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The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-α-1,3-galactose

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Abstract

Background—In 2009, we reported a novel form of delayed anaphylaxis to red meat, which is related to serum IgE antibodies to the oligosaccharide galactose-alpha-1,3-galactose (alpha-gal). Most of these patients had tolerated meat for many years previously. The implication is that some exposure in adult life had stimulated the production of these IgE antibodies.

Objectives—To investigate possible causes of this IgE antibody response, focusing on evidence related to tick bites, which are common in the region where these reactions occur.

Methods—Serum assays were carried out using biotinylated proteins and extracts bound to a streptavidin ImmunoCAP.

Results—Prospective studies on IgE antibodies in three subjects following tick bites showed an increase in IgE to alpha-gal of twenty-fold or greater. Other evidence included i) a strong correlation between histories of tick bites and IgE to alpha-gal (χ^2=26.8, p<0.001), ii) evidence that these IgE antibodies are common in areas where the tick *Amblyomma americanum* is common, and iii) a significant correlation between IgE antibodies to alpha-gal and IgE antibodies to proteins derived from *A. americanum* (r_s=0.75, p<0.001).

Conclusion—The results presented here provide evidence that tick bites are a cause, or possibly the only cause, of IgE specific for alpha-gal in this area of the United States. Both the number of subjects becoming sensitized and the titer of IgE antibodies to alpha-gal are striking. Here we report the first example of a response to an ectoparasite giving rise to an important form of food allergy.

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Keywords

ticks; anaphylaxis; oligosaccharide; alpha-gal; IgE antibody to CCD

INTRODUCTION

The monoclonal antibody (mAb) cetuximab, which is specific for epidermal growth factor receptor, was approved for use in treating cancer in 2005.[1,2] Shortly thereafter, it became clear that a significant number of patients were experiencing severe hypersensitivity reactions during their first infusion of this mAb. Surprisingly, those reports appeared to be restricted to an area of the southeast, including Tennessee, North Carolina, Arkansas, Virginia, and the southern half of Missouri.[3,4] In 2007, a study was published by the oncology groups at UNC and Vanderbilt suggesting that the prevalence of severe reactions to cetuximab was as high as 20%.[3] At that time, detailed investigation of serum antibodies established that these reactions were occurring in patients who had pre-existing IgE antibodies specific for the glycosylation on the Fab fragment of the mAb.[5] The relevant oligosaccharide is galactose-alpha-1,3-galactose (alpha-gal), which is a blood group substance of non-primate mammals.[6,7] This analysis was, in part, made possible because Dr. Zhou and his colleagues, working at ImClone, had published the full glycosylation of cetuximab[8].

After establishing the assay for IgE antibodies to cetuximab (i.e., alpha-gal), we screened large numbers of sera. The results established that these IgE antibodies were regionally distributed in a pattern consistent with the cases of cetuximab anaphylaxis. More importantly, the results led to the realization that IgE to alpha-gal was also associated with a novel form of food allergy.[5,9] Those patients reported that they developed generalized urticaria or frank anaphylaxis, starting 3-6 hours after eating beef, pork, or lamb; however, they reported tolerating chicken, turkey, or fish without difficulty.[9,10] Thus, the specificity of IgE antibodies present in their serum, which was known to be specific for a carbohydrate common to mammals but absent from poultry and fish, matched their symptoms.[6,9] In most cases, these patients were adults who had consumed red meat for many years before developing the delayed reactions. This history implies that some new exposure had triggered the production of IgE antibodies to alpha-gal.

