano-4-hydroxycinnamic acid on a stainless steel plate. The mass spectra of peptides were recorded in the reflectron mode of matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS; AB SCIEX TOF/TOF 5800 system, Framingham, MA, USA). The mass spectrometer was calibrated with angiotensin II, P14R, and adrenocorticotrophic hormone fragments 18–39 (Sigma, St. Louis, MO, USA). Data processing was carried out using Data Explorer software ver. 4.10 (Applied Biosystems/MDS Analytical Technologies, Foster City, CA, USA). For peptide mass fingerprinting (PMF) and the MS/MS ion search, the generated mass lists were searched against the protein data bank of the National Center for Biological Information (NCBI) using the data base search engine MASCOT (Matrix Science, London, UK) after excluding peak lists of known contaminants such as keratin and trypsin. The peptide mass tolerance and fragment mass tolerance were set at ± 100 ppm and ± 0.3 Da, respectively.

Table 1 Clinical features of 29 patients with beef allergy

Case No.	Age (year)	Gender	Symptom(s)*	Causative food(s)	Time to reaction (h)**	CAP-FEIA (kUa/l)			
						Beef	Pork	Chicken	Cetuximab
1	69	M	U, A	Beef innards and alcohol	3	7.83	6.95	<0.35	48.8
2	71	F	U	Beef meat	1	3.29	0.77	<0.35	26.3
3	69	F	U	Beef meat	2	39.1	32.6	<0.35	60.7
4	58	F	U, V, AS	Beef and pork meat	2	1.83	1.28	n.d.***	16.8
			U, AS	Pork meat	1.5				
			U	Beef and pork meat	Unknown				
			U	Beef meat	1.5				
5	47	F	U	Beef innards	3	3.35	3.65	<0.35	8.07
			U	Beef and pork meat	0.5				
			U, D	Beef meat	Unknown				
6	70	M	U	Beef and pork meat	Unknown	20.7	15.3	<0.35	75.5
7	59	M	U	Beef, pork, and alcohol	6	26.9	23.7	n.d.	86.4
8	68	M	U	Pork meat	5	26.1	21.3	<0.35	60.1
9	78	M	U	Boar meat	Unknown	2.62	1.23	<0.35	n.d.
10	70	F	U	Beef meat	1	1.93	1.72	<0.35	4.03
11	65	M	U	Beef and pork meat	Unknown	30	20.2	<0.35	122
12	68	F	A	Beef and pork meat	Unknown	5.64	4.10	<0.35	65.8
13	64	M	U, A, D	Beef innards	Unknown	2.23	1.94	<0.35	7.27
			U	Beef and pork meat	Unknown				
14	53	F	U, AS	Beef and pork meat	4	0.94	2.64	<0.35	31.0
			U, D	Beef and pork meat	4				
			A, D	Pork meat	7				
15	57	M	U	Chinese noodles	4	32.2	11.9	<0.35	67.6
			U	Pork meat and alcohol	2				
			U, V, A	Beef innards and alcohol	2.5				
16	70	F	D	Beef meat	Unknown	12.4	9.12	<0.35	75.3
17	86	M	U	Beef and pork meat	Unknown	6.27	6.28	n.d.	10.0
18	65	M	U	Raccoon dog meat	2	2.7	1.69	<0.35	30.0
19	67	M	U	Beef meat	2	8.49	8.17	<0.35	13.8
			U	Boar meat	4.5				
			U	Horse meat	Unknown				
			U	Boar meat	1.5				
			U	Beef meat	1–1.5				
20	60	F	U	Beef meat	Unknown	18.7	6.07	<0.35	89.7
			U	Beef meat	Unknown				
21	66	M	U	Beef innards	Unknown	1.86	1.29	<0.35	2.99
			U, D	Beef meat	Unknown				
			U	Pork meat	4				
			U	Beef and pork meat	Unknown				
			U	Pork meat	Unknown				
22	55	F	U	Beef meat and alcohol	5.5	31.2	14.5	<0.35	257
			U	Beef meat and alcohol	7.5				
23	64	F	U	Beef and pork	Unknown	0.94	0.69	<0.35	18.1
			U	Pork meat	Unknown				
24	64	F	U	Beef meat	4	16.4	7.44	<0.35	54.3
25	81	M	U	Beef meat	Unknown	6.37	5.32	<0.35	10.0
26	76	M	U	Beef and pork meat	Unknown	6.41	3.72	<0.35	20.3
			U	Pork meat	1				
			U	Beef meat	Unknown				

