

METHODS

Patient and control populations

A total of 39 patients reporting delayed allergic reactions after consumption of red meat; attending the Allergy Unit at Södersjukhuset, Stockholm, Sweden; and having IgE to α -Gal were enrolled in the study. All patients were examined by a physician experienced in allergic diseases and responded to a detailed questionnaire regarding symptoms of meat intake and exposure to ticks. Blood samples were drawn for analysis of IgE antibodies to a panel of different food and inhalant allergens. The patients were also blood typed according to routine methods (Blood Center, Karolinska University Hospital, Stockholm, Sweden). Sera from 143 healthy blood donors (Blood Center, Karolinska University Hospital) were tested for IgE antibodies to α -Gal. Sera from 207 patients with confirmed *Borrelia* species infection (IgG to *Borrelia burgdorferi*; median ratio, 2.22 arbitrary units (AU); range, 0.9–5.04 AU; cutoff, 0.5 AU [OXOID IDEIA *Borrelia burgdorferi* IgG, OXOID, Cambridge-shire, United Kingdom]; Department of Clinical Microbiology, Karolinska University Hospital) were also tested for IgE antibodies to α -Gal. Subjects in these 2 groups with α -Gal titers of 0.1 kU_A/L or greater were analyzed for total IgE and a panel of allergens. *Borrelia* species-positive sera having an α -Gal level of 0.1 kU_A/L or greater were blood typed. The study was approved by the local ethics committee.

Tick extract preparation

Adult pathogen-free *I ricinus* ticks (IS Insect Service GmbH, Berlin, Germany) were stored at -80°C until preparation of whole-body extracts. Tick extract was prepared by crushing frozen ticks with a tissue homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) in PBS (pH 7.4). After centrifugation, the supernatant was collected. In addition, extract of adult pathogen-free *A americanum* ticks (Oklahoma State Tick Rearing Facility, Stillwater, Okla) was prepared, as previously described.^{E1}

ImmunoCAP IgE determination

Total IgE levels and allergen-specific IgE antibody reactivity to α -Gal, beef, moose, pork, chicken, milk, dog, cat, the major cat allergen rFel d 1 and serum albumin from cat (Fel d 2), and beef (Bos d 6) were determined (ImmunoCAP, Phadia AB/Thermo Fisher Scientific). IgE antibodies against tick proteins (*I ricinus* and *A americanum*) were measured by coupling 5 μg of

biotinylated tick antigen to Streptavidin ImmunoCAP, as described by the manufacturer (Phadia AB/Thermo Fisher Scientific). This assay is not commercially available. All IgE determinations were analyzed by using the ImmunoCAP System (Phadia AB/Thermo Fisher Scientific), according to the manufacturer's instructions. The results are presented as kilounits of allergen per liter. The cutoff for total IgE was 2 kU/L or greater, and that for allergen-specific IgE was 0.1 kU_A/L or greater.

Absorption experiments

The capacity of *I ricinus* (2 mg/mL) or *A americanum* (2.6 mg/mL) extracts to inhibit IgE binding to solid phase-bound *I ricinus* (Streptavidin ImmunoCAP) was performed, essentially as described for the ImmunoCAP System (Phadia AB/Thermo Fisher Scientific). However, a prior step of 1-hour preincubation of a serum pool of 4 Swedish patients with meat allergy with 3-fold dilutions (0.01–81 $\mu\text{g}/\text{mL}$) of either extracts from *I ricinus* or *A americanum* was included.

Blood group distribution

The expected ABO blood group distribution was obtained from official national data in Sweden (available at <http://www.geblod.nu>).

Statistical analysis

Statistical analysis was performed with GraphPad Prism software (version 6; GraphPad Software, La Jolla, Calif). Quantitative measurements of IgE antibodies were compared by using Spearman rank correlation and Mann-Whitney *U* tests. Categorical measures were compared with the χ^2 test for trend. The frequency of B-positive subjects compared with a theoretical outcome was performed by using the binominal test. A *P* value of less than .05 was considered significant.

