

BRIEF COMMUNICATION

Identification of galactose- α -1,3-galactose in the gastrointestinal tract of the tick *Ixodes ricinus*; possible relationship with red meat allergy

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Keywords

galactose- α -1,3-galactose; IgE; red meat allergy; ticks.

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Abstract

Patients with IgE antibodies against the carbohydrate epitope galactose- α -1,3-galactose (α -Gal) have reported severe allergic reactions after consumption of red meat. Investigations have revealed associations between IgE to α -Gal and tick bites. We provide the first direct evidence that α -Gal is present within ticks thus potentially explaining the relationship between tick exposure and sensitization to α -Gal, with development of red meat allergy as a secondary phenomena. Serum from Swedish patients with delayed severe reactions to red meat was included in the study. A dose-dependent inhibition of IgE responses to α -Gal by the tick *Ixodes ricinus* is demonstrated. Furthermore, using cryostat-cut sections of *I. ricinus*, we show that both a monoclonal and a polyclonal antibody against α -Gal stains the gastrointestinal tract of the tick. The same pattern is seen when staining with patient sera IgE positive to α -Gal. These results confirm that the α -Gal epitope is present in *I. ricinus* and imply host exposure to α -Gal during a tick bite. This provides further evidence that tick bites are associated with IgE responses to α -Gal and red meat allergy.

IgE antibody responses to the oligosaccharide galactose- α -1,3-galactose (α -Gal) epitope were first identified when a subset of patients treated with cetuximab (a chimeric mouse-human monoclonal antibody targeting the epidermal growth factor) developed severe anaphylactic reactions at the first treatment (1). Further investigations revealed that pre-existing IgE antibodies directed to the α -Gal epitope on the Fab portion of cetuximab were found to be the cause of the severe hypersensitivity reactions (1).

The α -Gal epitope is abundant on glycolipids and glycoproteins of mammals with the exception of humans, apes and Old World monkeys, which as a consequence produce antibodies targeting α -Gal (2). IgG anti- α -Gal has been shown to constitute up to 1% of all circulating IgG antibodies (3) in humans and is a major obstacle in xenotransplantation (4). As α -Gal is present in nonprimate mammalian tissues, it has been proposed as a novel target in food allergy. Patients

reporting severe allergic reactions, anaphylaxis, urticaria or angioedema after consumption of red meat (beef, pork or lamb) have been found to be IgE sensitized to α -Gal (5–11). Most of the patients have reported a late in life onset of delayed reactions to meat, suggesting a recent development of IgE antibodies to α -Gal.

The association among tick bites, red meat allergy and IgE to α -Gal has been reported from three continents to date. First, Commins et al. described a relationship between allergic reactions to cetuximab and sensitization to α -Gal and noted a geographical variation in incidence and postulated an environmental factor (5). Second, Van Nunen and colleagues from Australia documented an association between large local reactions to bites from the tick *Ixodes holocyclus* and adult onset red meat allergy and hypothesized a pathogenic relationship (7). From the US where the geographical distribution of patients with IgE antibodies to α -Gal overlapped the region of

Rocky Mountain spotted fever, whose major vectors are the ticks *Amblyomma americanum* and *Dermacentor variabilis* Commins et al. reported an association between sensitization to α -Gal and ticks (8). The association was further supported in three patients where a time relationship between tick exposure and increased IgE to α -Gal was noted (8). In Europe, a history of tick bites has been reported in four of five patients with IgE antibodies to α -Gal from northern Spain (9). Recently, Mullins et al. described a relationship among red meat allergy, gelatin allergy, α -Gal sensitization and tick exposure as well as tick allergic patients with sensitization to red meat and α -Gal, without necessarily having red meat allergy (10).

The problem of severe food allergy to mammalian meat has also been highlighted in Sweden. In the greater Stockholm area, which is in the southern part of Sweden, the common European tick *Ixodes ricinus* is frequent, and we have previously identified IgE responses to α -Gal in this region (12). Here, we wanted to investigate whether Swedish patients with suspected red meat allergy have IgE antibodies to α -Gal and whether they also have IgE to the tick *I. ricinus* that can be linked to the α -Gal epitope.