Table 1 (Continued)

Case No.	Age (year)	Gender	Symptom(s)*	Causative food(s)	Time to reaction (h)**	CAP-FEIA (kUa/l)			
						Beef	Pork	Chicken	Cetuximab
27	78	M	U	Beef, pork, mutton, chicken meat and alcohol	Unknown	6.38	3.16	<0.35	n.d.
28	59	M	U	Beef liver and chicken meat	Unknown	58.4	n.d.	n.d.	103
29	57	M	U	Beef and alcohol	2	19.5	13.0	n.d.	84.1

*Symptom(s), U; urticaria, A; abdominal pain, V; vomiting, D; diarrhea, AS; anaphylactic shock.

**Time to reaction, time (h) taken for allergic symptom(s) to develop after eating causative food(s).

***n.d., not determined.

Sequence analysis

A homology search of proteins identified from the amino acid sequences was performed with BLASTp on the NCBI nonredundant protein sequence database. A conserved domain search was also performed on the NCBI platform.

Periodate treatment

Water-soluble beef proteins were separated on 7.5% SDS-PAGE under reducing conditions and electrophoretically transferred to a PVDF membrane. Membranes were incubated with a solution containing 50 mM sodium acetate (pH 4.5) and 20 mM sodium periodate for 1 h in the dark and further incubated with patient sera after washing with TBST. Bound IgE was detected as described above.

Inhibition assay by immunoblotting

Serum was diluted to 10% with blocking solution and incubated with different amounts of cetuximab (10, 100 µg) as an inhibitor, or PBS as control for 1 h at 37°C. The pre-incubated serum was reacted with water-soluble beef proteins electroblotted onto the PVDF membrane for 16 h at 28°C. Bound IgE was detected as described above.

Preparation of cetuximab CAP-FEIA

To prepare biotin-labeled cetuximab, cetuximab solution was dialyzed with PBS using the HiTrap desalting column (5 ml, GE Healthcare). The resultant solution, containing 3.3 mg of cetuximab, was labeled with 53.2 mmol biotin using the EZ-link sulfo-NHS-LC biotin reagent (Thermo Fisher Scientific, Waltham, MA, USA) for 30 min at 28°C. Subsequently, unreacted biotin was removed by passing through the HiTrap desalting column. Protein concentrations were determined using the RC DC protein assay kit (Bio-Rad). Cetuximab biotinylation was confirmed by dot blot analysis using HRP-conjugated streptavidin and the ECL kit. To construct cetuximab CAP-FEIA, 5 µg of biotinylated cetuximab was bound to the streptavidin CAP-FEIA system (9, 17). The IgE value to cetuximab was compared with that to beef using the Spearman's rank order correlation test. Statistical analysis was performed with SPSS software, version 20 (IBM Corp.,

Armonk, NY, USA). A two-sided *P* value of less than 0.05 was considered to indicate statistical significance.

Results

IgE immunoblot analysis of meat proteins

Serum IgE binding to water-soluble beef proteins was examined by immunoblotting in 29 patients with beef allergy. Multiple bands were detected in all patients, as shown in Fig. 1A, and two bands were commonly seen at 240 kDa and 140 kDa. When serum IgE binding to the water-soluble and water-insoluble proteins of pork and chicken were analyzed by immunoblotting using sera from four patients (case Nos 1, 3, 5, and 7), the 240-kDa band was detected commonly with the water-soluble proteins of pork, but not with those of chicken (Fig. 1B). In contrast, the 140-kDa band in water-soluble proteins of pork was detected only in the serum of patient No. 3. The 140-kDa band seen in the water-insoluble proteins of beef and pork was nonspecific because this band was also seen in sera of healthy subjects.