Initially, we investigated the possible role of a regionally important inhalant or fungal allergen, but those results were uniformly negative. The next possibility was that a regional helminth could have induced IgE antibodies to the oligosaccharide. However, both from studying the known distribution of helminths in the United States and from sharing sera with Dr. Amy Klion at the NIH, we excluded *Ascaris* and found little evidence for any other helminth.[11] By contrast, the known distribution of the immediate reactions to cetuximab was similar to the areas with high prevalence of Rocky Mountain spotted fever (RMSF).[12] In addition, this area is similar to the area for maximum prevalence of human ehrlichiosis.[13,14] We were also aware of patients who thought that their reactions to red meat started after receiving multiple tick bites. Finally, we were informed that Dr. van Nunen in Sydney, Australia and Dr. Deutsch in Georgia (personal communication, 2010) had reported to their local allergy meetings about patients who had become allergic to meat after experiencing multiple tick bites.[15] We report here the evidence that tick bites in the United States can induce IgE antibodies to alpha-gal. The evidence comes from i) prospective studies of the response to tick bites in three subjects, ii) epidemiological evidence that these IgE antibodies are present in areas where tick bites are common, iii) correlation between IgE antibodies to tick proteins and IgE antibodies to alpha-gal, and iv) evidence for an expanding range of the lone star tick, *Amblyomma americanum*.

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METHODS

Patient, control, and random populations

Clinic populations in Virginia—patients presenting to clinic in Charlottesville for evaluation of recurrent anaphylaxis or severe urticarial reactions (n=121), asthma (n=56), or controls (n=40) were enrolled and provided serum. Of these, the most recently enrolled 125 subjects responded to a full questionnaire, including questions about tick bites (questionnaire included in online repository, Form I). Patients who specifically presented with urticarial or anaphylactic reactions were enrolled as subjects and had their serum tested for IgE antibodies to alpha-gal. Other patients were enrolled sequentially upon their agreeing to the informed consent process.

Random populations in the southeastern United States where tick bites are common—The Tennessee control cohort was obtained as a random control population in a cancer-screening clinic at Vanderbilt University.[5] The North Carolina cohort was collected randomly from hospital patients at the University of North Carolina as part of a study on the genetics of this anaphylactic response.[16] The Virginia patients included 70 with acute asthma and 66 controls presenting to the ED at the University of Virginia.[17] In this last population, there was no difference in the prevalence of IgE to alpha-gal between the patients with asthma and the controls.

Populations in areas where tick bites are rare—Subjects in the Boston Women's Study were enrolled as part of a birth cohort, in a large urban center where tick bites are rare.[18] As part of a prospective study on asthma among children in the Norrbotten area of northern Sweden, sera were obtained from 963 subjects at age 18 years.[19] From these sera, all those with positive assays for IgE antibodies to cat epithelium and dander of class 2 or higher (n=150) were assayed for IgE to alpha-gal. Northern California was included because reactions to cetuximab are rare and although some ticks are present there, *A. americanum* is not.[13] The sera came from three cohorts: i) cancer patients at Stanford University,[5] ii) patients with acute asthma (n=60) and controls (n=57) presenting to Travis Air Force Base hospital near Sacramento,[20] and iii) asthma (n=102) and control (n=41) subjects enrolled in studies on asthma at the University of California San Francisco.

Populations from Kenya and the Esmeraldas Province of Ecuador (i.e., tropical areas where tick bites are common)—In a previous study in Kenya, we reported that IgE antibodies to cat were common among schoolchildren in the village of Kabati (n=131) and less common in the small town of Thika (n=123). In the village, helminth infections, tick bites, and other ectoparasites are extremely common and less so in the town.[21] Sera from children in the Esmeraldas Province of northern Ecuador (n=295) were collected as part of ongoing studies on the relevance of helminth infections to asthma and allergic disease.[22]

Approval for these studies was obtained locally in the area where subjects were enrolled and from the University of Virginia Human Investigation Committee. Further details regarding the individual cohorts may be found in the online repository Form II and in the indicated references.

Allergen source and preparation

Adult, pathogen-free *Amblyomma americanum* and *Dermacentor variabilis* ticks were purchased from the Oklahoma State Tick Rearing Facility (Stillwater, OK), and were maintained at 4°C until preparation of whole body extracts. The ticks were frozen with liquid nitrogen and crushed with a mortar and pestle. Proteins from the resultant powder

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were extracted overnight in borate buffered saline, pH 8.0, with added protease inhibitors (Sigma Aldrich, St. Louis, MO). After removal of the solid pellet, the protein solution was defatted using ethyl ether (Sigma Aldrich, St. Louis, MO), and after centrifugation at 3,000 rpm for 5 minutes, the sample separated into three layers, the bottom of which (the aqueous layer) was collected for analysis.[23,24]