REFERENCE

- E1. 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- α -1,3-galactose. *J Allergy Clin Immunol* 2011;127:1286-93.e6.

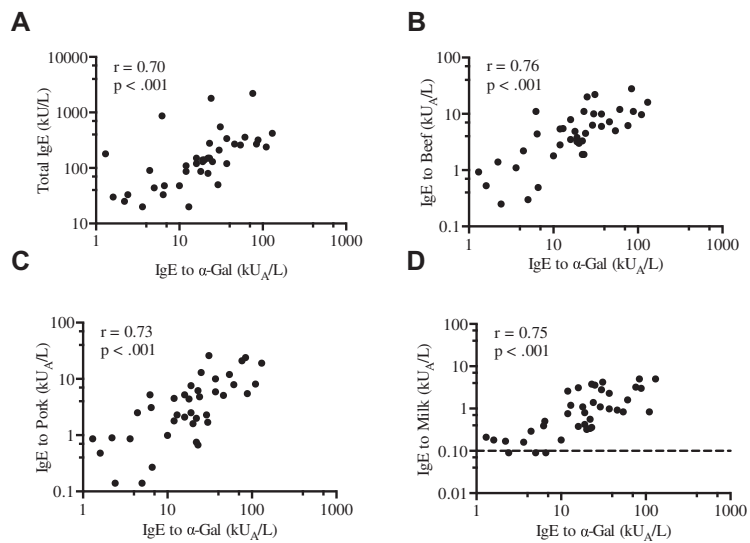


FIG E1. Correlations between IgE responses to α -Gal and total IgE (A), beef (B), pork (C), and cow's milk (D) in Swedish patients with red meat allergy.

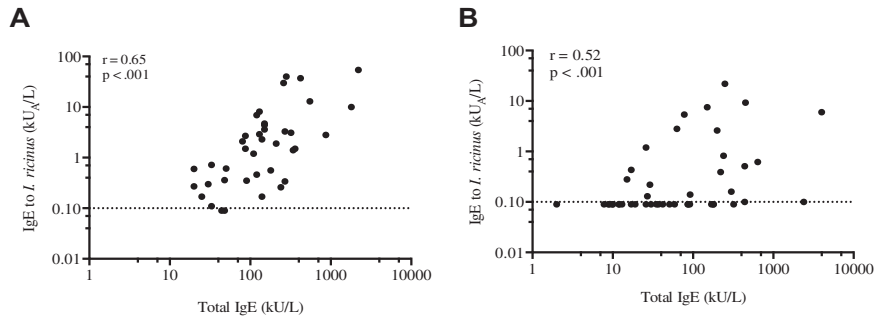


FIG E2. Correlation between IgE responses to *I ricinus* and total IgE levels in patients with meat allergy (**A**) and patients with Lyme disease with positive IgE levels to α -Gal (**B**).

