

Journal Pre-proof

On the cause and consequences of IgE to galactose- α -1,3-galactose: A Report from the National Institute of Allergy and Infectious Disease Workshop on Understanding IgE-Mediated Mammalian Meat Allergy

Thomas A.E. Platts-Mills, MD, PhD, FRS, Scott P. Commins, MD, PhD, Tilo Biedermann, MD, Marianne van Hage, MD, PhD, Michael Levin, MD, PhD, Lisa A. Beck, MD, Maria Diuk-Wasser, PhD, Uta Jappe, MD, MSc, Danijela Apostolovic, PhD, Michael Minnicozzi, PhD, Marshall Plaut, MD, Jeffrey M. Wilson, MD, PhD

PII: S0091-6749(20)30190-1

DOI: <https://doi.org/10.1016/j.jaci.2020.01.047>

Reference: YMAI 14415

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 26 November 2019

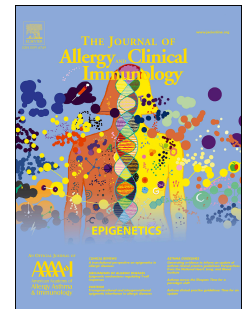
Revised Date: 29 January 2020

Accepted Date: 31 January 2020

Please cite this article as: Platts-Mills TAE, Commins SP, Biedermann T, van Hage M, Levin M, Beck LA, Diuk-Wasser M, Jappe U, Apostolovic D, Minnicozzi M, Plaut M, Wilson JM, On the cause and consequences of IgE to galactose- α -1,3-galactose: A Report from the National Institute of Allergy and Infectious Disease Workshop on Understanding IgE-Mediated Mammalian Meat Allergy, *Journal of Allergy and Clinical Immunology* (2020), doi: <https://doi.org/10.1016/j.jaci.2020.01.047>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology.



On the cause and consequences of IgE to galactose- α -1,3-galactose: A Report from the National Institute of Allergy and Infectious Disease Workshop on Understanding IgE-Mediated Mammalian Meat Allergy

Thomas A.E. Platts-Mills, MD, PhD, FRS¹, Scott P. Commins, MD, PhD², Tilo Biedermann, MD³, Marianne van Hage, MD, PhD⁴, Michael Levin, MD, PhD⁵, Lisa A. Beck, MD⁶, Maria Diuk-Wasser, PhD⁷, Uta Jappe, MD, MSc^{8*}, Danijela Apostolovic, PhD⁴, Michael Minnicozzi, PhD⁹, Marshall Plaut⁹, MD and Jeffrey M. Wilson, MD, PhD¹

1 Division of Allergy & Clinical Immunology, University of Virginia, Charlottesville, VA; 2 Departments of Medicine & Pediatrics, University of North Carolina, Chapel Hill, North Carolina; 3 Department of Dermatology and Allergy, Technical University of Munich and Unit Clinical Allergology (EKA), Helmholtz Zentrum München, Munich, Germany; 4 Division of Immunology and Allergy, Department of Medicine Solna, Karolinska Institutet and University Hospital, Stockholm, Sweden; 5 Division of Paediatric Allergy, University of Cape Town, Cape Town, South Africa; 6 Department of Dermatology, University of Rochester Medical Center, Rochester, NY; 7 Department of Ecology, Evolution, and Environmental Biology, Columbia University, New York, NY; 8 Division of Clinical and Molecular Allergology, Research Center Borstel, Airway Research, Center North (ARCN), Member of the German Center for Lung Research, Borstel, Germany; Interdisciplinary Allergy Outpatient Clinic, Department of Pneumology, University of Luebeck, Germany; 9 Division of Allergy, Immunology and Transplantation, Allergy, Asthma and Airway Biology Branch, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD

*Dr. Jappe could not attend the workshop but contributed to the manuscript

Correspondence to:

Thomas A.E. Platts-Mills, MD, PhD, FRS: Division of Allergy & Clinical Immunology, University of Virginia, P.O. Box 801355, Charlottesville, VA, 22908-1355, USA
Tel: (434) 924-5917; Fax: (434) 924-5779; E-mail: tap2z@virginia.edu

Disclosures/grant Support: T. Platts-Mills was supported by NIH grant R37-AI-20565, has received assay support from Thermo-Fisher/Phadia and has a patent on an IgE assay to α -Gal; S. Commins was supported by NIH grants R01AI135049, R56AI113095, K08AI85190, CDC grant IPA1908943, and has been on the speaker's bureau at Genentech and received author royalties from Up-to-Date; M. van Hage reports grants from H2020 FoodEnTwin GA No 810752, grants from Swedish Research Council, The Stockholm County Council (ALF project), The Swedish Asthma and Allergy Association's Research Foundation, The King Gustaf V 80th Birthday Foundation, The Swedish Heart-Lung Foundation, The Hesselman Foundation, The Swedish Cancer and Allergy Foundation, and the Swedish Association for Allergology, personal fees from Biomay AG, Vienna, Austria and Hycor Biomedical LLC, CA, US, personal fees from Thermo Fisher Scientific and ALK, outside the submitted work; L Beck is a consultant or Advisory Board Member for Abbvie, Allakos, Arena Pharma, Astra-Zeneca, Connect Biopharma, LEO Pharma, Lilly, Novan, Novartis, Pfizer, Regeneron, Sanofi, UCB and Vimalan, is or has recently been an investigator for an Abbvie, LEO Pharma, Pfizer and/or Regeneron clinical trial, and owns stock in Pfizer and Medtronic; D. Apostolovic reports grants from H2020 FoodEnTwin GA No 810752 and from Konsul Th C Berg Foundation, outside the submitted work; The rest of the authors declare they have no relevant conflicts of interest.