Identification of the 240-kDa and 140-kDa allergens as laminin γ -1 and the collagen α -1 (VI) chain

On 2D-PAGE, two dominant IgE-binding spots were detected using sera from two patients (case Nos 1 and 3) at 240 kDa and pI 5 (spot 1) and at 140 kDa and pI 5.5 (spot 2) (Fig. 2A). The position of each spot was identical in the sera of the patients tested. IgE-binding spots were not detected in the sera of two healthy subjects (data not shown). The 240-kDa and 140-kDa protein spots were analyzed with PMF and MS/MS ion search using MALDI-TOF MS. MAS-COT scores were significant in both PMF and MS/MS analyses (Table 2), and spots 1 (240 kDa) and 2 (140 kDa) were identified as laminin γ -1 and the collagen α -1 (VI) chain from *Bos taurus*, respectively. Other spots detected in the low molecular area by immunoblotting could not be identified because these proteins were not separated clearly (Fig. 2B).

Homology analysis showed that laminin γ -1 from *B. taurus* has significant homology with those from *Sus scrofa*, human, and *Gallus gallus* (Table 3). The collagen α -1 (VI) chain from *B. taurus* also has significant homology with those from human and *G. gallus* (Table 3). The homology between

© 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd

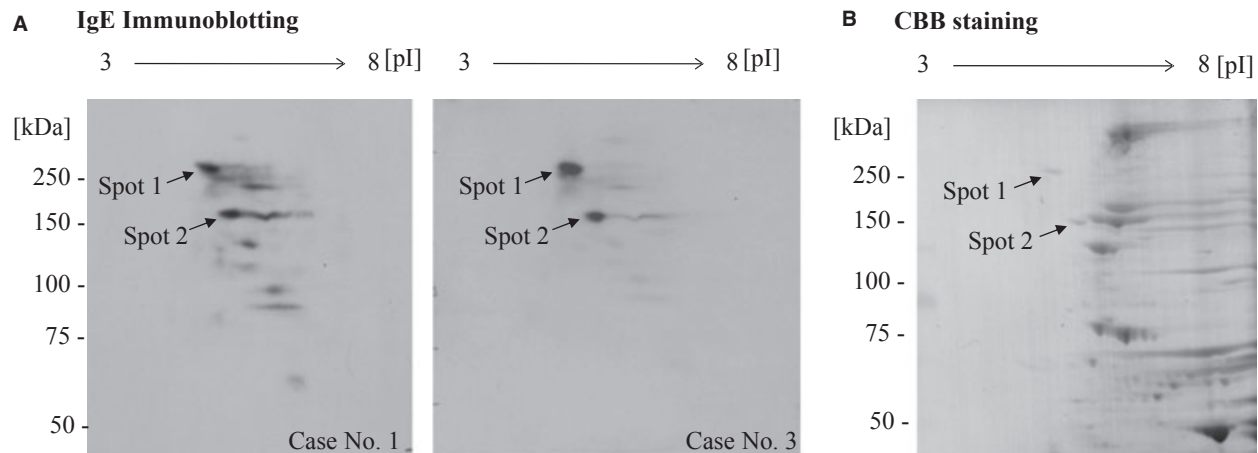


Figure 2 IgE immunoblotting of 2D-PAGE-resolved beef proteins. (A) The allergen-rich fraction, prepared from water-soluble beef proteins by ammonium precipitation, was developed by 2D-PAGE. The binding of IgE to proteins was analyzed by immunoblotting using the sera from two patients (case Nos 1 and 3) and two healthy

subjects after 2D-PAGE. Two dominant IgE-binding proteins were detected with IgE immunoblotting (spot 1 and spot 2, indicated by arrows). (B) Total protein developed by 2D-PAGE was stained by CBB. The protein spots were manually excised from the CBB-stained gel and subjected to MS analysis.

