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The alpha-gal story: Lessons learned from connecting the dots

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Activity Objectives

1. To describe the history and presenting symptoms of galactose-alpha-1,3-galactose (alpha-gal).
2. To describe the pathophysiology of the IgE-mediated response to alpha-gal in human subjects.
3. To list the diagnostic approach to alpha-gal.

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Anaphylaxis is a severe allergic reaction that can be rapidly progressing and fatal, and therefore establishing its cause is pivotal to long-term risk management. Our recent work has identified a novel IgE antibody response to a mammalian oligosaccharide epitope, galactose-alpha-1,3-galactose (alpha-gal). IgE to alpha-gal has been associated with 2 distinct forms of anaphylaxis: (1) immediate-onset anaphylaxis during first exposure to intravenous cetuximab and (2) delayed-onset anaphylaxis 3 to 6 hours after ingestion of mammalian food products (eg, beef and pork). Results of our studies and those of others strongly suggest that tick bites are a cause, if not the only significant cause, of IgE antibody responses to alpha-gal in the southern, eastern, and central United States; Europe; Australia; and parts of Asia. Typical immune responses to carbohydrates are considered to be T-cell independent, whereas IgE antibody production is thought to involve sequential class-switching that requires input from T cells. Therefore, establishing the mechanism of the specific IgE antibody response to alpha-gal will be an important aspect to address as this area of research continues. (J Allergy Clin Immunol 2015;135:589-96.)

Key words: *Anaphylaxis, delayed reaction to red meat, galactose-alpha-1,3-galactose*

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Hypersensitivity in the allergic setting refers to immune reactions stimulated by soluble antigens that can be rapidly progressing and, in the case of anaphylaxis, are occasionally fatal. Because the number of known exposures associated with anaphylaxis is limited, identification of novel causative agents is important in facilitating both education and other allergen-specific approaches that are crucial to long-term risk management. Within the last 10 years, several seemingly separate observations were recognized as related, all of which resulted from the development of antibodies to a carbohydrate moiety on proteins in which exposure differed from airborne allergens but

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Abbreviations used
Alpha-gal: Galactose-alpha-1,3-galactose
LDL: Low-density lipoprotein
RMSF: Rocky Mountain spotted fever
VLDL: Very low-density lipoprotein

that were nevertheless capable of producing anaphylactic and hypersensitivity reactions. Our recent work has identified these responses as being due to a novel IgE antibody directed against a mammalian oligosaccharide epitope, galactose-alpha-1, 3-galactose (alpha-gal).[1] This review will present the history and biology of alpha-gal and discuss the evidence that the IgE response to alpha-gal is different from typical IgE responses directed toward protein allergens.

CETUXIMAB-INDUCED HYPERSENSITIVITY REACTIONS

In 2004, ImClone and Bristol-Myers Squibb were investigating an mAb (cetuximab) specific for the epidermal growth factor receptor in clinical trials for the treatment of metastatic colorectal cancer. Early in those studies, it became clear that the antibody was causing hypersensitivity reactions; however, these reactions were occurring primarily in a group of southern US states (Table I). These reactions to cetuximab developed rapidly, and symptoms often peaked within 20 minutes after or during the first infusion of the antibody and occasionally proved fatal.[1-3] Because of delays in marketing, it was not until 2006 that the true severity of the reactions became obvious.[2]

At this time, our group began preliminary experiments examining the IgE response to this molecule. Dr Hatley, who was working in Bentonville, Arkansas, convinced our group to develop a new version of the IgE fluorometric enzyme immunoassay or CAP assay to cetuximab using the streptavidin technique. In this assay streptavidin is coupled to the solid phase of the CAP to provide a matrix for the binding of biotinylated novel or purified allergens.[4]

We were subsequently asked to investigate the reactions to cetuximab, in part because we had already developed the IgE assay to cetuximab. In collaboration with Dr Chung from Nashville, Dr Mirakhur from Bristol-Myers Squibb, and Dr Hicklin from ImClone, we demonstrated that the patients who had reactions to cetuximab also had IgE antibodies specific for this molecule before they started treatment.[1] The question remained as to what epitope the IgE antibody was recognizing on the cetuximab molecule.

Early work by Karl Landsteiner discovered that all human subjects had antibodies to a blood group "B-like" oligosaccharide found on nonprimate red blood cells.[5] That antigen was subsequently identified as alpha-gal, which represents a major transplantation barrier between primates and other mammals.[6-8] Antibodies against alpha-gal are present in all nonimmunocompromised human subjects, and some early studies suggested that the IgG antibodies against alpha-gal constituted about 1% of circulating immunoglobulins in human subjects, apes, and Old World monkeys.[9] Recent work in our laboratory with specific assays for IgG antibodies suggests that the percentages are not this high. As discussed below, the fact that all nonprimate mammals, including mice, can make oligosaccharides that are foreign to human subjects is an important component of our story.