ABSTRACT

The mammalian meat allergy known as the 'α-Gal syndrome' relates to IgE specific for galactose-α-1,3-galactose (α-Gal), an oligosaccharide which is present in cells and tissues of non-primate mammals. The recognition of delayed reactions to food derived from mammals in patients with IgE to α-Gal and also the association with tick bites has been increasing world-wide. In 2018, the National Institute of Allergy and Infectious Diseases, Division of Allergy, Immunology and Transplantation, sponsored a workshop on this emerging tick-related disease. International experts from the fields of tick biology, allergy, immunology, infectious disease and dermatology discussed the current state of our understanding of this emerging medical condition. The participants provided suggestions for specific research priorities and for the development of resources to advance our knowledge of the mechanisms, diagnosis, management and prevention of this allergic disease. This publication is a summary of the workshop and the panel's recommendations are presented herein.

Keywords

α-Gal; anaphylaxis; IgE; oligosaccharide allergen; glycolipids; mammalian meat allergy; ticks

Abbreviations

AGS – α -Gal syndrome

α -Gal – galactose alpha-1,3-galactose

BSA – bovine serum albumin

CDC – Centers for Disease Control and Prevention

IgA – immunoglobulin A

IgE – immunoglobulin E

IgG – immunoglobulin G

LDL – low-density lipoprotein

MMR – measles, mumps and rubella vaccine

NIAID - National Institute of Allergy and Infectious Diseases

RMSF – Rocky Mountain spotted fever

SFG - spotted fever-group rickettsiosis

INTRODUCTION

Recognition of the oligosaccharide galactose- α -1,3-galactose (α -Gal) as an important target for IgE antibodies came from the investigation of the often severe and occasionally fatal reactions during the first infusion of the monoclonal antibody cetuximab¹. Those studies established that the IgE antibodies present in the patients prior to their first exposure were specific for the α -Gal glycan present on the Fab portion of cetuximab (**Fig 1A and B**). The investigation was greatly helped by ImClone who had developed cetuximab. The company published the full glycosylation pattern of the monoclonal and also provided the molecule from an alternative cell line which did not express α -Gal². In addition, Bristol-Myers Squibb facilitated collaboration with the oncology group at Vanderbilt who provided the pretreatment sera and control sera from central Tennessee. Once the assay for IgE to α -Gal was available it became clear that there were patients in allergy clinics in Charlottesville, VA and also in Springfield, MO who reported delayed reactions to mammalian meat and who had IgE specific for the same oligosaccharide³. Over the first year it became clear that there were also cases in North Carolina, Tennessee, and Arkansas. This not only matched the distribution of cases of reactions to cetuximab but also resembled the map of the maximum density of Rocky Mountain Spotted Fever (RMSF) published by the CDC⁴. This provided a clue that this form of sensitization might be related to tick bites. Interestingly, two earlier abstracts had reported cases of mammalian meat allergy that were associated with preceding tick bites. The first was five cases in Athens, GA reported to the Georgia Allergy Society in 1989 by Anthony Deutsch (personal communication). The second was reported by Sheryl van Nunen to the Sydney Allergy Society in 2006 on patients who had been bitten by ticks in the North Shore of Sydney⁵. Dr. van Nunen published her cases in 2009 and related them to the tick *Ixodes holocyclus*⁶. Interestingly, investigators in Sweden had made another early observation that was relevant to the unfolding story. Marianne van Hage's group reported in 2007 that some patients in Stockholm had IgE antibodies specific for an oligosaccharide on cat IgA⁷, which was subsequently shown to be α -Gal⁸. Finally, Uta Jappe in Germany had been investigating allergic reactions to the consumption of pork-kidney as early as 2005, which in subsequent work with Tilo Biedermann were confirmed to be related to IgE to α -Gal⁹⁻¹¹. Thus, the discovery that α -Gal was an epitope for IgE on mammalian glycoproteins connected a series of previous observations that had not been recognized as related. It is important to remember that this glycan is also the 'B-like antigen' that Karl Landsteiner recognized on the red cells of mammals nearly a century ago, that it can also be present on glycolipids and that it is a major xenotransplantation barrier (**Fig 1A and C**)¹²⁻¹⁴.

In June of 2018 a workshop was convened in Bethesda, MD under the auspices of the National Institute of Allergy and Infectious Diseases (NIAID). This workshop was designed to evaluate the evidence that had been published since 2008, and in particular to lay out a background and plan for future research into the causes and consequences of IgE-mediated mammalian meat allergy and the contributions of α -Gal sensitization. The primary areas discussed included:

- i) Review of the clinical syndrome in North America and comparison with the European experience and emerging work from South Africa.

- ii) Current understanding of the mechanisms by which tick bites can induce IgE responses to this particular oligosaccharide.
- iii) Factors relevant to the severity and symptoms of allergic reactions in patients who are sensitized to α -Gal as well as the mechanism and significance of the delay in clinical responses after eating mammalian meat.

Review of Clinical Syndromes Related to α -Gal specific IgE

As an oligosaccharide of non-primate mammals and New World monkeys, α -Gal can be present on glycoproteins and glycolipids of a multitude of products derived from mammals. This can include skeletal muscle, organs, milk, and gelatin, but also other products such as biological drugs and vaccines that are prepared with mammalian cells or constituents¹⁵. As such the term ' α -Gal syndrome' (AGS) is often used to describe allergic reactions to mammalian meat and other α -Gal-containing products derived from mammals^{9,16}. In patients with an appropriate clinical history the main method of diagnosis is the measurement of IgE to α -Gal. Several α -Gal-bearing glycoproteins have been used on the solid-phase of the assay, but the two most widely reported have been beef thyroglobulin and cetuximab. Importantly, the performance characteristics of the assay has been similar with both glycoproteins^{3,17,18}. It is also important to highlight that not all subjects who are sensitized to α -Gal report allergic reactions to mammalian meat. For example, among 300 hunters and forest workers in Southwest Germany the prevalence of IgE to α -Gal was 19.3%, but among the 58 subjects who were positive (cut-off 0.35 IU/mL) only 5 had allergic symptoms to mammalian meat or innards¹⁹. Thus, as with other allergic diseases a good clinical history plays an important role in diagnosis of the syndrome.

