

Original Article

SENSITIZATION TO THE MAMMALIAN OLIGOSACCHARIDE GALACTOSE-ALPHA-1,3-GALACTOSE (ALPHA-GAL): EXPERIENCE IN A FLEMISH CASE SERIES

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ABSTRACT

Background. Recent observations have disclosed that the galactose- α (1,3)-galactose (α -gal) moiety of non-primate glycoproteins can constitute a target for meat allergy.

Objective. To describe adults with allergic reactions to mammalian meat, dairy products and gelatin. To investigate whether patients could demonstrate sensitization to activated recombinant human coagulation factor VII ectapog alpha that is produced in baby hamster kidney cells.

Methods. Ten adults with mammalian meat, dairy products and gelatin allergies were examined using quantification of specific IgE and/or skin prick test for red meat, milk, milk components, gelatin, cetuximab and eptacog alpha.

Results. Most patients demonstrate quite typical clinical histories and serological profiles, with anti- α -gal titers varying from less than 1% to over 25% of total serum IgE. All patients demonstrate negative sIgE for gelatin, except the patient with a genuine gelatin allergy. All patients also demonstrated a negative sIgE to recombinant milk components casein, lactalbumin and lactoglobulin. Specific IgE to eptacog was positive in 5 out of the 9 patients sensitized to α -gal and none of the 10 control individuals.

Conclusion. This series confirms the importance of the α -gal carbohydrate moiety as a potential target for allergy to mammalian meat, dairy products and gelatin (oral, topical or parenteral) in a Flemish population of meat allergic adults. It also confirms in vitro tests to mammalian meat generally to be more reliable than mammalian meat skin tests, but that diagnosis can benefit from skin testing with cetuximab. Specific IgE to gelatin is far too insensitive to diagnose α -gal related gelatin allergy. IgE binding studies indicate a potential risk of α -gal-containing human recombinant proteins produced in mammals.

Key words: Anaphylaxis, galactose- α -1,3-galactose (α -gal), cetuximab, conestat alpha, eptacog alpha, gelatin, meat allergy

INTRODUCTION

Recent observations disclosed that sIgE antibodies to galactose- α (1,3)-galactose (α -gal) moieties present on asparagine at position 88 in the murine heavy chain Fab portion of the chimeric mouse-human IgG1 mAb cetuximab produced in a murine myeloma cell culture (1) are able to trigger life-threatening and even fatal reactions (2). Alpha-gal is formed by α (1,3)-galactosyltransferase that places a terminal galactose residue in an α -linkage to another galactose. Alpha(1,3)-galactosyltransferase displays a particular pattern in mammals, where its expression has been suppressed in primates including humans, apes and New World monkeys (3-5), all of which produce large amounts of natural anti-Gal antibodies against the α -galactosyl epitope that are responsible for the hyperacute rejection of porcine heart xenografts in baboons (6, 7). Because α -gal is present on proteins of most non-primates, Chung *et al* (2) also performed IgE quantification for cat, dog, beef and pork proteins and identified several patients who reported having had episodes of delayed anaphylaxis or serious angioedema up to 6 hours after eating beef or pork. These data were confirmed by others (8-11) and most recently also in two series described by Mullins *et al* (12) and Morisset *et al* (13). The mechanism(s) behind the delayed onset of these reactions remain(s) poorly understood, but might relate to the digestion time of meat (8) or the amount of ingested allergen (13).

The rationale of this study was to evaluate the potential importance of α -gal sensitization in Flemish adults patients with allergic reactions to one or more kinds of mammalian meat, dairy products and gelatin (oral, parenteral and topical), and to evaluate to potential risk of a recombinant protein

(human coagulation factor VII ectapog alpha) produced in non-primate mammalian.

MATERIALS AND METHODS

Patients

The demographics and characteristics of the patients are summarized in the table. Ten healthy control individuals were enrolled to assess the eptacog allergosorbent ImmunoCAP assay. All patients and control individuals gave informed consent approved by the local ethics committee of the University of Antwerp.

Quantification of total and specific IgE

Total and specific IgE (sIgE) quantification for pork, beef, rabbit, milk, bovine serum albumin, casein, lactalbumin, lactoglobulin, gelatin, α -gal and recombinant human coagulation factor VII ectapog alpha that is produced in baby hamster kidney cells (NovoSeven®, Novo Nordisk BV, AV Alphen aan de Rijn, The Netherlands) was performed using an ImmunoCAP technique (ThermoFisher Phadia, Uppsala, Sweden) according to the manufacturers' recommendation.