Table 2 PMF analysis and MS/MS ion search results

Spot No.	Accession number	Protein name (organism)	PMF analysis			MS/MS ion analysis		
			Mascot score	Matched peaks/ searched peaks	Sequence coverage (%)	Mascot score	Matched peaks/ searched peaks	Sequence coverage (%)
1	XP_611689	Laminin γ -1 (<i>Bos taurus</i>)	82	16/45	11	101	4/52	3
2	DAA32939	Collagen α -1 (VI) chain (<i>B. taurus</i>)	100	9/11	9	58	3/15	9

Table 3 Homology to laminin γ -1 and the collagen α -1 (VI) chain from *Bos taurus*

Organism	Laminin γ -1				Collagen α -1 (VI) chain			
	Identity (%)*	Similarity (%)*	Accession number	Amino acids	Identity (%)**	Similarity (%)**	Accession number	Amino acids
<i>Sus scrofa</i>	96	97	NP_001258644	1608	Unknown	Unknown	Not recorded	Unknown
Human	94	97	NP_002284.3	1609	91	95	NP_001839	1028
<i>Gallus gallus</i>	79	89	XP_001234659	1603	70	80	NP_990438	1019

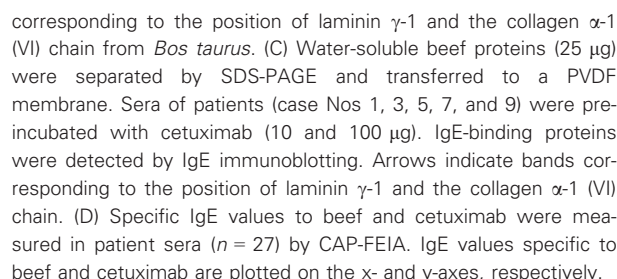
*Homology to laminin γ -1 from *B. taurus* (accession number, XP_611689).

**Homology to collagen α -1 (VI) chain from *B. taurus* (accession number, DAA32939).

IgE from patient sera reacted to pork proteins, but not to chicken proteins, which is consistent with the patient's history of allergic episodes.

Whereas all patients' sera reacted commonly with the 240-kDa and 140-kDa proteins, multiple bands were detected in the low molecular area of the patients' sera (Fig. 1A). These bands were not recognized by anti- α -Gal antibody (Fig. 3A), but were diminished by peroxidase treatment and cetuximab treatment (Fig. 3B,C). We speculate that these are carbohydrate moieties mimicking α -Gal. These results indicate that laminin γ -1 and the collagen α -1 (VI) chain are the predomi-

nant IgE-reactive proteins, whereas other proteins might be involved in causing beef allergy. BSA, bovine IgG, and actin have been reported as allergens in patients with atopic dermatitis who experienced allergic reactions after consuming cow's milk and meat such as beef or pork (5, 8, 21). In the present study, IgE-reactive bands corresponding to bovine IgG (50 kDa) and actin (42 kDa) were observed in several patients upon IgE immunoblotting (Fig. 1), suggesting that bovine IgG may also be involved in the IgE-mediated allergic reactions seen after the ingestion of beef in these patients because the presence of α -Gal in bovine IgG has been reported (22).



The actual molecular size of laminin γ -1 (240 kDa) was much larger than the estimated molecular size from its amino acid sequence (140 kDa), indicating heavy glycosylation with N-linked oligosaccharides, including α -Gal (24), which is

According to EST profiles in the NCBI UniGene database, each gene encoding laminin γ -1, the collagen α -1 (VI) chain, and α -1,3-galactosyl transferase is expressed in the kidney, liver, muscle, and intestine, suggesting that almost all organs derived from *B. taurus* are allergenic. These data also explain

the clinical finding that some patients with beef allergy had an allergic reaction upon ingestion of grilled beef intestine.

Commings et al. described that characteristic features of red meat allergy are adult onset and have a delayed (3–5 h) appearance of the allergic reaction to mammalian meats (12). These features are consistent with our patients; 21 of 29 patients were over 60 years of age, and 10 of the 20 patients reacted to mammalian meats with a delay. We do not clarify the reason of this characteristic features in this study. Although some patients had an allergic reaction within 2 h, we speculate that the time to the reaction depends on the condition of the patients, mainly the condition of gastrointestinal tract, and not on the specific features of the patient. Similar observations were reported by Morisset et al. (14), where some patients developed an anaphylactic reaction within 2 h after ingesting meat or kidney.