Anaphylaxis during treatment with cetuximab

These reactions which occur during the first infusion of the drug can be very severe, rapid and clearly relate to pre-existing IgE antibodies to α -Gal^{1,2}. Screening patients with an IgE assay could reduce the risk of reactions, however severe reactions to cetuximab can also occur in subjects who are not sensitized to α -Gal. The predictive value of the test is expected to vary in different patient populations, but the α -Gal IgE test has shown a sensitivity of 75-92% and a specificity of 90-92%^{1,20}. To date, use of an IgE assay specific for α -Gal has not been recommended as a screening procedure for use of this drug²⁰. There have been several consequences following the initial description of the syndrome. The most important is the increased awareness of the possible relevance of glycosylation on recombinant molecules to allergic or other reactions during treatment, including consideration given to the cell line used for generation of the molecules²¹⁻²³. In keeping with that, many recently developed monoclonal antibodies have the α -Gal glycosylation site on the Fab region engineered out. A related question is whether the immune response to tick bites can induce significant IgE to other relevant oligosaccharides²⁴. To date, limited studies in subjects who are positive for IgE to α -Gal (and had histories of tick bites) have not revealed significant IgE to other oligosaccharides^{25, 26}.

Allergic reactions to gelatin-containing vaccines and other mammalian-related pharmaceuticals

Recent evidence suggests that some patients can react to vaccines which are prepared in culture systems or with excipients that are derived from non-primate mammals. For example, Stone et al. reported a case with a history of delayed anaphylaxis to mammalian meat who experienced anaphylaxis upon receipt of Zostavax²⁷. In a subsequent report the same group described a similar pediatric case who reacted to the measles, mumps and rubella vaccine (MMR)²⁸. In both cases the patient's serum had IgE that bound to preparations of the relevant vaccine and, importantly, the *in vitro* activity was lost when α -Gal-specific IgE was depleted. Further investigation suggested that gelatin in the vaccine preparation was likely the source of the α -Gal. Taken together, individuals with histories of severe reactions to mammalian meat or gelatin could be good candidates for allergy consultation and consideration to graded administration of relevant vaccines. However, it is important to realize that many individuals who are sensitized to α -Gal have tolerated gelatin-containing vaccines (which in the USA currently includes MMR, Zostavax, Yellow Fever, Rabies, oral Typhoid and FluMist)^{29, 30}. It is possible that measuring IgE to gelatin could be useful for stratifying risk to gelatin-containing vaccines but we are unaware of studies that have addressed this.

In addition to vaccines there are other pharmaceutical products and devices that have the potential to contain α -Gal epitopes. For example, Fischer et al. and others have reported on a putative link between α -Gal IgE and immediate reactions to anti-venom^{31, 32}. As addressed in a recent review there have also been case reports relating AGS to heparin, magnesium stearate, pancreatic enzyme replacement (e.g. pancrelipase), gelatin-containing vaginal capsules and porcine-derived heart valves^{15, 33-38}. Further research is needed to determine if these products carry α -Gal, either consistently or sometimes, and whether the concentration is sufficient to cause clinical reactions.

Reactions related to meat and other mammalian products which are ingested

There are multiple surprising features of AGS but the two most important are that it can develop at any age and, in contrast to the immediate reactions to infused cetuximab, there is almost always some delay after ingestion before symptoms start. In a recent analysis of 261 adults and children presenting for evaluation of allergic symptoms to mammalian meat in central Virginia, 248 cases consistent with AGS were identified³⁹. Patients' age ranged from 5-82 years and 84% reported symptoms beginning at least two hours after ingestion of mammalian meat (median 240 minutes). This delay in symptom onset, which has also been reported from Europe⁴⁰, was consistent with the results of challenge studies that Commins et al. reported in 2014 on a similar population where all patients had a delay of at least 150 minutes⁴¹. The extent of delay has not been unanimous across all studies, however. Mabelane *et al*, described a median onset of symptoms of 100 minutes in challenges conducted in a cohort in rural South Africa⁴². In this cohort the likely cause of the sensitization was locally prevalent ticks, but the study was also notable for a large proportion of children who reacted to challenge. Tilo Biedermann's group in Germany reported similar kinetics when using pork kidney for the challenges¹⁰. The explanation for the differences in timing between the studies could relate to the specific patient populations investigated or the type of product used for challenge (i.e. beef muscle vs pork kidney). Regardless, we would highlight that most reactions occurred >1 hour after ingestion in all the aforementioned studies (**Fig 2A**). The

allergic reactions have not differed by age, with children as likely as adults to report urticaria, gastrointestinal distress and/or anaphylaxis with an equally delayed response^{39, 42, 43}.

Most α -Gal meat allergy reactions reported in the literature focus on the presentation of delayed pruritus, urticaria, angioedema and anaphylaxis³. More recently, and at the Workshop, researchers have described reactions that manifest with abdominal pain – both in conjunction with skin reactions or as isolated gastrointestinal reactions. For example, Marianne van Hage reported that 74% of the 128 participants in her Swedish cohort reported gastrointestinal symptoms (unpublished data), so did 64% in the Virginia cohort³⁹. Michael Levin and colleagues reported a large case series of challenge-proven α -Gal allergy with high prevalence of isolated abdominal reactions (approximately 20%); surprisingly, the time to onset of symptoms did not differ between individuals with isolated abdominal symptoms compared to those with skin manifestations and there were no differences in α -Gal IgE levels between the groups⁴². Anecdotally, there are patients in whom the initial symptoms were restricted to the gastrointestinal tract but over a period of weeks or months, in some cases following additional tick bites, the symptomatology expanded to include the skin and/or anaphylaxis. It is, therefore, possible that isolated abdominal pain is an under-reported and underdiagnosed feature of AGS⁴⁴.