Sera from five Swedish patients (four males) reporting anaphylaxis or urticaria following consumption of red meat and a history of several tick bites, and one nonmeat-allergic atopic control, were analysed for IgE to α -Gal, beef and chicken using the ImmunoCAP System (Phadia AB/Thermo Fisher Scientific, Uppsala, Sweden). All patients were IgE positive to α -Gal and beef, but IgE negative to chicken due to the absence of α -Gal. The control was IgE negative to α -Gal, beef and chicken (Table 1). Investigation of IgE responses to the tick *I. ricinus* (IS Insect Services GmbH, Berlin, Germany) was performed by Western blotting and competition ELISA. Extracts of *I. ricinus* [prepared by crushing of frozen ticks in phosphate buffer saline using a tissue homogenizer (Bertin Technologies, Cedex, France) and removal of the solid pellet] were separated on a 15% SDS-PAGE and blotted onto polyvinylidene fluoride membranes. Blots were probed with sera (diluted 1 : 4), followed by rabbit anti-human IgE (1 : 2000, MIAB, Uppsala, Sweden), alkaline phosphatase (AP) conjugated goat anti-rabbit (1 : 1000, Jackson ImmunoResearch, West Grove, PA, USA) and AP

substrate (BioRad, Hercules, CA, USA). Patterns of strong IgE binding to several tick proteins were detected in the patients but not in the control (Fig. 1A). Preincubation with α -Gal (100 μ g/ml, Sigma-Aldrich, St. Louis, MO, USA) resulted in a significant reduction of IgE binding in two patients, indicating the presence of α -Gal on several tick proteins. The result also demonstrates that the remaining patients have strong α -Gal-independent IgE responses to tick proteins. In the next step, competition ELISA was performed by preincubation of sera with threefold dilutions (0.27 μ g/ml to 600 μ g/ml) of *I. ricinus* extract followed by detection of IgE binding to α -Gal (Sigma-Aldrich)-coated plates using rabbit anti-human IgE (1 : 2000, MIAB), AP conjugated goat anti-rabbit (1 : 1000, Jackson ImmunoResearch) and AP substrate (BioRad). A dose-dependent inhibition of IgE to α -Gal was obtained in all meat-allergic patients, but not in the atopic control (Fig. 1B). A maximum inhibition of 68–97% was obtained using 600 μ g/ml of tick extract. Two patients had extremely high IgE levels to α -Gal (≥ 100 k_A/L), which might explain the lower degree of inhibition observed in these patients.

Immunohistochemistry was used to further explore serum responses to *I. ricinus* as well as to detect the presence of α -Gal in *I. ricinus* by murine anti- α -Gal antibodies. Cryostat-cut sections of *I. ricinus* nymphs (8 μ m) were probed with a monoclonal anti- α -Gal antibody (diluted 1 : 10, Enzo Life Sciences, Farmingdale, NY, USA), a polyclonal anti- α -Gal antibody [diluted 1 : 1000, in-house production from α -1,3-Galactosyl-transferase (–/–) C57Bl/6 mice (13)], patient sera (undiluted) or with an irrelevant polyclonal antibody (anti-Fel d 1, diluted 1 : 1000, in-house production) and stained using peroxidase (ABCelite, Vector Laboratories, Burlingame, CA, USA). ELISAs using natural and synthetic glycoproteins containing α -Gal and other glycans were used to demonstrate the specificity and minimal cross-reactivity of the polyclonal anti- α -Gal antibody (Supporting Information).

Patient sera IgE positive to α -Gal (Fig. 2A), the anti- α -Gal polyclonal antibody (Fig. 2B) and the anti- α -Gal monoclonal antibody (Figure S2) resulted in comparable results, predominantly staining the gut. Sections probed with patient sera IgE negative to α -Gal (Fig. 2C) and the irrelevant antibody (Fig. 2D) gave no specific staining. The results demonstrate

Table 1 Subject characteristics

Patient ID	Age (y)/sex	Reaction	Time to reaction*	IgE**				Tick bites	
				α -Gal	Beef	Chicken	Total	2011	Total
1	69/M	U, AE, GI	4	23	11	<0.1	280	10	>100
2	43/M	U, AE, GI, ANA	8	88	11.4	<0.1	320	15	>100
3	70/M	U, AE	6	128	16.2	<0.1	420	5	>100
4	60/M	U, AE, ANA	6–7	100	27	0.4	2200	15	>100
5	54/F	U, ANA	5–6	80	14	<0.1	130	100	>100
C	43/F	N/A	N/A	<0.1	<0.1	<0.1	51	0	0

C, control; M, male; F, female; U, urticaria; AE, angioedema; ANA, anaphylaxis; GI, gastrointestinal symptoms; N/A, not applicable.

*Time to reaction expressed in hours.