CARBOHYDRATE ANALYSIS OF CETUXIMAB

Glycosylation of proteins is a posttranscriptional modification that can play key roles in many processes, including protein folding, protein stability, intracellular trafficking, and cellular adhesion, as reviewed by Hurtado-Guerrero and Davies.[10] Characterization of cetuximab glycosylation, as measured based on peak area on time-of-flight mass spectrometric spectra, revealed 21 distinct oligosaccharide structures, of which approximately 30% have 1 or more alpha-1,3–linked galactosyl residues.[11] Analysis of the IgE antibodies to cetuximab demonstrated that these antibodies were specific for the oligosaccharide residues on the heavy chain of the Fab portion of the mAb. From the known glycosylation of the molecule at amino acids 88 and 299 (Fig 1),[11] alpha-gal was identified as the relevant epitope. Of the total alpha-gal in cetuximab, most of it is located in the Fab domain (Fab domain: 990 nmol alpha-gal/μmol IgG vs Fc domain: 140 nmol alpha-gal/μmol IgG).[11] Recent mass spectrometric analysis indicates that glycosylation of cetuximab might be more complex than previously thought, containing both dianternary and trianternary structures.[12] Synthesis of alpha-gal requires the gene encoding alpha-1, 3-galactosyltransferase. In human subjects and higher primates this gene is not functional, and therefore these species cannot produce alpha-gal, which in turn makes it possible for these animals to initially make IgG and IgM antibodies directed toward this oligosaccharide.[7,13] How IgE to alpha-gal gets made and the nature of the IgE response will be considered later.

Of considerable importance to the development of biologics, in particular mAbs, is the observation that murine cell lines, such as NS0 and Sp2/0, can synthesize galactose in an alpha-1,3 linkage such that alpha-gal is present on the molecules. Sp2/0 was the cell line used to produce cetuximab. In those subjects with IgE to alpha-gal (≥0.35 IU/mL), reactions are likely to occur directed against this mAb.[3]

THE RED MEAT CONNECTION

During this same time period (2006-2008), we evaluated a number of patients, most of whom spent a significant amount of time outdoors, who had presented with episodes of generalized urticaria, angioedema, or recurrent anaphylaxis. The importance of the time spent outdoors was not clear at that time. There was no obvious immediate cause for the symptoms, but in several cases the patients reported that they believed the reactions might be due to consumption of meat 3 to 5 hours earlier. Skin prick tests were performed with commercial extracts of beef, pork, or lamb and produced small wheals only 2 to 4 mm in diameter that often would be interpreted as negative results. However, given the compelling history described by the patients, we extended our analysis to intradermal skin testing with commercial meat extracts or skin prick tests with fresh meat extracts, both of which demonstrated strong positive results.[14] These results were confirmed with blood tests for specific IgE antibody to red meats.[14]

Although not published, similar sensitivity to red meat had been previously noted in Georgia. Starting in 1989, Mrs Sandra Latimer, together with Dr Antony Deutsch from Athens, Georgia,