Patients with α -Gal food allergy report marked intra-individual variability in the dose that causes a reaction⁴⁵. Many describe mild or no reactions with some exposures, yet have severe symptoms on repeat exposure to the same food served in a similar amount and preparation. This variation in susceptibility, at least in part, appears to be modulated by co-factors⁴⁶. In keeping with many other forms of food allergy, the most prominent co-factors associated with α -Gal consumption reactions are exercise, alcohol and non-steroidal anti-inflammatory medications^{10, 45, 47}. Theoretically, exposure to these co-factors may enhance absorption of the antigen, may decrease the threshold of response, and/or contribute to the severity of the allergic responses to α -Gal⁴⁸. Another possible explanation for the variability is that the amount of α -Gal varies in different foods. Unfortunately this is a topic that has not been systematically examined, however α -Gal content appears to be particularly high in foods derived from internal organs⁴⁹. In addition, consumption of lipid-laden mammalian meat has also been associated with more consistently severe hypersensitivity responses in α -Gal allergic subjects⁵⁰. There are also marked inter-individual differences in susceptibility to α -Gal. This runs the gamut from patients who are sensitized to α -Gal but are consistently asymptomatic when ingesting mammalian meat to those who react to minor quantities of α -Gal present in milk and gelatin-containing medications and sweets^{19, 51, 52}. For example, α -Gal patients with concomitant mastocytosis, can react to very small amounts of pork kidney (3g)⁵³.

What Explains the Delay in Symptom Onset after Ingestion of Mammalian Meat?

The observations about the symptom delay from patient reports and challenge studies is reinforced by a study of basophils collected from patients who underwent mammalian meat challenge. In this study, in which the basophils did not receive any *ex vivo* stimulation, basophils collected at early time points did not exhibit appreciable activation but the activation marker CD63 increased significantly over baseline in blood that was drawn between 3 and 5 hours after

challenge (**Fig 2B**)⁴¹. Understanding the mechanism that explains the delay remains an important objective. Differences in timing following the ingestion of mammalian meat and organs, as reported by Tilo Biedermann's group, suggests that differences in the quantity or "quality" of α -Gal present in different tissues could be an important element in explaining the delay¹⁰. An important qualitative consideration is that α -Gal can be present on not only glycoproteins, but also glycolipids. It is well established that α -Gal bearing glycosphingolipids are an important source of the glycan in non-primate mammalian cells, tissues and organs (including kidneys)⁵⁴⁻⁵⁶. The argument for this 'glycolipid hypothesis' revolves around the fact that the digestion, absorption and processing of lipids occurs with kinetics very similar to the delay in symptom onset⁴⁸. Lipid processing relies on transit via chylomicrons through the thoracic duct before arriving into the systemic circulation at the left subclavian vein. The lipid cargo subsequently transitions to progressively smaller lipoprotein particles, including low-density lipoprotein (LDL) particles, which are sufficiently small to passively filter through the vasculature into the interstitium (i.e. ~ 20 nM)⁵⁷. Studies with radiolabeled lipids have demonstrated that the peak lipid level at the thoracic duct occurs 4 hours after a meal, and the peak level in tissue (e.g. muscle) 5 hours after the meal⁵⁸. This kinetic pattern corresponds with the clinical experience and the result of the *ex vivo* basophil testing. Recent studies using an *in vitro* Caco-2 gut barrier model have provided additional support for this hypothesis as beef-derived α -Gal glycolipids, but not glycoproteins, passed through the epithelial cells and were packaged into chylomicrons⁵⁹.

Relevance Beyond Traditional Allergic Disease?

In addition to the isolated gastrointestinal symptoms which were previously discussed, preliminary evidence reported by Wilson et al. suggests that α -Gal sensitization could be a risk factor for coronary artery disease⁶⁰. Among a population of 118 adults in Virginia who underwent coronary catheterization at the discretion of their cardiologist, 26% had measurable IgE to α -Gal (cut-off 0.1 IU/mL). Using highly sensitive intravascular ultrasound imaging, the sensitized group was found to have a greater burden of atherosclerotic plaques and their plaques had high-risk features, as assessed by the extent of atheroma calcification and necrosis (**Fig 3**). Importantly, the association was significant in multiple variable regression analyses accounting for traditional cardiovascular risk factors. Although a connection between allergic disease and atherosclerosis may not be obvious to most physicians, there is already good evidence in the cardiology literature for a link between both mast cells and IgE with atherosclerosis development and severity^{61, 62}. Cardiac anaphylaxis, commonly known as Kounis syndrome, further supports a link between allergic disease and coronary artery disease^{63, 64}. In thinking about a putative mechanism it may also be relevant to consider that α -Gal can exist as a glycolipid antigen. Although dietary and allergic history of the study participants in the report by Wilson et al. was not available, it is expected that many and possibly the majority of study participants were asymptomatic and, thus, in all likelihood were continuing to consume mammalian products. This inference is based on our understanding of differences in the prevalence of α -Gal sensitization in the community (and high-risk populations) as compared to the

prevalence of subjects who report symptoms to mammalian meat¹⁹. The association between α -Gal IgE and coronary artery disease does not offer evidence of causality and requires confirmation in larger populations.⁶⁵

The Tick Connection: What controls the distribution of cases of the α -Gal syndrome and how is it likely to change?