**ImmunoCAP IgE results. A positive result was defined as ≥ 0.10 k_A/L.

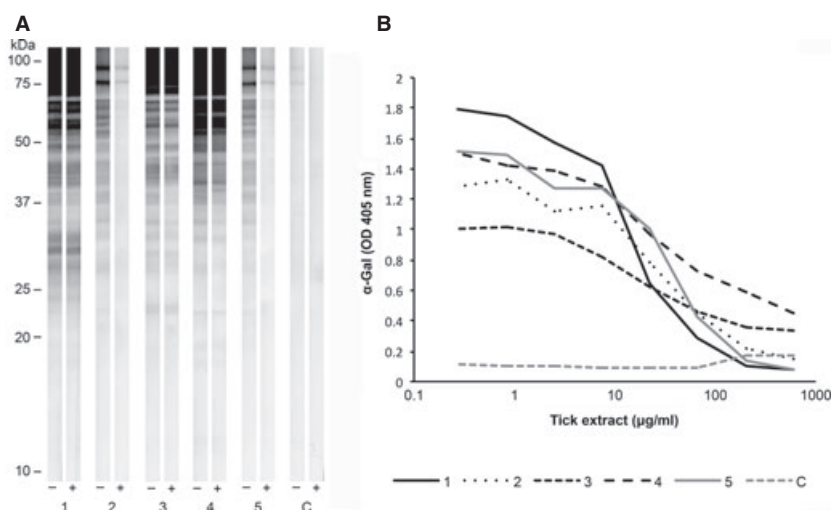


Figure 1 (A) Immunoblot showing IgE reactivity to *Ixodes ricinus* (tick) extracts in serum from five red meat-allergic patients, without (–) and with (+) preincubation with α -Gal (100 μ g/ml). (B) Inhibition assay demonstrating dose-dependent inhibition of serum IgE

responses to α -Gal using threefold dilutions of tick extract (0, 27–600 μ g/ml). Inhibition was shown in sera from all meat-allergic patients, with no effect in the control serum.

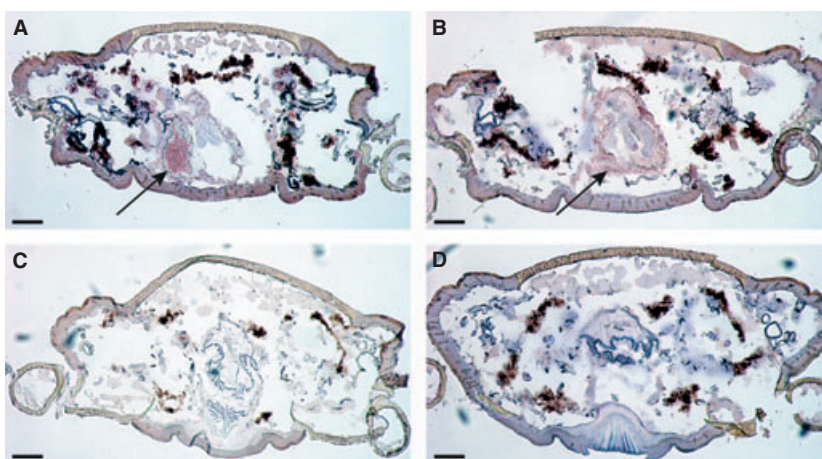


Figure 2 Cryostat-cut lateral sections of *Ixodes ricinus* nymph probed with (A) patient serum IgE positive to α -Gal, (B) a polyclonal mouse anti- α -Gal antibody, (C) patient serum IgE negative to α -Gal

and (D) an irrelevant polyclonal mouse antibody (anti-Fel d 1). Red areas represent antibody binding. Arrows show staining of the gut. Bar = 100 μ m.

that the α -Gal epitope is present in the gastrointestinal tract of *I. ricinus*, which implies host exposure to α -Gal during a tick bite.

In conclusion, we have for the first time shown that the tick *I. ricinus* contains the α -Gal epitope, which can be localized to the gastrointestinal tract of the tick. In addition, patients with delayed severe reactions to red meat in Sweden have IgE responses to beef and *I. ricinus* that can be linked to the α -Gal epitope. Most of these patients also have strong α -Gal-independent IgE responses to *I. ricinus*, indicating genuine sensitization to the tick. This provides further evidence of the tick as an initiator of red meat allergy. As exposure to ticks in Northern Europe will probably increase due to expanding deer and rodent populations (major tick vectors)

as well as climate changes; more people will be at risk of developing red meat allergy.

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Conflict of interest

None of the authors has any conflict of interest to declare.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Specificity of the monoclonal (mAb) and polyclonal (pAb) anti- α -Gal antibody indicating that the monoclonal is specific for α -Gal, whereas the polyclonal displays minor cross-reactions to other glycans.

Figure S2. Cryostat-cut lateral section of *I. ricinus* nymph probed with a monoclonal anti- α -Gal antibody. Red areas represent antibody binding. Arrow shows staining of the gut. Bar = 100 μ m.