A possible relationship of exposure to tick bites and adult-onset red meat allergy has been reported in the United States and Europe (29, 30). The report from the United States shows that the distribution of patients with Rocky Mountain spotted fever matches those of patients with red meat allergy who have specific IgEs against α -Gal (29); thus, bites from adult or larval ticks of the species *Amblyomma americanum* are suspected. Recently, Hamsten et al. (30) have reported that Swedish patients with red meat allergy have an IgE response to α -Gal, and the α -Gal epitope is present in the gastrointestinal tract of *Ixodes ricinus*, a common European tick. In Japan, Japanese spotted fever and Tsutsugamushi disease are well known as being due to tick infestation and are distributed widely. In this study, however, patient clinical information regarding tick bites was insufficient to examine the association between tick bites and beef allergy.

In conclusion, the carbohydrate moiety (α -Gal) on laminin γ -1 and the collagen α -1 (VI) chain are possibly common IgE-reactive proteins in the Japanese patients with beef allergy. Although the involvement of ticks in beef allergy has not been established in Japan, clinicians need to be aware of a possible association between tick bites and beef allergy.

Acknowledgments

This work was supported in part by Health and Labor Sciences Research Grants from the Ministry of Health, Labor, and Welfare of Japan (H24-Research on rare and intractable diseases-005 to EM). The authors thank Dr. Koji Kamiya (Department of Dermatology, Okayama University Graduate School of Medicine, Okayama, Japan) and Dr. Akihisa Yamamoto (Department of Dermatology, Kobe Rosai Hospital, Kobe, Japan) for providing patient sera and Mrs. Kiyoe Ueda and Mrs. Kanako Yano for technical assistance.

Author contributions

All authors contributed to the manuscript. Hitoshi Takahashi performed most experiments and wrote the article. Yuko Chinuki and Eishin Morita evaluated the clinical findings, collected serum, and made diagnoses. Akira Tanaka measured the amount of serum-specific IgE values by CAP-FEIA. Eishin Morita thoroughly supervised the work and participated in writing of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest for the work presented in this study.

References

- Urisu A, Ebisawa M, Mukoyama T, Morikawa A, Kondo N. Japanese guideline for food allergy. *Allergol Int* 2011;**60**:221–236.
- Ayuso R, Lehrer SB, Lopez M, Reese G, Ibanez MD, Esteban MM et al. Identification of bovine IgG as a major cross-reactive vertebrate meat allergen. *Allergy* 2000;**55**:348–354.
- Fiocchi A, Restani P, Riva E. Beef allergy in children. *Nutrition* 2000;**16**:454–457.
- Fuentes MM, Palacios R, Garces MM, Caballero ML, Moneo I. Isolation and characterization of a heat-resistant beef allergen: myoglobin. *Allergy* 2004;**59**:327–331.
- Han GD, Matsuno M, Ito G, Ikeuchi Y, Suzuki A. Meat allergy: investigation of potential allergenic proteins in beef. *Biosci Biotechnol Biochem* 2000;**64**:1887–1895.
- Restani P, Ballabio C, Tripodi S, Fiocchi A. Meat allergy. *Curr Opin Allergy Clin Immunol* 2009;**9**:265–269.
- Tanabe S, Shibata R, Nishimura T. Hypoallergenic and T cell reactive analogue peptides of bovine serum albumin, the major beef allergen. *Mol Immunol* 2004;**41**:885–890.
- Werfel SJ, Cooke SK, Sampson HA. Clinical reactivity to beef in children allergic to cow's milk. *J Allergy Clin Immunol* 1997;**99**:293–300.
- Chung CH, Mirakhor B, Chan E, Le QT, Berlin J, Morse M et al. Cetuximab-induced anaphylaxis and IgE specific for galactose- α -1,3-galactose. *N Engl J Med* 2008;**358**:1109–1117.
- Commings SP, Platts-Mills TA. Anaphylaxis syndromes related to a new mammalian cross-reactive carbohydrate determinant. *J Allergy Clin Immunol* 2009;**124**:652–657.
- Commings SP, Platts-Mills TA. Allergenicity of carbohydrates and their role in anaphylactic events. *Curr Allergy Asthma Rep* 2010;**10**:29–33.
- Commings SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD et al. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose- α -1,3-galactose. *J Allergy Clin Immunol* 2009;**123**:426–433.
- Jacquet S, Moneret-Vautrin DA, Bihain BE. Mammalian meat-induced anaphylaxis: clinical relevance of anti-galactose- α -1,3-galactose IgE confirmed by means of skin tests to cetuximab. *J Allergy Clin Immunol* 2009;**124**:603–605.
- Morisset M, Richard C, Astier C, Jacquet S, Croizier A, Beaudouin E et al. Anaphylaxis to pork kidney is related to IgE antibodies specific for galactose- α -1,3-galactose. *Allergy* 2012;**67**:699–704.
- Saleh H, Embry S, Nauli A, Atiyas S, Krishnaswamy G. Anaphylactic reactions to oligosaccharides in red meat: a syndrome in evolution. *Clin Mol Allergy* 2012;**10**:5.
- Sekiya K, Fukutomi Y, Nakazawa T, Taniguchi M, Akiyama K. Delayed anaphylactic reaction to mammalian meat. *J Invest Allergol Clin Immunol* 2012;**22**:446–447.
- Sander I, Kespohl S, Merget R, Goldscheid N, Degens PO, Bruning T et al. A new method to bind allergens for the measurement of specific IgE antibodies. *Int Arch Allergy Immunol* 2005;**136**:39–44.