Results of specific IgE (sIgE) are expressed as kUA/L (normal <0.35).

Skin prick tests

Skin prick tests involved raw pork and beef, cetuximab (Erbix®; Merck, Overijse, Belgium) and succinylated bovine gelatin (Gelofusin®, 40 mg/mL gelatin, B. Braun Melsungen AG, Germany). Skin prick test results are expressed in wheal and flare reactions. Normal <3/3 mm. For the drugs results are shown for undiluted stock solution. Except for patient V.M., prick test for cetuximab 1/100 (see table).

RESULTS

The most relevant data of confirmatory testing in our patients are displayed in the table. Most patients demonstrate quite typical clinical histories and serological profiles, with anti- α -gal titers varying from less than 1% to over 25% of total serum IgE. Note that all patients demonstrate negative sIgE for gelatin, except the patient with a genuine gelatin allergy that was included for comparison (patient 10). All patients also demonstrated a negative sIgE to recombinant

Table: Characteristics of patients allergic to red meat, dairy or gelatin.

Parameter	PT1	PT2	PT3	PT4	PT5	PT6	PT7	PT8	PT9	PT105
Seks/age	M/55 ¹	F/55	M/31 ¹	F/55	F/79	M/44 ¹	F/20	M/19	F/19	F/51
Clinics	Ur	Ur	Ur, A	Ur	Ur,B	Ur,A	Ur,A	Ur, A	Ur, A	Ur, A, B
Causes	Pork, Beef, Lamb	Pork Beef Milk Gel ²	Pork Beef Milk	Pork Beef Milk	Various meats	Pork	Gel ³	Pork	Beef Hair products ⁴	Gel
Interval (hours)	4-6	2-6	<4	3	3	4-6	1-1.5	4-6	4	10 min
Tick bites	Yes	U	Yes	U	U	U	Yes	Yes	U	U
Total IgE	1922	664	554	237	201	183	814	170	49	494
Specific IgE (sIgE, normal <0.35 kUA/L)										
sIgE α -gal	>100	27.1	>100	19.60	3.44	1.32	1.13	45.5	3.19	<0.35
sIgE gelatin	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	3.70
sIgE pork	21.00	6.30	20.90	22.30	5.24	3.10	1.11	5.12	1.65	<0.35
sIgE beef	21.90	5.96	45.70	25.90	6.38	0.57	1.14	8.05	2.24	<0.35
sIgE rabbit	5.29	3.40	11.80	N.A.	2.54	<0.35	0.56	2.24	N.A.	<0.35
sIgE milk	9.33	7.14	6.97	4.15	3.02	0.59	0.42	2.01	<0.35	<0.35
sIgE BSA	1.25	0.36	1.93	<0.35	<0.35	<0.35	<0.35	0.40	N.A.	<0.35
sIgE eptacog	1.59	1.64	3.77	0.40	<0.35	<0.35	<0.35	0.69	<0.35	<0.35
Skin prick tests (SPT, wheals and flares expressed in mm)										
Pork	3/5	0/2	N.A.	3/8	2/5	0/0	3/6	8/17	2/4	0/0
Beef	2/10	N.A.	N.A.	5/10	N.A.	0/0	0/0	2/3	1/3	0/0
Cetuximab	6/13	3/5	3/23	4/6	9/14	8/15	0/0	5/27	2/6	0/0
Gelofusin®	5/6	5/15	0/0	4/5	8/15	0/0	4/12	0/0	0/0	5/13

Ur: urticaria; B: bronchospasm; A: angioedema. Gel: gelatin-based colloid plasma (PT2), gelatin-containing marshmallow (PT7, PT10). U: unknown, N.A.: not available.

¹ denotes an atopic status

² patient 2 had generalized urticaria and significant bronchospasm starting immediately after infusion of a bovine gelatin-based colloid plasma substitute (Gelofusin®, 40 mg/mL gelatin, B. Braun Melsungen AG, Germany) during general anesthesia for knee arthroscopy.