Table S1. Proteins used for evaluation of the polyclonal anti- α -Gal antibody.

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SUPPORTING INFORMATION

Identification of galactose- α 1,3-galactose in the gastrointestinal tract of the tick *Ixodes ricinus*; possible relationship with red meat allergy

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Short title: The tick *Ixodes ricinus* and red meat allergy

Specificity and cross-reactivity of the polyclonal anti- α -Gal antibody

An ELISA was used to evaluate the polyclonal anti- α -Gal antibody in parallel to the commercially available monoclonal anti- α -Gal antibody (Enzo Life Sciences, Farmingdale, NY). Table S1 lists the natural and synthetic glycoproteins (and specific glycan structures) used to coat wells at a concentration of 5 μ g/ml. The obtained results (Figure S1) displayed minor reactivities to the non- α -Gal glycan structures for the polyclonal antibody that were absent for the monoclonal antibody. However, the polyclonal antibody had much higher reactivity to the natural α -Gal containing glycoprotein (bovine thyroglobulin) compared to the monoclonal antibody despite the difference in dilution (1:300 for the polyclonal and 1:10 for the monoclonal). Both antibodies have similar reactivity to the synthetic α -Gal containing glycoprotein (α -Gal-BSA).

Table S1. Proteins used for evaluation of the polyclonal anti- α -Gal antibody

Glycoprotein	Glycan structure
Bovine thyroglobulin	α -Gal (Gal α 1-3Gal β 1-4GlcNAc-R)
α -Gal-BSA	α -Gal (Gal α 1-3Gal β 1-4GlcNAc-R)
N-Acetyllactosamine-BSA	Gal β 1-4GlcNAc-R
Bovine fetuin	SA-Gal β 1-4GlcNAc-R and SA-Gal β 1-3GlcNAc-R
Bovine asialofetuin	Gal β 1-4GlcNAc-R and Gal β 1-3GlcNAc-R

Figure S1. Specificity of the monoclonal (mAb) and polyclonal (pAb) anti- α -Gal antibody indicating that the monoclonal is specific for α -Gal whereas the polyclonal displays minor cross-reactions to other glycans.

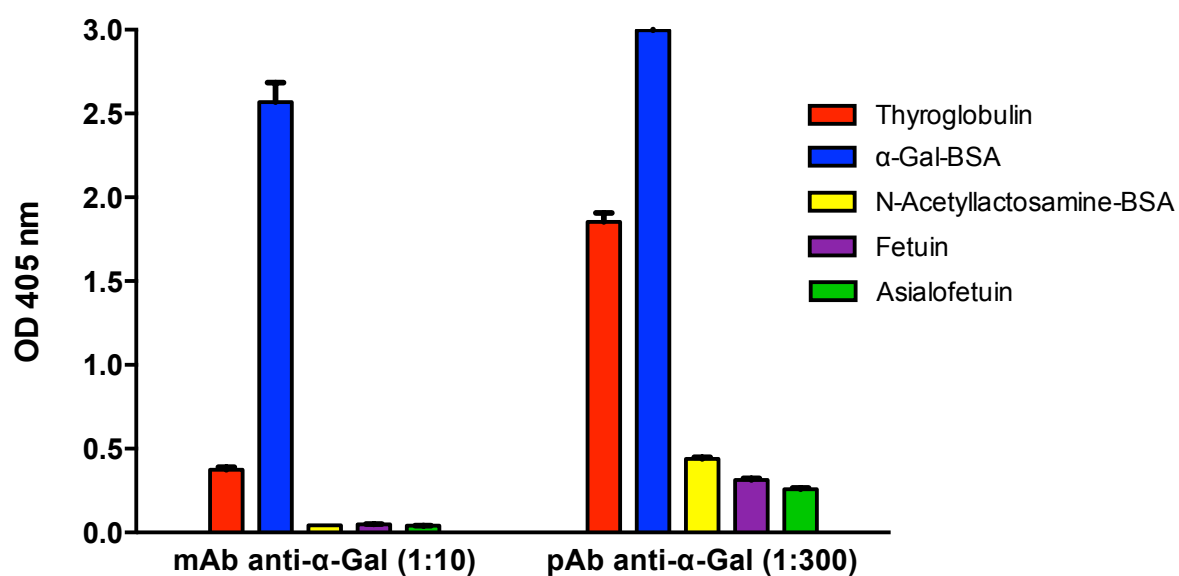


Figure S2. Cryostat-cut lateral section of *I. ricinus* nymph probed with a monoclonal anti- α -Gal antibody. Red areas represent antibody binding. Arrow shows staining of the gut. Bar = 100 μ m.