ImmunoCAP IgE assays

Total and specific IgE antibodies were measured by using either commercially available ImmunoCAP (Phadia US, Portage, MI) or a modification of the assay with streptavidin on the solid phase.[25] The assays were performed with the ImmunoCAP 250 instrument, and the results were expressed as international units per milliliter, where the international unit both for specific and total IgE is approximately 2.4 ng. For specific assays, the standard cutoff point for a positive reaction was 0.35 IU/mL. The streptavidin CAP technique was used to measure IgE antibodies to alpha-gal and tick proteins (*A. americanum* and *D. variabilis*), where approximately 5 μg of biotinylated antigen was added to each CAP before adding 40 μL of undiluted serum. This assay is now available commercially. Selected sera were tested with commercially available assays for IgE antibodies to dust mite (*D. pteronyssinus*; d1), dog epithelium (e2), ryegrass pollen (g5), beef (f27), *Trichophyton rubrum* (m205), common silver birch pollen (t3), and German cockroach (i6).

Statistical analyses

We compared quantitative measures of IgE antibodies by using the Spearman rank correlation and compared qualitative measures of tick bite severity and presence of IgE antibodies by using the χ^2 test for trend. A 2-sided *P* value of less than 0.05 was considered to indicate statistical significance. Statistical analyses were performed with SPSS software, version 18.0 (SPSS Inc, Chicago, IL), and GraphPad Prism, version 4 (GraphPad Software, La Jolla, CA).

RESULTS

Rapid development of IgE antibodies to alpha-gal following tick bites in three subjects

Over the last three years, we have had the opportunity to follow serum IgE responses prospectively in three individuals whose serum was available from before they experienced multiple tick bites. In each case, IgE antibodies to alpha-gal rose over twenty-fold after tick bites and there was a parallel, though not identical, rise in total IgE (Fig 1). Furthermore, two of these individuals (#1 and #3 in Fig 1 and Table I) experienced an episode of generalized urticaria three to four hours after eating red meat, something that had not occurred prior to the tick bites. In these two cases, the IgE antibodies to alpha-gal represented ≥30% of the total IgE. By contrast, in subject #2, IgE to alpha-gal rose from <0.35 to 8 IU/ml, but this accounted for less than 1% of the total IgE, and this individual has yet to report any allergic symptoms after eating mammalian meat. IgE antibodies to several other allergens were measured in serial samples from each of these individuals (Table I). The results show that none of the three developed new specificities of IgE antibodies, other than those that could be explained by IgE antibodies to alpha-gal (e.g., cat, dog, beef, and milk). While IgE antibodies to inhalants in sera from subject #2 did increase, these increases were much less than the increase in IgE to alpha-gal. In cases 1 and 2, the ticks responsible for the bites were identified as *A. americanum*.

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Relationship between histories of tick bites and serum IgE antibodies to alpha-gal on a local and regional level

From questionnaire results, it was clear that a large proportion (>90%) of the subjects with serum IgE antibodies to alpha-gal had a history of tick bites. However, tick bites are now common in central Virginia. Among 125 subjects, including patients with delayed anaphylaxis, as well as patients with asthma and controls, a positive response to questions, including "Have you ever had local reactions to tick bites last for weeks or longer?" correlated highly significantly with the presence of IgE antibodies to alpha-gal ($p<0.001$) (Fig 2). We have now assayed sera or received results on over 300 subjects who presented with anaphylaxis or urticaria, and about whom we knew where they lived at the time of their first reaction. Although this is by no means a random sample, the results are interesting. Plotting positive cases on a map of the United States showed a striking similarity to the known distribution of *A. americanum*.[13]

Although ticks are ubiquitous in temperate and tropical regions, there are major regional differences in species and also regions or populations where tick bites are rare or absent (Table II). These include large cities and areas where the climate prevents survival of ticks or their natural hosts (e.g., deer, small mammals). In keeping with the absence of ticks in the inland areas of northern Sweden, assays of sera from Norrbotten were negative for IgE antibodies to alpha-gal (Table II).[20] In contrast, we have assayed sera from Africa and Ecuador, which show a significant number of positive sera among rural populations. The positive results from Africa and Ecuador can only be interpreted as providing evidence that ticks *could* be the cause of these IgE antibodies, because other ectoparasites or helminths could be relevant (Table II). Of note, the generally negative data from Norrbotten, Boston, and northern California argue strongly that eating food containing this oligosaccharide (e.g., beef or milk) does not induce sensitization to alpha-gal.