18. Timpl R, Rohde H, Robey PG, Rennard SI, Foidart JM, Martin GR. Laminin—a glycoprotein from basement membranes. *J Biol Chem* 1979;**254**:9933–9937.
19. Tzu J, Marinkovich MP. Bridging structure with function: structural, regulatory, and developmental role of laminins. *Int J Biochem Cell Biol* 2008;**40**:199–214.
20. Fujiwara S, Shinkai H, Timpl R. Structure of N-linked oligosaccharide chains in the triple-helical domains of human type VI and mouse type IV collagen. *Matrix* 1991;**11**:307–312.
21. Fiocchi A, Restani P, Riva E, Qualizza R, Bruni P, Restelli AR et al. Meat allergy: I-Specific IgE to BSA and OSA in atopic, beef sensitive children. *J Am Coll Nutr* 1995;**14**:239–244.
22. Thall A, Galili U. Distribution of Gal alpha 1—3Gal beta 1—4GlcNAc residues on secreted mammalian glycoproteins (thyroglobulin, fibrinogen, and immunoglobulin G) as measured by a sensitive solid-phase radioimmunoassay. *Biochemistry* 1990;**29**:3959–3965.
23. Galili U. The alpha-gal epitope and the anti-Gal antibody in xenotransplantation and in cancer immunotherapy. *Immunol Cell Biol* 2005;**83**:674–686.
24. Arumugham RG, Hsieh TC, Tanzer ML, Laine RA. Structures of the asparagine-linked sugar chains of laminin. *Biochim Biophys Acta* 1986;**883**:112–126.
25. Maruyama S, Cantu E 3rd, Galili U, D'Agati V, Godman G, Stern DM et al. alpha-galactosyl epitopes on glycoproteins of porcine renal extracellular matrix. *Kidney Int* 2000;**57**:655–663.
26. Zhang RZ, Pan TC, Timpl R, Chu ML. Cloning and sequence analysis of cDNAs encoding the alpha 1, alpha 2 and alpha 3 chains of mouse collagen VI. *Biochem J* 1993;**291**(Pt 3):787–792.
27. Hesse H, Engvall E. Type VI collagen. Studies on its localization, structure, and biosynthetic form with monoclonal antibodies. *J Biol Chem* 1984;**259**:3955–3961.
28. Nayak BR, Spiro RG. Localization and structure of the asparagine-linked oligosaccharides of type IV collagen from glomerular basement membrane and lens capsule. *J Biol Chem* 1991;**266**:13978–13987.
29. Commins SP, James HR, Kelly LA, Pochan SL, Workman LJ, Perzanowski MS et al. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-alpha-1,3-galactose. *J Allergy Clin Immunol* 2011;**127**:1286–1293.
30. Hamsten C, Starkhammar M, Tran TA, Johansson M, Bengtsson U, Ahlen G et al. Identification of galactose-alpha-1,3-galactose in the gastrointestinal tract of the tick *Ixodes ricinus*; possible relationship with red meat allergy. *Allergy* 2013;**68**:549–552.