In the USA, cases of immediate reactions to cetuximab and delayed anaphylaxis to mammalian meat have been strikingly regional, and it was the overlap of those cases with the CDC map of cases of Rocky Mountain Spotted Fever (RMSF) that helped establish the initial tick connection. Ten years later, it is now clear that the CDC map is actually most consistent with a family of spotted fever-group rickettsiosis (SFG), and not with cases of *bona fide* RMSF⁶⁶. In keeping with this, the tick *Amblyomma americanum*, commonly known as the lone star tick, rarely carries *Rickettsia rickettsii* (the cause of RMSF), but commonly carries a non-pathogenic or minimally pathogenic SFG called *Rickettsia amblyommatis*⁶⁷⁻⁷⁰. The abundance of α -Gal cases in VA, NC, TN, AR, OK and MO, and also the eastern tip of Long Island, NY are highly consistent with the area where *Amblyomma americanum* is endemic^{71, 72}. The connection between α -Gal sensitization and tick bites is further supported by a number of experimental studies and also the observation that subjects with AGS who avoid recurrent tick bites, but not those who report ongoing tick bite(s), experience a decline in their α -Gal IgE titers⁷³⁻⁷⁵. In Sweden AGS cases are also regional and match the distribution of *Ixodes ricinus*⁴⁰. In particular, cases are not reported in or north of Umeå. In France, Germany and Spain cases have been identified in wide areas and may relate primarily to outdoor activity¹⁹. In these areas the evidence also relates to *Ixodes ricinus*⁹. In Australia the cases of delayed anaphylaxis to mammalian meat are restricted to a region north and south of Sydney extending as far inland as Canberra, an area which is the primary distribution of the tick *Ixodes holocyclus*⁷⁶. Formal studies characterizing the incidence and prevalence of AGS have been lacking, but it is clear from reports in the US and Europe that sensitization to α -Gal can reach 10-20% of the population in some regions^{4, 19, 77}. Notably, these levels of community sensitization (i.e., 10—20%) only occur in areas where ticks are common and are much higher than the prevalence found in areas where ticks are rare.¹

Ongoing efforts to determine the distribution of AGS in the USA have generally confirmed that cases are most common in areas where *Amblyomma americanum* (but not *Ixodes scapularis*, the primary vector for Lyme disease) is established, nonetheless unpublished evidence presented at the workshop by Thomas Platts-Mills has suggested two anomalies (**See Fig 4A and B**)⁷⁸. First is the presence of a cluster of over 20 cases reported in northern Minnesota. This is interesting because *Amblyomma americanum* is not known or expected to be established in this region because of climatic conditions^{79, 80}. These cases suggest that North American ticks beside *Amblyomma americanum* could be relevant to α -Gal sensitization and/or that agents other than ticks could contribute to α -Gal sensitization⁸¹. A second anomaly is the lack of AGS cases in the Gulf Coast and Texas, an area where the CDC reports that *Amblyomma americanum* is established^{79, 80}. This finding, while preliminary, suggests that lone star tick populations could be decreasing in abundance in the Gulf Coast and Texas. There are some predictions that such a pattern could be expected as part of

future climate change, but it is not clear this has been a factor to date⁷⁹. Dr. Platts-Mills suggested an alternative possibility, which relates to the steady expansion of an established tick predator in the Gulf Coast region - the imported fire ant⁸². In support of this idea, evidence was presented for a negative correlation between cases of fire ant anaphylaxis and cases of the AGS in the Deep South. Relatedly, during the workshop, there was discussion about a connection between rising tick populations and increasing numbers of white-tailed deer in urban and suburban environments. Indeed, the significance of this increase in deer populations in relation to tick-borne zoonoses associated with the lone star tick was described as 'ecological havoc' by Paddock and Yabsley in 2007⁸³.

How do bites from ticks of many species induce IgE to α -Gal ?

Evidence has been published relating mammalian meat allergy to bites of *Amblyomma americanum*⁴, *Ixodes ricinus*^{19, 73}, *Ixodes holocyclus*,⁶ *Haemophysalis longicornis*⁸⁴ and most recently *Amblyomma testudinarium*⁸⁵. In each case, there was a dominant IgE response specific for galactose- α -1,3-galactose. However, the evidence does not exclude other ticks. Most importantly, we do not know the mechanism whereby a tick bite can lead to induction of IgE specific to α -Gal. Speaking to the question of why only certain ticks have been associated with α -Gal IgE sensitization Maria Diuk-Wasser discussed the possibility that differences in tick habitat, questing behavior and/or anatomy could be relevant. She also highlighted the unexplained observation that although *Ixodes ricinus* is the dominant cause of sensitization to α -Gal in Europe, its phylogenetic North American relative *Ixodes scapularis* is almost certainly not a major cause of sensitization in the USA.

In regards to mechanism, tick saliva is suspected to play an important role, which is consistent with the remarkable complexity of tick saliva and the view that ticks represent venomous ectoparasites⁸⁶⁻⁸⁸. Despite the fact that α -Gal has been identified in the midgut and saliva of several species of hard ticks, the source of the α -Gal remains uncertain. A possibility is that ticks can intrinsically produce the oligosaccharide, but this has been questioned because the enzyme that catalyzes the generation of α -Gal in vertebrates (α 1,3-galactosyltransferase) has not been identified in ticks⁸⁹. Two alternative possibilities include: i) α -Gal could be present in ticks because of a prior blood meal on a non-primate mammal, or ii) Rickettsiales or other symbionts present in tick saliva could express α -Gal and be transferred to a human host during a tick bite. Evidence for these competing hypotheses are detailed in **Table 1**, though notably emerging evidence increasingly suggests that ticks can intrinsically generate the α -Gal glycan⁸⁹⁻⁹². Crispell et al. reported that α -Gal was not detectable in the salivary glands of unfed adult *Amblyomma americanum*, however the α -Gal glycan was detectable after the ticks were fed using an artificial feeding model where a silicone membrane covered human blood⁹¹. Furthermore, Apostolovic et al have reported that larval ticks contain α -Gal-carrying proteins and these larvae were not fed on the host, which supports that α -Gal-carrying proteins originate from ticks⁹⁰.