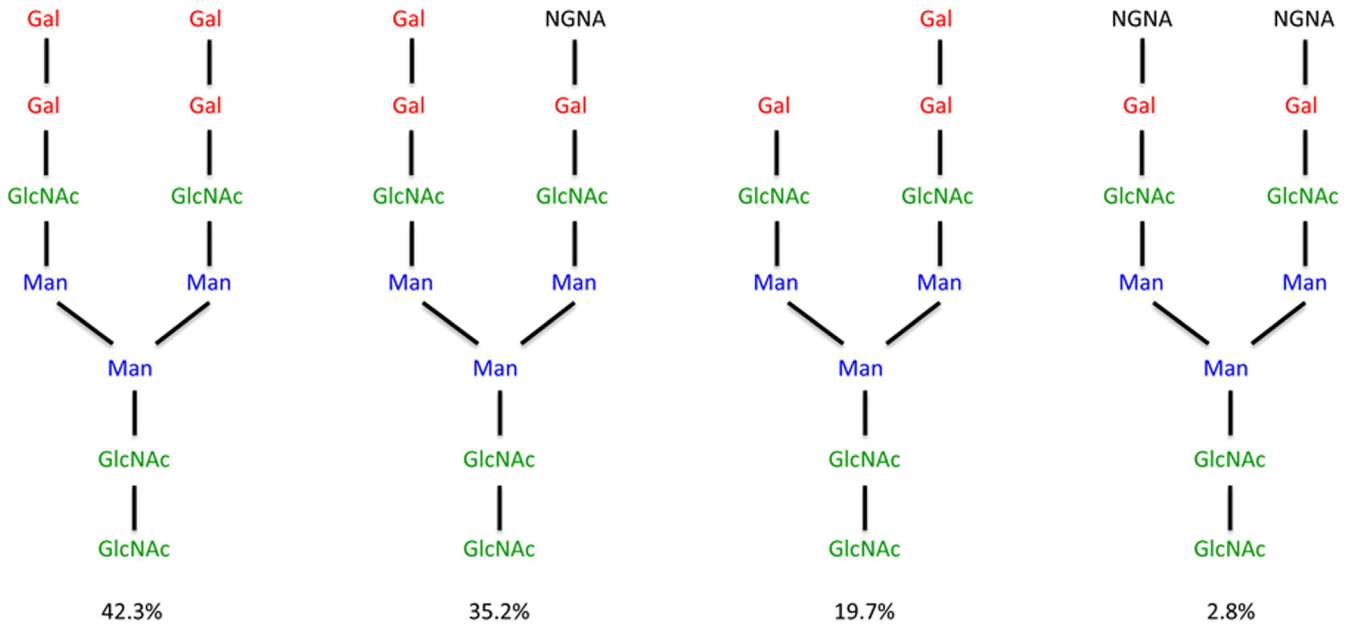

FIG 1. Carbohydrate structure of N-linked glycans on the Fab portion of cetuximab, as determined by using mass spectrometry. Alpha-gal *(red)*, mannose *(blue)*, N-acetylglucosamine *(green)*, and N-glycolyl neuraminic acid *(black)* are shown. Percentages represent the proportion of the indicated structures found on the Fab region of cetuximab. Adapted from Qian et al.[11]

collected 10 cases of delayed reactions to mammalian meat and made a connection with the occurrence of tick bites several weeks or months before the first episode of hives or anaphylaxis. They presented these findings to the Georgia Allergy Society and to the US Centers for Disease Control and Prevention in 1991, but no additional reports or statements were issued by either of these organizations.

The characteristics of red meat allergy are different from typical allergic reactions. Common complaints include both gastrointestinal symptoms and urticaria, but unlike most allergic reactions, patients do not have any symptoms for at least 2 hours after eating red meat, whereas many reactions are delayed for 3 to 5 hours or even longer. Nonetheless, symptoms can be severe or even life-threatening. Many of the patients described nausea, diarrhea, or indigestion before a reaction; however, the most common symptom reported was itching. The presence of symptoms before a severe reaction is common but not a requirement. Many patients do not have any symptoms, and symptoms do not occur with every exposure to red meat even in those who have had them previously. All of the patients had consumed red meat without complications for many years before the onset of the syndrome. Although some patients had a prior history of allergy, most of them had no previous allergic symptoms, and thus an atopic disposition does not appear to predispose patients to this kind of IgE response.

Three observations led us to investigate whether IgE antibodies to alpha-gal were present in the sera of adult patients reporting reactions to beef. Alpha-gal is known to be present on both tissues and meat from nonprimate mammals,[15] the antibodies causing reactions to cetuximab were directed against alpha-gal, and the geographic distribution of the reactions to cetuximab overlapped the same geographic area where the red meat–induced reactions were occurring. Not surprisingly, the patients' sera had positive results for IgE to beef, pork, lamb, cat, and dog but not to nonmammalian meat, such as turkey, fish, or chicken.[14,16] The presence of alpha-gal was confirmed by using 2 different absorption assays, one with alpha-gal on human serum albumin and the other with mammalian or beef thyroglobulin, which is heavily decorated with alpha-gal. The glycosylated antigens were bound to sepharose beads.[14] In each case, levels of the specific IgE binding to beef, pork, lamb, cat, and dog were reduced by greater than 75%. More recently, evidence has been obtained from a study examining beef extracts using 2-diemnsional gel electrophoresis. The authors demonstrated 7 alpha-gal–containing IgE-binding proteins, 4 of which survived heating the beef extract.[17]

How alpha-gal is structurally expressed on red meat remains unclear. Also unclear is whether differences in the structure exist and whether these differences affect IgE binding. The terminal carbohydrate residue on red meat is likely alpha-gal based on the binding of IgE from sera of patients with red meat allergy to cetuximab, which, as discussed, also has terminal alpha-gal residues. However, one can envision a difference in carbohydrate structure, such that only a single exposed alpha-gal–binding site is present in the oligosaccharide chain on meat, contrasting the 2 found predominately on cetuximab. The majority of alpha-gal found on cetuximab has a dianternary structure (Fig 1). The structure on meat has not been determined. Whether having 2 alpha-gal residues on the terminus of the carbohydrate structure has an effect on the strength of IgE binding is unknown.