IgE antibodies to tick proteins

Studies from both Europe and Australia have reported the presence of IgE antibodies to tick salivary proteins in sera from patients who experienced anaphylactic reactions to tick bites.[26-28] Using whole body extracts of *A. americanum* or *Dermacentor variabilis*, we assayed sera with or without IgE antibodies to alpha-gal for IgE antibodies specific for tick proteins. The results show a strong correlation between IgE antibodies to *A. americanum* and IgE antibodies to alpha-gal (Fig 3). The titers of IgE antibodies to tick proteins were lower than the IgE antibodies to alpha-gal. When sera were absorbed using a solid phase coated with alpha-gal, half of the sera retained (>80% of the IgE to ticks(online repository, Table eI). Four of the 12 sera had over 80% of the IgE that bound the tick extract removed by absorption with alpha-gal coated beads. Performing the same experiments with blank sepharose beads or as a mock experiment caused less than a 1% change alpha-gal or *A. americanum* IgE binding (data not shown). The results for *D. variabilis* did not show a significant relationship with IgE antibodies to alpha-gal (online repository, Figure eI). Thus, our evidence suggests that bites from *A. americanum* ticks can induce both IgE antibodies to tick proteins and IgE antibodies to alpha-gal. The sera from the inland town of Kiruna in the Norrbotten province of Sweden were uniformly negative for IgE antibodies to ticks.

DISCUSSION

The results presented here strongly suggest that tick bites are a cause, if not the only significant cause, of IgE antibody responses to alpha-gal in the southeastern United States. This evidence includes following the response prospectively in three cases, a strong correlation with histories of tick bites, epidemiological evidence that these antibodies are not found in regions where tick bites are rare, and the correlation with IgE antibodies specific

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for tick proteins. As recently as January of 2006, we were totally unaware of this phenomenon. Our present results in Virginia, North Carolina, and Tennessee suggest that as many as 20% of the population in these states have serum IgE antibodies to alpha-gal. In 2009-2010, delayed anaphylaxis to red meat is a common reason for presentation to the clinics of this predominantly rural or suburban area of central Virginia. Nevertheless, we do not mean to suggest that 15% of the population in these states has an allergy to mammalian meat. We are aware of many individuals with IgE to alpha-gal (and beef) who do not have reactions after eating red meat. In addition to the high prevalence, the titers of IgE antibodies to alpha-gal can be very high. In many cases, patients have greater than 100 IU/ml of IgE antibodies to alpha-gal, often in conjunction with high total IgE. Indeed, our results suggest that tick bites can be an important cause of high total serum IgE. That the saliva of ticks is a potent immunogen is well known;[29-31] however, it has not previously been reported that ticks could induce IgE antibodies to a cross-reactive oligosaccharide.

Our original observation was that the distribution of anaphylactic reactions to cetuximab (Fig 4A) was similar to the maximum prevalence of Rocky Mountain spotted fever (Fig 4B). The major vectors of RMSF in this region are the ticks *D. variabilis* and *A. americanum*, and the geographic range of *A. americanum* has been expanding over the last 30 years, probably in parallel with the massive increase in the deer population (Fig 4C and D).[13,14,32,33] Many people are not aware that in 1950, deer had been hunted close to extinction in the southeastern United States. Indeed, at about that time, the Virginia state game commission "reintroduced" deer to sections of the Blue Ridge Mountains. Over the last 20 years, the deer population has expanded to become a major pest of suburban areas. This further increase may have been made possible by the introduction of leash laws for dogs. Our focus has been on *A. americanum* because i) two of our cases involved this species, ii) the cases match the known distribution of this tick (Fig 4C and D), and iii) this species has a well-deserved reputation for being "aggressive."[14,32-34] Both adult and larval forms of this tick are very willing to bite humans as well as other mammals. The larval ticks are about 1 mm in diameter, and are generally not seen or appear as small black dots. This creates a confusion that is important in taking histories. The term used for larval ticks is "seed ticks;" however, the term "chiggers" is better known, and it is not easy to identify either seed ticks or chiggers correctly. Chiggers are correctly the larval forms of trombiculid mites, which can also bite humans, but are less common than seed ticks in the region in question.[35]