Another important question that was the focus of comments by Lisa Beck relates to why tick bites are so effective at inducing IgE responses in humans and whether the skin plays an important role in facilitating this response. For example, we recognize that the skin is an important route for IgE responses to parasites, venomous insects and also to foods such

as peanut⁹³⁻⁹⁵. High-titer IgE is also a hallmark of atopic dermatitis⁹⁶. Interestingly, however, in the experience of the panel, AGS does not appear to be over-represented among subjects with atopic dermatitis. This suggests that intrinsic barrier defects in the skin are not a prerequisite for the syndrome. Perhaps this is not surprising given that tick ‘mouthparts’ can penetrate through the epidermis and generate a local inflammatory response in the dermis⁹⁷. Nonetheless, the skin may represent a tissue microenvironment that strongly favors the development of IgE (and type-2 immunity generally), a position that is supported by recent work in mice which demonstrated that GATA-3, a ‘master’ regulator of type-2 immunity, is particularly abundant in effector and regulatory T cells of the skin⁹⁸. The idea that IgE responses to tick depend on presentation via the skin is also supported by a recent report which showed that subcutaneous but not intraperitoneal inoculation of tick extracts elicited a robust specific IgE response⁹⁹.

Animal models and investigations of the tick-host interface and host immune response

Despite increased awareness, the mechanism of several aspects of mammalian meat allergy remain unknown. The link between tick bites and the development of IgE to α -Gal is strong, though notably the IgE response does not develop in all individuals bitten by ticks^{4, 75, 85, 100, 101}. To investigate the mechanisms driving IgE responses to α -Gal as well as the delayed reaction, investigators recognize the need for an animal model of mammalian meat allergy. Barriers to a mouse model include the fact that mice naturally remove ticks and also that mice express α -Gal (hence they do not naturally make anti-Gal antibodies). To overcome the first obstacle, Susan Little and Brian Herrin at Oklahoma State University developed a small chamber to allow prolonged exposure and tick feeding of mice. In multiple experiments, *Amblyomma americanum* ticks feeding on CH3/HeN mice for 10-14 days induced a significant increase in total IgE compared to control mice. The magnitude of the IgE response appeared to be dose-dependent as mice with greater numbers of ticks which were attached and feeding had higher total IgE levels. While these experiments demonstrated the feasibility of using mice to examine the IgE response to tick bites, the inherent variability in dosing associated with live tick feedings has led to the development of tick-independent methods for delivery of tick-associated factors to mice. The second barrier has been addressed with the use of α -Gal ‘knock-out mice’, in which the gene that encodes for α 1,3-galactosyltransferase has been genetically modified. An initial report by Araujo et al. demonstrated that subcutaneous inoculation of saliva from *Amblyomma sculptum*, or direct feeding of the same ticks, induced an IgE response in α -Gal deficient mice that bound α -Gal epitopes presented on virus-like particles⁷⁴. Similarly, Scott Commins presented studies at the workshop that used a model where salivary gland extract from partially fed *Amblyomma americanum* were repeatedly inoculated into a different transgenic model of α -Gal deficient mice. These mice produced an α -Gal directed IgE response and also demonstrated systemic allergic reactions, as measured by a decrease in body temperature, following injection of α -Gal containing antigen (cetuximab) or gavage with pork^{102, 103}. As stated above, a recent paper by Chandrasekhar and colleagues provides additional evidence that the skin plays an important role in sensitization to tick proteins⁹⁹. Subcutaneous inoculation of *Amblyomma americanum* larval extracts (whole body), but not intraperitoneal injection of the same extracts, elicited a robust tick-specific IgE response in wild-type mice⁹⁹. The same investigators also

looked at α -Gal specific IgE responses using the α -Gal knock-out mice. Cutaneous sensitization using a tick extract which was modified to include α -Gal-BSA led to an induction of IgE specific to α -Gal as well as an increase in the frequency of activated basophils following beef thyroglobulin challenge⁹⁹.

It is anticipated that further development and investigation using mouse models will help identify specific constituents of the tick 'sialome' that act as adjuvants to promote IgE and type 2 immune responses⁸⁶. Putative candidates within the tick that could have this activity include prostaglandins, phospholipases, and lipocalins^{86, 87, 104, 105}. Animal models will also be helpful to characterize the nature of the host immune response that contributes to the generation of IgE to α -Gal. There are reasons to think that basophils may play an important role in promoting IgE induction to α -Gal. For several decades it has been known that mammalian hosts can develop acquired immunity that protects against subsequent tick infestation, and it is becoming increasingly clear that basophils, as well as IgE, play an important role in this process¹⁰⁶⁻¹⁰⁸. In an area of Japan where *Amblyomma testudinarium* is common, a recent publication demonstrated that IgE to α -Gal was common in subjects with recurrent tick bites and that the number of basophils at the site of tick penetration increased with repeated tick exposures⁸⁵. Tilo Biedermann reported that in his cohort in Germany he and his colleagues have also observed elevated levels of basophils at the site of tick bites. Interestingly, his group has also recently reported that urticaria at the site of a prior tick bite can be an early sign of an allergic reaction in individuals with AGS¹⁰⁹. While basophils are not expected to remain in the skin long after a tick exposure, this finding of 'recall urticaria' suggests the presence of long-lived IgE antibodies bound to mast cells at the site of a prior bite.