ARE TICK BITES RESPONSIBLE FOR THE INDUCTION OF IgE ANTIBODY TO ALPHA-GAL?

In 2008, as the specificity of the IgE antibodies to alpha-gal that caused reactions to cetuximab became clearer, the number of reports describing delayed reactions to red meat was also increasing. A relationship between mammalian meat allergy and tick bites had already been suggested in Australia[18]; however, the role of alpha-gal was not known, and the tick connection was

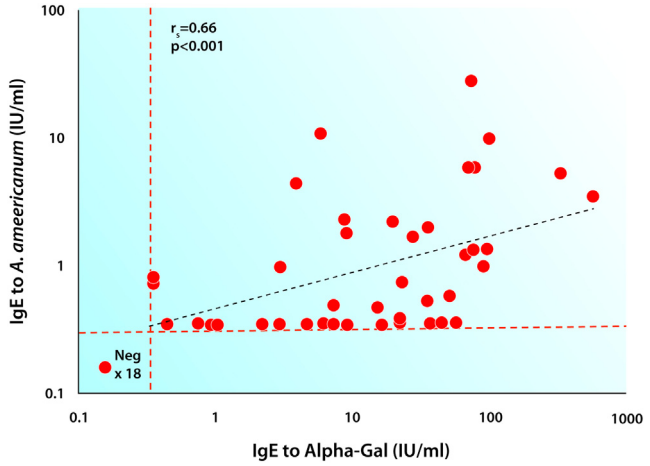

FIG 2. Relationship of IgE to alpha-gal with IgE to *Amblyomma americanum* (lone star tick). Levels of specific IgE to alpha-gal and *A americanum* were measured by using ImmunoCAP, and the correlation between the 2 types of IgE was determined by using Spearman correlation (r_s = 0.66, P < .001).

not yet obvious in the United States. What caught our attention was that both cetuximab reactions and delayed reactions to red meat were being reported from the same region of the country, a group of southeastern states. However, it was not clear why these cases were geographically localized, and the only area that was comparable was the maximum incidence of Rocky Mountain spotted fever (RMSF).

At this time, red meat allergy developed in 3 members of our group, and each one distinctly remembered being bitten by ticks weeks or months before the development of symptoms. Sera from these persons that had been obtained before the tick bite were compared with sera collected after the bite, and it was found that serum levels of IgE to alpha-gal had increased dramatically (4- to 10-fold).

Following up on this connection, we started to ask patients about tick bites and rapidly became aware that most of those with delayed anaphylaxis had experienced recent bites from adult or larval ticks. Examination of US Centers for Disease Control and Prevention maps of the distribution of the tick *Amblyomma americanum* (lone star tick) revealed an overlap with the region of both cetuximab sensitivity and red meat allergy. Additional indications that tick bites are involved in the development of specific IgE to alpha-gal include histories of bites that have itched for 2 or more weeks, a significant correlation between IgE antibodies to alpha-gal and IgE to lone star tick (Fig 2), and prospective data on the increase in IgE levels to alpha-gal after known lone star tick bites.[19] Allergy to red meat is now being reported in other countries, but the ticks giving rise to this response are not the same species as in the United States. In Europe *Ixodes ricinus* has been implicated, whereas in Australia the relevant tick is *Ixodes holocyclus*.[18,20,21] It appears that *Ixodes scapularis*, the main vector of Lyme disease (*Borrelia burgdorferi* infection) in the United States, does not induce IgE to alpha-gal, and unlike bites of the lone star tick, bites of *I scapularis* that transmit Lyme disease are not associated with itching.[22]

Given that tick bites represent the most important cause of alpha-gal sensitization in the United States, Sydney, and Stockholm, why has our recognition of this problem increased so dramatically over the past 10 years? The increase in lone star ticks parallels the increase in the deer population, a major carrier of these ticks, throughout the United States over the last 30 to 40 years,[23,24] making it more likely that persons who walk in the woods or in long grass will be bitten at some point. The increasing deer population can also be linked to the enactment of leash laws for dogs, a decrease in the number of hunters, and movement of deer into suburban areas. This last point is important because the deer provide a means for the ticks to be transported over large geographic areas quickly. Clearly, the increase in tick exposure is one plausible explanation for the increase in the number of cases. However, the data from different countries demonstrate that not all tick bites *per se* or tick bites from one particular species result in the problem (Table II).