TABLE E1. Further characteristics of patients with meat allergy

Patient no.	IgE*							rFel d 1
	Pork	Moose	Chicken	Milk	Dog	Cat		
1	12	0.84	<0.10	0.84	2.2	1.5	<0.10	
2	2.1	0.5	<0.10	0.38	0.83	0.4	0.37	
3	2.3	0.3	<0.10	1.2	0.28	0.45	<0.10	
4	6.2	2.0	<0.10	3.8	3.4	0.96	<0.10	
5	3.1	1.4	<0.10	0.5	1.0	0.24	<0.10	
6	1.7	1.0	1.4	2.8	0.61	1.1	<0.10	
7	0.9	0.7	<0.10	0.17	0.3	0.64	0.41	
8	5.2	2.2	<0.10	3.1	5.3	3.1	<0.10	
9	5.5	1.6	0.22	3.0	2.6	1.5	<0.10	
10	4.4	1.5	<0.10	1.1	0.98	0.64	<0.10	
11	2.5	0.12	<0.10	0.29	0.26	<0.10	<0.10	
12	19	3.6	<0.10	5	4.4	1.9	<0.10	
13	5.2	0.48	<0.10	0.39	2.4	0.93	<0.10	
14	4.8	6.1	0.39	1.4	14	20	12	
15	5.1	0.8	<0.10	0.92	1.4	0.41	<0.10	
16	26	6.2	0.13	4.2	9.1	3.3	0.15	
17	13	2.1	<0.10	3.6	3.1	2.0	0.44	
18	1.6	0.28	<0.10	0.32	0.16	0.3	<0.10	
19	2.5	1.1	<0.10	0.8	1.1	0.4	<0.10	
20	0.86	0.19	<0.10	0.16	0.27	0.11	<0.10	
21	0.48	0.21	<0.10	0.18	0.17	<0.10	<0.10	
22	2	0.82	0.13	0.56	0.65	0.28	0.16	
23	21	0.92	0.4	3.2	6.3	3.5	0.47	
24	7.6	0.61	<0.10	0.42	2.7	0.78	<0.10	
25	1.8	0.85	<0.10	0.76	2.9	0.53	<0.10	
26	2.3	1.0	<0.10	1.1	0.69	0.2	<0.10	
27	0.27	<0.10	<0.10	<0.10	0.12	<0.10	<0.10	
28	0.99	0.31	<0.10	0.18	0.47	2.4	1.6	
29	0.14	<0.10	<0.10	<0.10	0.21	<0.10	<0.10	
30	4.5	3.9	<0.10	2.6	1.8	1.3	<0.10	
31	5.9	0.89	0.12	0.98	0.61	0.31	<0.10	
32	0.14	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	
33	10	1.8	<0.10	2.3	1.1	0.72	<0.10	
34	8.1	0.69	<0.10	0.84	0.73	0.35	<0.10	
35	24	5.4	<0.10	5.0	5.9	2.0	0.23	
36	7.9	1.9	<0.10	1.6	1.4	1.9	1.9	
37	0.86	0.33	<0.10	0.21	0.21	0.12	<0.10	
38	0.75	0.47	<0.10	0.34	0.56	3.0	2.3	
39	0.67	0.44	<0.10	0.36	0.53	2.9	2.3	

*ImmunoCAP IgE results are presented in kilounits of allergen per liter.

TABLE E2. Characteristics of healthy blood donors with IgE responses of 0.1 kU_A/L or greater to α-Gal

Sample ID	Age (y)/sex	IgE*									Blood type	
		Total	α-Gal	Beef	Tick	Milk	Dog	Cat	rFel d 1	Chicken		
H23	67/M	150	0.16	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
H26	38/F	61	0.14	<0.10	<0.10	<0.10	0.10	<0.10	<0.10	<0.10	<0.10	A
H28	29/M	18	0.12	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
H34	36/M	18	0.7	0.3	0.16	0.11	0.10	<0.10	<0.10	<0.10	<0.10	A
H40	58/M	120	0.14	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
H45	22/M	10	0.13	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
H48	23/M	860	0.59	<0.10	<0.10	0.32	<0.10	<0.10	<0.10	<0.10	<0.10	B
H51	47/M	15	1.2	0.27	<0.10	<0.10	0.11	0.26	0.28	<0.10	<0.10	A
H65	46/F	20	0.2	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
H96	65/F	18	0.13	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	O
H114	19/F	9	0.12	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	AB
H118	35/F	110	0.19	0.8	0.13	1.8	6.2	9.8	0.22	<0.10	<0.10	O
H119	42/M	180	0.1	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	O
H128	55/M	60	0.62	0.14	0.15	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
H136	62/F	11	0.37	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A

*ImmunoCAP IgE results (total IgE, kilounits per liter; allergen-specific IgE, kilounits of allergen per liter).