³ large amount of marshmallows containing bovine gelatin (patient is vegetarian)

⁴ history taking revealed these reactions to occur after topical application of several hair care products and conditioners applied on the scalp. Careful revision of the product leaflets disclosed some of these products to contain collagen and gelatin.

⁵ nausea, vomiting, facial erythema, angioedema of the throat and wheezing about 10 minutes after eating some marshmallows containing bovine gelatin. History revealed an IgE-mediated latex allergy with a sensitization to Hev b 5 and Hev b 6. A diagnosis of a genuine IgE-mediated gelatin allergy unrelated to α -gal was made.

milk components casein, lactalbumin and lactoglobulin that lack the α -gal moiety (data not shown). Specific IgE to eptacog was positive in 5 out of the 9 patients sensitized to α -gal and none of the 10 control individuals.

DISCUSSION

This series confirms the importance of the α -gal carbohydrate moiety as a potential target for allergy to mammalian meat, dairy products and gelatin in an adult Flemish population. Furthermore, our case clinics confirm this particular kind of food allergy to display distinctive characteristics that makes an α -gal-related meat allergy clearly distinctive from "traditional" meat allergy that results from a sensitization to protein components. First, unlike traditional meat allergy, it is confirmed that this α -gal related meat allergy (that involves muscle meat as well as viscera) to be characterized by a delayed onset of symptoms (up to 6 hours). Second, in our series, patients generally present generalized reactions that vary from urticaria to anaphylactic shock. So far no cases with an isolated OAS have been described. Third, not all mammalian meat-containing meals will elicit symptoms. The reason for this observation remains elusive but has been hypothesized that it could relate to the variability in meat digestion and matrices. It appears that reactions to α -gal could also relate to the meat preparation (marinating) or the amount that is consumed. Alternatively, explanations might relate to the α -gal content of the food and the presence of additional risk factors such as intake of alcohol, exercise or use of β -blocking agents (13). Fourth, patients are generally allergic to several meat species including pork, beef, horse, lamb and rabbit. Non-mammalian meat such as poultry is tolerated.

The novelty in our series is that patients sensitized to α -gal might also demonstrate IgE seroreactivity to recombinant human proteins that are produced in non-primate mammals. Actually, about half of our patients sensitized to α -gal displayed a positive sIgE result for activated recombinant human coagulation factor VII (rhFVII, Ectapog alpha (NovoSeven®) that is produced in baby hamster kidney cells. Although the clinical relevance of this observation remains elusive, it might indicate that prudence should be called upon the use of other drugs that might contain α -gal moieties such as conestat alpha (Rucanest®, a recombinant analogue of human C1-esterase inhibitor purified from transgenic rabbit milk) and Thymoglobuline® (a purified rabbit anti-thymocyte immunoglobulin, obtained by the immunization of rabbits with human thymocytes).

Finally we confirm that patients sensitized to α -gal might also demonstrate sensitization to gelatin and even clinically react to gelatin-containing drugs, foods (12) and probably also to bovine collagen present in hair care products and conditioners (14, 15). Moreover, it appears that the α -gal related gelatin allergy clearly differs from "traditional" gelatin allergy that was diagnosed in our last patient. In parallel to α -gal related meat allergy, α -gal related gelatin allergy has a delayed onset of symptoms and not all gelatin-containing foods will trigger symptoms. The mechanisms underlying this retardation are currently subject of speculation and intense research. Alternatively, note that succinylated bovine gelatin (Gelofusin®) administered intravenously almost instantaneously triggered

an anaphylactic reaction in one of our α -gal sensitized patients. These findings parallel the observations by Mullins *et al* (12), who reported their patients to tolerate oral challenges with 10 g of gelatin, but to present urticaria and bronchospasm during intravenous challenges at doses as low of 0.024 g. Last but not least, our findings confirm that diagnosis of α -gal related gelatin sensitization and allergy seems readily overlooked by a conventional sIgE assay and needs additional skin testing (12). The reason why the *in vitro* test is uniformly negative remains elusive, but could relate to a too low sensitivity.

Correct diagnosis of an α -gal-mediated meat allergy starts with thorough history that should take into account the afore-mentioned clinical particularities complemented by appropriate *in vitro* and *in vivo* tests. These comprise measurement of sIgE antibodies against mammalian meat (e.g. beef and pork) and epitope resolved diagnosis including determination of anti- α -gal sIgE (available from ThermoFisher Phadia, Uppsala, Sweden). Alternatively, although some of the patients reported symptoms to cow's milk and all display positive sIgE results for cow's milk, sIgE for the recombinant major cow's milk components α -lactalbumin, β -globulin and casein that lack the α -gal moiety was negative (data not shown).