The epidemiologic evidence in the United States would suggest that the increase in the deer population has played an important role. However, it is important to remember that there are at least 3 theories about how tick bites give rise to an IgE response:

1. the response is induced by the normal (ie, tick-derived) constituents of their saliva;
2. residual mammalian glycoproteins or glycolipids are present in the tick from a previous blood meal and they are responsible for inducing the response to alpha-gal; and
3. the response is induced by another organism that is present in the tick.

The best recognized organisms present as commensals on ticks are *Rickettsia* species, such as those that cause RMSF, or bacteria, such as *B burgdorferi*, which is not found in the lone star tick *(A americanum)*. Other organisms are possible, but none have been recognized.

It might be thought that the IgE response seen with seed ticks would argue against either residual mammalian proteins or other organisms; however, transovarial transmission of RMSF is well recognized. Sorting out these possibilities is the subject of ongoing investigation.

A BROADER UNDERSTANDING OF ALPHA-GAL

The early reports of alpha-gal sensitivity were mostly from adults, with very few reports of affected children. However, children often have urticaria, angioedema, or recurrent anaphylaxis for which the cause is unknown. We identified 51 children aged 4 to 17 years with symptoms consistent with possible delayed allergic reactions to mammalian foods and measured IgE levels to alpha-gal in their sera. Serum IgE levels to alpha-gal were high in 45 of the subjects, and there was a strong correlation with beef IgE levels, as previously observed in adults.[16] When questioned, these children gave a history of symptoms 3 to 6 hours after ingestion of meat, and many could recall recent tick bites. The geographic distribution of affected children matches that of adults, namely the southeastern United States.

For protein allergens, there is a strong correlation between atopic sensitivity and asthma.[25-27] It is unknown whether this same relationship exists when an oligosaccharide is the target of the IgE response. Three populations were examined: one with high levels of IgE to alpha-gal and anaphylaxis, angioedema, or acute urticaria after ingestion of red meat; another of persons admitted to the emergency department for an acute asthma

TABLE I. Time course of the alpha-gal story

Year: Events leading to our understanding of red meat allergy
~2000: At least 2 groups reported cases of meat allergy that started after tick bites.
2003: IgE to cat allergens is common in an African village but not related to symptoms.
2005: There are reports of hypersensitivity reactions to first infusion of cetuximab in clinical trials.
2007: Severe reactions to cetuximab are common in Tennessee, North Carolina, Arkansas, Missouri, and Virginia.
2007: Two cases in Virginia of adult-onset delayed anaphylaxis occurring 3 to 6 hours after eating beef are reported.
2008: Alpha-gal is identified as the epitope on cetuximab.
2009: Twenty-four cases of delayed anaphylaxis to red meat are found in the United States.
Multiple cases of meat allergy after tick bites are reported in Sydney, Australia.
2010: There is a range of evidence that ticks are responsible for the IgE response in the United States.
2011: There is extensive evidence that the IgE response is not related to asthma, despite cross-reactions with dog and cat.
2014: Open challenge tests confirm the delay in reactions to red meat.

exacerbation; and a cohort of children in Kenya who showed sensitization to cat despite limited cat exposure. These studies involved extensive investigation of lung function, exhaled nitric oxide levels, histories of asthma symptoms, and serum assay results for IgE antibodies to alpha-gal. Taken together, those studies showed no association between sensitization to alpha-gal and asthma.[28] One caveat is that persons develop this syndrome differently than the typical areoallergen sensitivity because alpha-gal does not appear to be airborne, and it might be that if given enough time after the onset of symptoms, these persons would have asthma. This would either require a prospective study following patients and seeing how their lung function changes over time when they have a high alpha-gal IgE titer or a retrospective study many years from now after patients have had the disease for years comparing the new lung function with lung function before development of disease.

Previously, we studied an asthmatic cohort from Sweden and found a strong correlation between atopic sensitization to cat allergens and asthma.[29] However, when we examined a population from rural Kenya, we saw high-titer IgE to cat allergens but no association with asthma or atopic disease. What we did not understand was why the level of sensitization to cat was so high in Kenya while exposure to cats was low. A clue to that riddle was provided when we found that patients in the United States with delayed anaphylaxis to red meat had positive skin prick test responses and serum assay results to cat. Further investigation revealed that the alpha-gal–positive patients also had positive results to cat epithelium extracts but not to Fel d 1.[28] On re-examination of the Swedish and Kenyan cohorts, it was discovered that for Sweden, there was a strong correlation between IgE levels to Fel d 1 and IgE levels to cat dander, whereas in the Kenyan population there was no correlation.[28] Instead, there was a strong correlation between IgE levels to cat dander and IgE levels to alpha-gal, thus explaining the apparent high levels of serum IgE to cat we had observed in the Kenyan cohort. Care must be taken when interpreting skin test or serum results in patients who present with symptoms of urticaria, angioedema, and idiopathic anaphylaxis.