The role of B cells in the AGS is another area that would benefit from further research. It has long been appreciated that 'natural' anti- α -Gal antibodies of the IgM, IgG and IgA isotype are abundant in immunocompetent humans¹¹⁰⁻¹¹³. In fact, Galili has estimated that up to ~1% of the IgG repertoire (and B cells in circulation) were specific for α -Gal¹⁴. The source of α -Gal specific natural antibodies in mouse models has been reported to be B-1b B cells, although in humans the lineage of the B cell is less clear^{114, 115}. An interesting possibility, and one that could distinguish the allergic response to α -Gal from the response to traditional environmental allergens, is that induction of α -Gal IgE could involve class switch of existing memory B cells rather than a *de novo* humoral immune response. It is anticipated that advanced tools such as polychromatic flow cytometry and deep sequencing of antibody VDJ will provide useful insights into this question.

Another question is whether the IgE response to α -Gal requires T cell help, or whether the class-switch occurs independent of T cells¹¹⁶. Because the α -Gal epitope is a non-zwitterion carbohydrate, it is unlikely that it can directly participate in MHC II priming of CD4 T cells. Nonetheless, there are reports from animal models that suggest that antibody responses to α -Gal depend on CD4+ T cell help^{117, 118}. This is in keeping with the fact that the immune response to α -Gal that occurs in subjects with mammalian meat allergy has some Th2-related features in addition to IgE class-switch. For example, IgG1 specific to α -Gal is consistently elevated in AGS subjects. IgG4 specific to α -Gal has not been consistently reported but this could relate to the fact that the exposure which causes α -Gal sensitization (i.e., tick bites) is usually an intermittent and not a chronic event¹¹⁹⁻¹²². Marianne van Hage presented evidence that dendritic cell

processing and presentation of α -Gal bearing glycoproteins was delayed compared to non-glycosylated proteins in an *in vitro* model, a finding that could also have implications for T cell activation¹²³. An interesting possibility is that the glycolipid form of α -Gal could be present in ticks and be recognized as an antigen by non-canonical T cells, such as NKT cells. There is evidence that α -Gal-bearing lipids can be presented by CD1d on antigen-presenting cells, that blockade of CD1d impairs anti- α -Gal antibody formation, and that invariant NKT cells are present in higher numbers in subjects with AGS than in healthy controls¹²⁴⁻¹²⁶.

An additional area of interest which is relevant to understanding the host response relates to the observation that subjects who have ABO blood group B (i.e., have B or AB blood) may be partially protected from developing the syndrome^{39, 100, 121, 127}. This is likely explained by mechanisms governing tolerance: i) the B-antigen and α -Gal are structurally similar (see Fig 1), ii) subjects with A and O blood groups have antibodies which recognize both the B-antigen and α -Gal, and iii) subjects with group B blood have lower titers of antibodies which recognize α -Gal than subjects with group A or O blood^{39, 121, 128}.

Workshop Recommendations

It has now been ten years since the initial publications describing the oligosaccharide galactose- α -1,3-galactose as an IgE-binding epitope and a causal antigen in anaphylaxis to mammalian products^{1,3}. The ' α -Gal syndrome' can involve immediate reactions to drugs which are delivered intravascularly, but characteristically manifests with a delay of 3-6 hours upon oral ingestion of mammalian products such as meat, organs and/or dairy¹²⁹. Anti- α -Gal IgM, IgG and IgA, which arguably represent natural antibodies, are produced by all immunocompetent humans, but it is increasingly clear that bites from certain species of hard ticks are the dominant cause of IgE sensitization to α -Gal. The NIAID Workshop on Understanding IgE-Mediated Mammalian Meat Allergy recommends that future research focuses on the following topics:

- **Epidemiologic and observational research to**

- o determine the prevalence of α -Gal IgE sensitization in the general population and its association with i) mammalian meat allergy, ii) other allergic reactions (e.g. vaccine reactions), iii) other medical conditions (e.g. atherosclerotic disease)
- o confirm the association between α -Gal IgE sensitization and tick bites and identify specific tick species that can induce sensitization and environmental conditions that modify the sensitization process
- o describe the natural history of α -Gal IgE sensitization and determine whether early symptomatology can be identified that develops over time into more severe, anaphylactic reactions

- **Pathophysiologic research using human samples and animal models to**

- o understand the mechanism(s) of the delayed allergic reactions to α -Gal and the factors that modify the time interval between oral ingestion and reaction

- o determine the nature of the host immune response that contributes to the induction of α -Gal specific IgE
- o identify the source of the α -Gal in tick saliva
- o identify possible contributions of other tick-associated factors that may act as Type-2 adjuvants
- o investigate efficacy of biologics in mouse models

Although there are many ways that α -Gal could be considered to 'break the rules', investigation into this unusual allergen is likely to reveal novel insights into the causes and consequences of all allergic diseases. It is hoped that these proceedings can be used as a guide for research proposals that will move this field forward.