TABLE E3. Characteristics of patients with Lyme disease with IgE responses of 0.1 kU_A/L or greater to α-Gal

Sample ID	IgE*									Blood type
	Total	α-Gal	Beef	Tick	Milk	Dog	Cat	rFel d 1	Chicken	
L1	240	8.5	3.2	0.82	0.66	0.34	0.2	<0.10	<0.10	A
L4	35	0.53	0.14	<0.10	0.1	<0.10	<0.10	<0.10	<0.10	A
L6	250	0.85	<0.10	22	0.19	<0.10	<0.10	<0.10	<0.10	A
L14	440	11	1.2	0.51	0.37	0.28	0.11	<0.10	<0.10	AB
L17	12	0.23	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
L28	29	0.13	<0.10	0.22	0.22	<0.10	<0.10	<0.10	<0.10	O
L39	17	1.7	0.30	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	O
L44	30	0.12	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
L45	320	0.17	0.17	<0.10	<0.10	0.14	<0.10	<0.10	<0.10	A
L68	27	0.24	<0.10	0.13	<0.10	<0.10	<0.10	<0.10	<0.10	O
L71	59	0.12	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
L72	7.8	0.12	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
L74	13	0.19	0.17	<0.10	0.14	<0.10	<0.10	<0.10	<0.10	O
L75	15	0.12	<0.10	0.28	<0.10	<0.10	<0.10	<0.10	<0.10	O
L81	180	0.26	<0.10	<0.10	0.77	4.7	3.1	2.8	<0.10	O
L95	180	0.11	0.13	<0.10	0.68	0.31	0.68	0.60	<0.10	O
L96	78	0.27	0.11	5.4	<0.10	0.10	<0.10	<0.10	<0.10	O
L99	17	2.7	0.79	0.43	0.26	0.18	<0.10	<0.10	<0.10	O
L100	92	0.36	<0.10	0.14	0.21	NA	NA	<0.10	<0.10	O
L102	450	0.11	<0.10	9.3	<0.10	5.7	8.8	8.5	<0.10	O
L103	9.1	0.18	<0.10	<0.10	0.16	<0.10	<0.10	<0.10	<0.10	B
L106	10	0.46	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	O
L111	63	0.41	0.23	2.8	0.13	0.20	<0.10	<0.10	<0.10	O
L112	150	0.16	<0.10	7.5	0.18	0.25	1.2	1.4	<0.10	O
L116	640	3.0	0.10	0.62	2.4	NA	NA	<0.10	0.13	B
L122	42	0.17	0.16	<0.10	0.77	<0.10	<0.10	<0.10	<0.10	O
L125	220	0.6	0.49	0.39	0.23	1.0	3.1	1.4	<0.10	O
L128	9.9	0.11	<0.10	<0.10	0.15	<0.10	<0.10	<0.10	<0.10	O
L139	12	0.13	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	B
L140	37	0.24	<0.10	<0.10	NA	NA	NA	NA	NA	AB
L144	2	0.21	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	O
L147	91	0.4	0.18	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
L149	19	0.25	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	O
L154	4000	200	67	6	17	16	15	7.8	0.14	A
L155	26	0.34	0.17	1.2	<0.10	<0.10	<0.10	<0.10	<0.10	A
L164	87	0.63	0.25	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
L166	200	0.72	<0.10	2.6	<0.10	<0.10	<0.10	<0.10	<0.10	AB
L171	51	0.47	0.13	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	B
L175	170	0.14	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	O
L178	26	0.52	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	B
L188	440	0.49	<0.10	0.10	<0.10	<0.10	<0.10	<0.10	<0.10	B
L191	8.8	0.23	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	A
L192	NA	0.21	NA	1.7	NA	NA	NA	NA	NA	B
L195	300	0.59	0.32	0.16	0.12	<0.10	<0.10	<0.10	<0.10	A
L202	2400	0.16	0.76	0.10	0.82	74	>100	>100	<0.10	O
L205	86	0.12	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	O

NA, Not available.

*ImmunoCAP IgE results (total IgE, kilounits per liter; allergen-specific IgE, kilounits of allergen per liter).