TABLE II. Ticks that commonly bite human subjects in countries where IgE to alpha-gal has been reported

	Tick	Outcome
United States	*Ixodes scapularis* (deer tick)	Lyme disease
	Dermacentor variabilis (dog tick)	RMSF
	Amblyomma americanum (lone star tick)	IgE to alpha-gal, ehrlichiosis, and RMSF
Australia	*Ixodes holocyclus*	IgE to alpha-gal and/or tick bite–induced anaphylaxis
Europe	*Ixodes ricinus*	IgE to alpha-gal and Lyme disease
	Argas reflexus (pigeon tick)	Anaphylactic reactions to tick bite

In Zimbabwe Dr Elopi Sibanda, working with colleagues in Austria and Sweden, identified a group of patients who had IgE to cat allergens, which was explained by IgE to alpha-gal.[30] In that report they focused on the potential for IgE to alpha-gal to produce "false-positive" or confusing results for cat allergy. However, equally interesting was the observation that these patients did not report allergic reactions to red meat. In fact, given the evidence that many children and adults in Africa have IgE to alpha-gal, there are remarkably few reports of delayed or other allergic reactions to meat on that continent. Whether this reflects (1) a difference in IgE response, (2) some aspect of the fat content of meat or digestion of meat, or (3) a difference in the response of mast cells is not clear.

MECHANISMS OF ANAPHYLAXIS

Currently, it is our belief that the initial step in sensitization to the oligosaccharide alpha-gal is through tick bites. The patients have IgE antibodies to this hapten that is present on all nonprimate mammalian food products. This is comparable with the sensitization that occurs to inhaled plant oligosaccharides, such as MUXF3, a hapten on the glycoproteins of many plant species.[31-33] Unlike alpha-gal, IgE antibodies to these plant-derived cross-reactive carbohydrate determinants have not been shown to contribute to symptoms related to pollen exposure.[31,34] Patients with IgE to alpha-gal typically report symptoms beginning 3 to 5 hours after eating meat. Despite detailed and aggressive questioning, the patients do not recognize any oral or gastrointestinal symptoms less than 2 hours after eating a meal. Similarly, in challenge studies with pork, hives and other symptoms are delayed at least 2 hours after meat ingestion.[35] This is different than the reactions to cetuximab that develop rapidly, in which symptoms often peak within 20 minutes of initial administration of the drug.[1-3] This rapid time frame is similar to the *in vitro* responses of basophils after activation with glycoproteins, such as beef thyroglobulin or cetuximab, which can be detected within 25 minutes. Skin test responses to cetuximab, beef extract, pork sausage, or beef thyroglobulin are also rapid. Thus the delay in response after eating meat does not reflect a delayed response or inability of basophils or mast cells to be activated by these glycoproteins. The obvious explanation is that the oligosaccharide is absorbed from the gut in a form that enters the circulation slowly. Given that alpha-gal is present on both glycoproteins and glycolipids (including chylomicrons), it is our belief that the most