Skin test prick tests generally used several raw and cooked mammalian meat species. However, as also emerges from our data, such skin testing seems actually less sensitive than *in vitro* testing in picking up sensitization to α -gal (8, 10). Actually, only about half of our patient demonstrated a positive skin test for mammalian meat. In this context, and in parallel to others (10, 13), we show that diagnosis of α -gal sensitization can benefit from skin tests with cetuximab that was recently demonstrated to contain 10.2 μ g α -gal/5 mg (12). In our series, skin prick testing with undiluted cetuximab (Erbix®) was positive in all patients, except one.

With respect to diagnosing α -gal related gelatin sensitization and allergy, it is confirmed that the commercially available *in vitro* IgE assay for gelatin is far too insensitive (12). Actually correct diagnosis of α -gal related gelatin sensitization and allergy seems to need skin prick or intradermal testing using undiluted bovine gelatin-derived colloids such as Gelofusine® or Haemacel® (35 mg/mL gelatin, Aventis Pharma) that contain 0.52 $\pm$ 0.1 μ g and 0.44 $\pm$ 0.2 μ g/g, respectively. As illustrated by one of our patients, anaphylaxis during anaesthesia that could relate to gelatin-containing colloids needs appropriate skin testing in order not to overlook a potential life-threatening allergy.

The observation that many patients with cetuximab allergy already demonstrated their reactions during the first administration (2, 16) and that the majority of these patients had pre-existent anti- α -gal antibodies (2) raised the question about the possible cause(s) of this IgE response. Recently, Commins *et al* (17) presented data strongly suggesting that tick bites could be the responsible of the IgE antibody responses to α -gal in the southeastern United States where the lone star tick *Amblyomma americanum* is common. Maps of the USA that highlight the geographical areas of reactions to cetuximab, sensitization to α -gal and occurrence of delayed meat allergy are shown elsewhere (11). In a survey by Van Nunen *et al* (9) in Sydney Australia, 24 out of 25 patients with a mammalian meat allergy reported tick bites. The suspected tick here is *Ixodes holocyclus*, commonly

known as the paralysis tick, that is highly prevalent in the northern beaches region of Sydney. A history of thick bite was present in 4 out of 5 Spanish patients residing in the rural area of Galicia (18) and 6 out of the 14 French patients (13). In both regions the *Ixodes ricinus* is the predominant tick species. In our case series exposure to tick bites was confirmed in 4 patients. How tick bites contribute to α -gal sensitization remains highly speculative.

We present a case series of a recently described mammalian meat allergy that is related to sensitization to the α -gal glycan moiety of non-primate glycoproteins. The novel finding of our series relates to the observation that these patients might demonstrate IgE seroreactivity to human recombinant proteins produced in mammals. Although the clinical relevance of this finding remains elusive, together with the findings about infusion of cetuximab, it indicates that α -gal sensitization constitutes a contra-indication for such drugs. Furthermore, it is confirmed that a subset of patients sensitized to the α -gal glycan moiety of mammalian might demonstrate sensitization and eventually severely react to gelatin, particularly when administered intravenously. In the absence of controlled challenges, it remains unclear whether sensitization to α -gal constitutes a significant risk of clinical reactivity to oral gelatin and which oral dose might be required to trigger an allergic reaction. Our clinical practice currently is to advise patients with mammalian meat allergy and gelatin sensitization to avoid ingestion of substantial quantities of red meat (particularly the species they reacted to and viscera) and gelatin. We discourage intravenous administration of gelatin-based colloids. Alternatively, recommendation about avoidance of dairy products is restricted to patients with a suggestive history. It appears that patients sensitized to α -gal might also suffer from contact reactions towards collagen and gelatin present in hair conditioners and styling products.

ACKNOWLEDGEMENTS

The authors would like to thank Mrs. C. Mertens for her skin full technical. D.G.E is a Senior Clinical Researcher of the Research Foundation Flanders (FWO) (1800609N).

CONFLICT OF INTEREST: None.

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