Our evidence about the regional distribution of these reactions does not favor a role for *Ixodes scapularis*. Indeed, the Lyme disease literature has very few mentions of multiple bites or severe pruritic reactions to bites. This may well be because persons who consistently report itching in association with tick bites are less likely to experience an episode of Lyme disease than do those who fail to react against tick bites of *I. scapularis*.[36] In the same study from Block Island where Lyme disease is highly endemic, it was found that the frequency of itch increased as the number of reported tick bites increased, suggesting that tick-related itch was associated with an acquired cutaneous hypersensitivity response.[36] Of note, the larval forms of the ticks that carry *Borrelia burgdorferi* are not known to bite humans. In other areas of the world, different ticks have been associated with allergic reactions in humans. In Australia, bites of *Ixodes holocyclus* can precede subsequent allergic reactions to beef.[15] In Europe, the pigeon tick, *Argas reflexus*, is known to cause sensitization and anaphylactic reactions to subsequent bites from ticks of the same species.[27,28] That tick has not been associated with allergic reactions to red meat, and the IgE antibodies have been shown to be specific for a protein.[28] In the Stockholm area of southern Sweden, ticks, particularly *Ixodes ricinus*, are a major problem, and IgE antibodies to alpha-gal have been reported.[37,38] At present, it seems likely that several different ticks can cause an IgE antibody response to alpha-gal; however, the behavior of the ticks (e.g., propensity to bite humans) may also be important.

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The severity of the pruritic symptoms that our patients report has been impressive. Although many different patterns of tick bites have been reported, three forms stand out:

1. A few bites from adult ticks that persist for weeks or months, remaining pruritic. The most severe case of this kind reported having two tick bites removed surgically 6 months after the original contact.
2. Repeated bites often around the ankles in subjects who work outside or hunt regularly. In a few cases, the local reactions to the ticks have been so severe as to preclude further work outside.
3. Multiple bites from larval ticks, generally 10 or more, but often hundreds, which are again severely pruritic, but generally do not last more than a few weeks.

We have seen high or very high (>100 IU/ml) titers of IgE antibodies to alpha-gal in patients with histories of each of these types. Surprisingly, despite the severity of pruritic reactions locally and the presence of IgE antibodies to tick proteins, we are not aware of any cases of anaphylactic reactions to subsequent tick bites among patients we have seen. There is, however, one case report of anaphylaxis to a tick bite in the United States.[39]

The number of cases of delayed anaphylaxis being diagnosed in central Virginia and the surrounding areas has increased dramatically, suggesting that we might be looking at an epidemic. However, there were some cases of this condition being seen, if not correctly diagnosed, twenty years ago; in fact, two patients have recently come to see us and reported that they had told us the same story 15 and 18 years previously! Equally, we know that IgE antibodies to alpha-gal were present in this area in 1988, because sera from an ED study at that time have recently been assayed for IgE to alpha-gal.[40] If the incidence has increased, there are several possible causes related to ticks. There is good evidence that *A. americanum* has increased its range, and that its primary host has increased dramatically in numbers.[14,33] It is also possible that some factor about the tick has changed; for example, a minor variation in the composition of saliva or the presence of a symbiotic organism. This species is known to carry multiple species of rickettsiae, including *R. amblyommii* and the organism responsible for human ehrlichiosis, *Ehrlichia chaffeensis*.[41,42] Although the results of absorption experiments provide clear evidence for antibodies that can be absorbed by alpha-gal and those that cannot, there is not an obvious explanation for the tick protein IgE (Figure 3 and online Table eI). Future studies will be directed at the possibilities for this, which include a symbiotic organism, bystander polyclonal IgE induced in the alpha-gal response, or a concomitant sensitization to tick salivary protein(s) or other oligosaccharide.