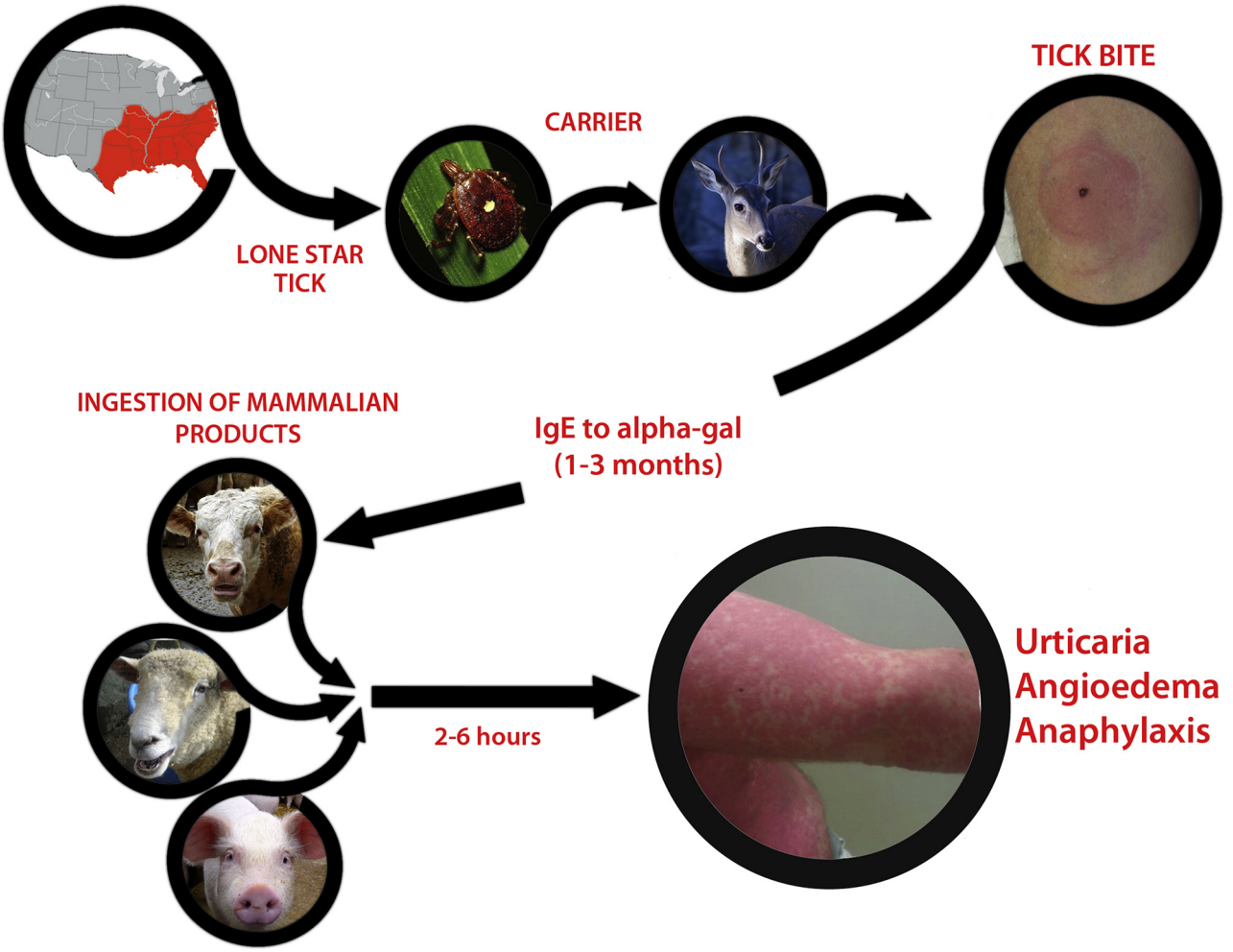

FIG 3. Summary of alpha-gal sensitization leading to clinical symptoms of red meat allergy. The southeastern section of the United States is where most of the reactions to red meat have been reported. This region overlaps with the distribution of the lone star tick. The current hypothesis is that persons are bitten by lone star ticks carried by deer into rural and urban areas. After a period of time, IgE to alpha-gal develops. Once IgE to alpha-gal reaches sufficient levels, ingestion of red meat can trigger reactions. Several of the images used in this figure are licensed under a Creative Commons CC BY-NC 2.0 (Attribution-NonCommercial 2.0 Generic) license (Cow: https://flic.kr/p/adgjhp by user Plashing Vole; Deer: https://flic.kr/p/jeZwq7 by user Cherry Bream; Sheep: https://flic.kr/p/4WirD by user Lauren; Tick: https://flic.kr/p/cdnNaY by user Katja Schulz; Pig: https://flic.kr/p/N7gpc by user Anne).

likely explanation for the delay in symptoms is due to a delay in the appearance of the antigen in the circulation. Because chylomicrons enter the circulation through the thoracic duct after a several-hour process of absorption, repackaging, and transit, mediator release triggered by the accumulated metabolic products (eg, very low-density lipoprotein [VLDL] or low-density lipoprotein [LDL]) might account for the now documented delay. Our studies have shown that during a challenge, circulating basophils assessed *ex vivo* upregulate the expression of CD63 in a time frame similar to that of patients having symptoms.[35] Surprisingly, a proportion of nonallergic control subjects also demonstrated upregulation of CD63, although they do not experience any symptoms. Evidence that basophils and mast cells have receptors for LDL was reported many years ago.[36,37] We postulate that the likely explanation for this enigmatic finding is that although VLDL or LDL can cause basophils to upregulate CD63, the quantity of histamine released in nonallergic control subjects is not sufficient to cause symptoms. The implication is that LDL particles with alpha-gal on the surface can cause mast cell mediator release but only in subjects with IgE antibodies to alpha-gal. In keeping with this model, 3 of the challenge patients but none of the control subjects had tryptase in their circulation after challenge.[35]