Taken together, the evidence pointing towards tick bites as the primary cause of IgE antibody responses to alpha-gal creates a compelling argument. In addition to the three cases reported in detail here, there is epidemiological evidence that i) these IgE antibodies are found in areas where tick bites are common, ii) the responses correlate with reports of tick bites, and iii) IgE antibodies to alpha-gal correlate with the presence of IgE antibodies to tick proteins. Furthermore, the currently known distribution of delayed anaphylactic reactions to red meat is similar to the known distribution of *A. americanum* (Fig 4C and D). Predictions are that populations of both deer and ticks will continue to increase their range, particularly in suburban areas. Thus, the number of Americans exposed to these ticks and their larval forms will, in all probability, continue to increase.

Key Messages

- IgE antibodies to the oligosaccharide alpha-gal are related to two forms of anaphylaxis that are most common in the southeastern United States (e.g. anaphylaxis during cetuximab infusion; anaphylaxis after eating mammalian meat)

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- Tick bites are directly related to the presence of these IgE antibodies as assessed prospectively, by history of tick bites, and by analyses of the areas where these IgE antibodies are found

- This IgE antibody response provides a model of an ectoparasite-specific response which has significant clinical consequences

Capsule Summary

Two forms of anaphylaxis are related to the presence of IgE antibodies to the oligosaccharide alpha-gal. We report here the evidence that these IgE antibodies are induced by ticks that are regionally distributed.

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Abbreviations Used

Alpha-gal	galactose-alpha-1,3-galactose
ED	Emergency Department
RMSF	Rocky Mountain spotted fever

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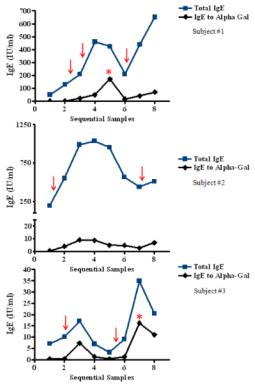

Fig 1.

Time course of IgE antibodies to alpha-gal and total IgE collected as sequential samples following episodes of multiple tick bites (red arrows) in three subjects. The time intervals between samples vary considerably, and the specific times can be seen in Table I. Subjects #1 and #3 experienced episodes of generalized urticaria starting 3-4 hours after eating mutton and beef, respectively (indicated by red asterisk). In each case, this was the first such episode.

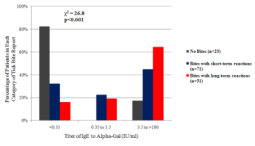

Fig 2.

Prevalence of IgE antibodies to alpha-gal among 125 patients with or without histories of tick bites. Histories of bites were analyzed by length of time that reactions persisted at the site. Chi square analysis on three bite categories versus assays for IgE antibodies >0.35 IU/ml.

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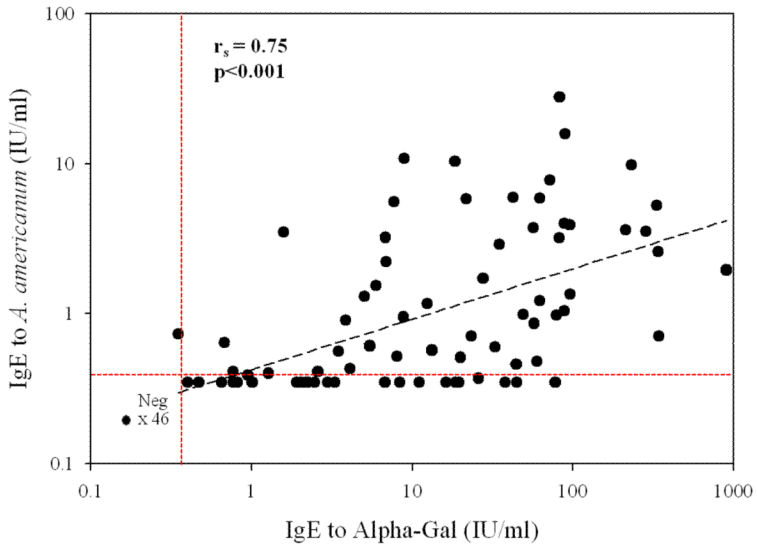

Fig 3.

Correlation between IgE antibodies to alpha-gal and IgE antibodies to proteins derived from the tick *Amblyomma americanum* in 125 sera obtained from patients living in Virginia.

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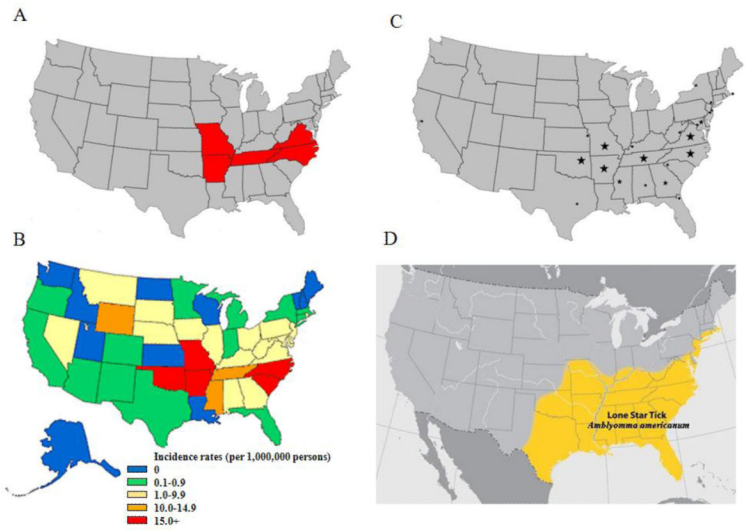

Fig 4.

Comparison of the states where anaphylactic reactions to cetuximab were reported in 2006 (A) and the reported prevalence of Rocky Mountain spotted fever (B). Distribution of known cases of patients with delayed anaphylaxis to red meat whose serum contained IgE antibodies to alpha-gal (C). Markers indicate the number of cases: • single cases; smaller black stars indicate 5-24 cases within a state, and larger black stars indicate states with ≥25. Data in panel A from Bristol-Myers Squibb; data in panel B from CDC website. Numbers of cases in states with stars: 65 Arkansas, 14 Georgia, 8 Maryland, 5 Mississippi, 30 Missouri, 35 North Carolina, 25 Oklahoma, 40 Tennessee, >200 Virginia. In panel D, the known distribution of the tick *Amblyomma americanum* is shown: data from CDC website.

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Table I

Changes in IgE Antibodies (IU/ml) Following Tick Bites in Three Subjects (Black Lines Indicates Occurrence of Tick Bites)

#1		**Apr-02**	**Oct-06**	**May-07**	**Aug-07**	**Jan-08**	**Feb-10**	**Aug-10**	**Sep-10**
	Dust Mite	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Cockroach	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Rye Grass	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Silver Birch	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Trichophyton	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Dog Epithelium	**0.46**	**0.38**	**10.2**	**29.3**	**64.3**	**11.5**	**29.9**	**41.1**
	Beef	**0.56**	**0.40**	**10.3**	**28.0**	**65.0**	**13.3**	**34.7**	**45.2**
	Alpha-Gal	**0.51**	**0.49**	**20.2**	**48.3**	**170**	**14.7**	**40.1**	**69.2**
	Total IgE	49	127	208	460	425	209	439	652
#2		**Jul-07**	**Jun-09**	**Jul-09**	**Jul-09**	**Aug-09**	**Jan-10**	**Feb-10**	**Nov-10**
	Dust Mite	**0.62**	**0.55**	**0.68**	**1.28**	**1.26**	**0.80**	**0.65**	**0.6**
	Cockroach	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Rye Grass	**6.83**	**5.30**	**6.02**	**7.21**	**6.69**	**5.68**	**5.03**	**6.86**
	Silver Birch	**30.4**	**28.3**	**31.0**	**39.4**	**35.5**	**33.5**	**31.4**	**36.1**
	Trichophyton	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Dog Epithelium	0.35	**1.21**	**2.37**	**2.46**	**2.35**	**1.28**	**1.12**	**1.63**
	Beef	0.35	**1.47**	**2.53**	**3.04**	**2.59**	**1.37**	**1.25**	**2.15**
	Alpha-Gal	0.35	**3.88**	**8.94**	**8.71**	**4.87**	**4.67**	**2.62**	**6.84**
	Total IgE	192	551	989	1036	954	566	440	510
#3		**Jun-96**	**Jun-99**	**Aug-08**	**Aug-09**	**Mar-10**	**Sep-10**	**Oct-10**	**Nov-10**
	Dust Mite	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Cockroach	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Rye Grass	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Silver Birch	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Trichophyton	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
	Dog Epithelium	0.35	0.35	**3.36**	**0.78**	0.35	**0.67**	**9.3**	**4.66**
	Beef	0.35	0.35	**4.15**	**1.00**	0.35	**0.85**	**12.0**	**5.9**
	Alpha-Gal	0.35	0.35	**7.29**	**1.24**	0.35	**1.18**	**16.2**	**11**
	Total IgE	7.01	10.1	17.1	6.95	3.25	9.05	34.9	20.5