In the United States, delayed allergic reactions are almost uniformly related to eating beef, pork, or lamb, with a minority of cases reporting reactions to milk or cheese. However, in most cases the reactions have followed consumption of more than 100 g of mammalian meat. By contrast, in Europe it is normal to eat meat and organs from a much wider range of mammals. This includes not only horse, goat, and rabbit but also liver, heart, tripe (intestine), or kidney. Two separate groups have reported that reactions to pork kidney can be both severe and more rapid (ie, 2 hours rather than 4 hours).[38,39] In addition, there are increasing anecdotal reports from both the United States and

Europe that drinking alcohol at the same time as eating red meat or kidney can increase the probability of a reaction.

NATURE OF ALPHA-GAL IgE DEVELOPMENT

Although our data and those of others support the theory that bites from ectoparasitic ticks initiate the development of an IgE response to alpha-gal in human subjects, the mechanistic aspects of this response have not yet been elucidated. There is already extensive evidence that (1) IgE antibody responses can occur outside mature germinal centers,[40,41] (2) that the switch to IgE can occur in B cells locally in the nose,[42] and (3) that antibody responses to oligosaccharides can be or normally are relatively T-cell independent.[43,44] Thus there is a real possibility that the IgE response to alpha-gal involves switching that occurs outside germinal centers, and it is possible that the skin is the site of such a switch. It will be important to establish the extent of rearrangement in the complementarity-determining region, which could provide additional clues regarding the antigen or antigens involved.[45] Previously, it has been documented that persons bitten by the pigeon tick *(Argas reflexus)* can have specific IgE to extracts made from the whole tick body.[46] From preliminary experiments, we have noted that in subjects with IgE to alpha-gal, there appears to be proliferation of a subset of plasmablasts in response to tick extract that was not present in control subjects. In keeping with the observed decreases in IgE levels and reactivity to alpha-gal over time in patients who avoid further tick bites,[14] the formation of plasmablasts could occur in this setting without the development of long-lived plasma cells. Overall, the alpha-gal IgE response has some features resembling an IgM response to an oligosaccharide. Certainly, understanding why exposure to one antigen leads to a long-lived IgE response when another exposure does not would be an important and potentially therapeutically manipulable insight.

CONCLUSION

The finding that IgE to alpha-gal explains 2 novel forms of anaphylaxis has not only changed several established rules about allergic disease but has also opened up at least 2 new areas of research. The results provide evidence that (1) IgE responses to an oligosaccharide can induce significant or severe allergic symptoms, (2) demonstration of sensitization to this epitope by skin tests often requires both intradermal and skin prick tests, (3) ticks can induce high-titer food-specific IgE responses in adult life, and (4) eating mammalian products carrying this epitope does not give rise to any symptoms during the first hour or more. Like so many new findings, this area of research provides both challenges and opportunities. The delay in onset of symptoms after eating red meat is best explained by delayed arrival of the relevant form of antigen in the circulation, but the question remains as to what form of glycoprotein or, more likely, glycolipid takes 3 hours or more to appear in the circulation.

Finally, the often-rapid production of IgE antibodies to alpha-gal after tick bites provides a striking model of a parasite-induced IgE response (Fig 3). This parasite only enters through the skin, and the tick saliva contains a wide variety of agents that could act as antigens, adjuvants, or both. However, it remains a striking challenge to identify why the response is so strong and why it is directed so consistently against the alpha-gal carbohydrate residue.

What do we know?

- **IgE antibodies specific for the mammalian oligosaccharide alpha-gal are common in a large area of the southeastern United States.**
- **These IgE antibodies are causally associated with 2 novel forms of allergic reactions: (1) anaphylaxis or urticaria during the first infusion of cetuximab and (2) urticaria, angioedema, or anaphylaxis starting 3 to 5 hours after eating red meat.**
- **These IgE antibodies in the United States are caused predominantly, if not exclusively, by bites of larval or adult lone star ticks.**

What is still unknown?

- **Although deer are the major vector for the relevant ticks in both the United States and Sweden, the increase in deer populations might not be the only or the major cause for an increase in the disease.**
- **Can the IgE response to tick bites be explained simply by the normal contents of tick saliva, or is it possible that some other symbiotic organism, such as a new *Rickettsia* species, is involved?**
- **Although the best explanation for the delayed food-induced response to red meat is that it relates to the absorption of lipid particles, it is not clear what form of particle carrying glycolipids or glycoproteins is present in the circulation after 3 to 5 hours.**

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