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[1]Values for serum IgE antibodies and total IgE following episodes of multiple tick bites. Values are shown for five allergens unrelated to alpha-gal: dust mite, cockroach, ryegrass, silver birch, and the fungus *Trichophyton rubrum*. Values for IgE antibodies that are related to the oligosaccharide alpha-gal are shown in a shaded area.

[2]Patient #2 was allergic to mite, grass, and birch prior to tick bites.

[3]The timing of episodes of multiple tick bites are shown as vertical black lines. In each case, the bites occurred within ten days of the subsequent blood draw.

[4]Patients #1 and #3 experienced generalized hives 3-4 hours after eating red meat. In each case, this was their first such episode and occurred when IgE antibody titer to alpha-gal was high (Case #1: November 20, 2007 and Case #3: October 10, 2010).

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Table II

Prevalence of IgE antibodies to galactose-alpha-1,3-galactose in different populations related to local prevalence of tick bites.

Virginia Clinic Populations	Prevalence*	Percentage
Patients presenting with delayed anaphylaxis to red meat § [9]	117/121 (99)	97%
Asthma in the clinic ‡	6/56 (3)	11%
Random Populations in the Southeast Δ		
Tennessee [5]	24/107 (11)	22%
North Carolina [16]	15/75 (9)	20%
Virginia (ER) [17]	25/136 (8)	18%
Southeastern Populations, Total	64/318 (28)	20%
Areas Where Tick Bites are Rare		
Boston Women's Study (adults) [18]	2/341 (0)	<1%
Norrbotten, Sweden (age 18) [19] Northern California	1/150 (0)	<1%
- Stanford University cancer patients [5]	3/49 (0)	6%
- Travis Air Force Base asthmatics and controls [20]	1/117 (0)	1%
- UCSF asthmatics and controls	3/136 (1)	2%
Northern California, Total	7/302 (1)	2%
Tropical Areas Where Tick Bites are Common ¶		
Kabati, Kenya (rural) [21]	100/131 (50)	76%
Thika, Kenya (moderately sized industrial town) [21]	36/123 (10)	29%
Esmeraldas Province, Ecuador [22]	110/295 (36)	37%

*Values are the number of sera with greater than 0.35 IU/ml, and the values in parentheses are the number of sera greater than 3.5 IU/ml. Also shown is the percentage of positive sera.

§Patients presenting to the clinic with a history of recurrent anaphylaxis, angioedema, or generalized urticaria occurring 3-5 hours after eating mammalian meat [9]

‡Patients evaluated in the University of Virginia Allergic Disease clinic for asthma, including 30 with severe or moderately severe asthma.

ΔThree studies in which enrollment was unrelated to histories that could be related to IgE antibody responses to alpha-gal.

¶Tick bites of several species are known to be common in each of these areas, but there are also multiple helminths and other ectoparasites that could be relevant.