REFERENCES

1. Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *N Engl J Med*. 2008;358(11):1109-17.
2. Qian J, Liu T, Yang L, Daus A, Crowley R, Zhou Q. Structural characterization of N-linked oligosaccharides on monoclonal antibody cetuximab by the combination of orthogonal matrix-assisted laser desorption/ionization hybrid quadrupole-quadrupole time-of-flight tandem mass spectrometry and sequential enzymatic digestion. *Anal Biochem*. 2007;364(1):8-18.
3. Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD, et al. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose-alpha-1,3-galactose. *J Allergy Clin Immunol*. 2009;123(2):426-33.
4. 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-alpha-1,3-galactose. *J Allergy Clin Immunol*. 2011;127(5):1286-93 e6.
5. van Nunen S, O'Connor K, Fernando SL, Clarke LR, Boyle RX. The Association Between Ixodes holocyclus Tick Bite Reactions and Red Meat Allergy. *Internal Medicine Journal* 2007; 39 (Suppl5): A132. *Internal Medicine Journal*. 2007;39 (Suppl5):A132.
6. Van Nunen SA, O'Connor KS, Clarke LR, Boyle RX, Fernando SL. An association between tick bite reactions and red meat allergy in humans. *Med J Aust*. 2009;190(9):510-1.
7. Adedoyin J, Gronlund H, Oman H, Johansson SG, van Hage M. Cat IgA, representative of new carbohydrate cross-reactive allergens. *J Allergy Clin Immunol*. 2007;119(3):640-5.
8. Gronlund H, Adedoyin J, Commins SP, Platts-Mills TA, van Hage M. The carbohydrate galactose-alpha-1,3-galactose is a major IgE-binding epitope on cat IgA. *J Allergy Clin Immunol*. 2009;123(5):1189-91.
9. Fischer J, Yazdi AS, Biedermann T. Clinical spectrum of alpha-Gal syndrome: from immediate-type to delayed immediate-type reactions to mammalian innards and meat. *Allergo J Int*. 2016;25:55-62.
10. Fischer J, Hebsaker J, Caponetto P, Platts-Mills TA, Biedermann T. Galactose-alpha-1,3-galactose sensitization is a prerequisite for pork-kidney allergy and cofactor-related mammalian meat anaphylaxis. *J Allergy Clin Immunol*. 2014;134(3):755-9 e1.
11. Jappe U, Kreft B, Ludwig A, Przybilla B, Walker A, Becker W, et al. Allergy to red meat/offal is associated with IgE to a new mammalian cross-reactive carbohydrate determinant also found in cetuximab and cat IgA. *Allergy*. 2010;65:164-5.
12. Landsteiner K, Miller CP. Serological Studies on the Blood of the Primates : lii. Distribution of Serological Factors Related to Human Isoagglutinogens in the Blood of Lower Monkeys. *J Exp Med*. 1925;42(6):863-72.
13. Galili U. The alpha-gal epitope and the anti-Gal antibody in xenotransplantation and in cancer immunotherapy. *Immunol Cell Biol*. 2005;83(6):674-86.
14. Galili U. Anti-Gal: an abundant human natural antibody of multiple pathogeneses and clinical benefits. *Immunology*. 2013;140(1):1-11.
15. Platts-Mills TA, Li RC, Keshavarz B, Smith AR, Wilson JM. Diagnosis and management of patients with the alpha-Gal syndrome. *J Allergy Clin Immunol Pract*. 2019.
16. Platts-Mills TA, Commins SP. Emerging antigens involved in allergic responses. *Curr Opin Immunol*. 2013;25(6):769-74.
17. Jappe U, Minge S, Kreft B, Ludwig A, Przybilla B, Walker A, et al. Meat allergy associated with galactosyl-alpha-(1,3)-galactose (alpha-Gal)-Closing diagnostic gaps by anti-alpha-Gal IgE immune profiling. *Allergy*. 2018;73(1):93-105.
18. Wilson JM, Platts-Mills TAE. Meat allergy and allergens. *Mol Immunol*. 2018;100:107-12.
19. Fischer J, Lupberger E, Hebsaker J, Blumenstock G, Aichinger E, Yazdi AS, et al. Prevalence of type I sensitization to alpha-gal in forest service employees and hunters. *Allergy*. 2017;72(10):1540-7.
20. Maier S, Chung CH, Morse M, Platts-Mills T, Townes L, Mukhopadhyay P, et al. A retrospective analysis of cross-reacting cetuximab IgE antibody and its association with severe infusion reactions. *Cancer Med*. 2015;4(1):36-42.
21. Borrebaeck CK, Malmberg AC, Ohlin M. Does endogenous glycosylation prevent the use of mouse monoclonal antibodies as cancer therapeutics? *Immunol Today*. 1993;14(10):477-9.

22. Sheeley DM, Merrill BM, Taylor LC. Characterization of monoclonal antibody glycosylation: comparison of expression systems and identification of terminal alpha-linked galactose. *Anal Biochem.* 1997;247(1):102-10.
23. Lammerts van Bueren JJ, Rispens T, Verploegen S, van der Palen-Merkus T, Stapel S, Workman LJ, et al. Anti-galactose-alpha-1,3-galactose IgE from allergic patients does not bind alpha-galactosylated glycans on intact therapeutic antibody Fc domains. *Nat Biotechnol.* 2011;29(7):574-6.
24. Homann A, Schramm G, Jappe U. Glycans and glycan-specific IgE in clinical and molecular allergology: Sensitization, diagnostics, and clinical symptoms. *J Allergy Clin Immunol.* 2017;140(2):356-68.
25. Amoah AS, Asuming-Brempong EK, Obeng BB, Versteeg SA, Larbi IA, Aryeetey Y, et al. Identification of dominant anti-glycan IgE responses in school children by glycan microarray. *J Allergy Clin Immunol.* 2018;141(3):1130-3.
26. Apostolovic D, Tran TA, Sanchez-Vidaurre S, Cirkovic Velickovic T, Starkhammar M, Hamsten C, et al. Red meat allergic patients have a selective IgE response to the alpha-Gal glycan. *Allergy.* 2015;70(11):1497-500.
27. Stone CA, Jr., Hemler JA, Commings SP, Schuyler AJ, Phillips EJ, Peebles RS, Jr., et al. Anaphylaxis after zoster vaccine: Implicating alpha-gal allergy as a possible mechanism. *J Allergy Clin Immunol.* 2017;139(5):1710-3 e2.
28. Stone CA, Jr., Commings SP, Choudhary S, Vethody C, Heavrin JL, Wingerter J, et al. Anaphylaxis after vaccination in a pediatric patient: further implicating alpha-gal allergy. *J Allergy Clin Immunol Pract.* 2019;7(1):322-4 e2.
29. Pinson ML, Waibel KH. Safe administration of a gelatin-containing vaccine in an adult with galactose-alpha-1,3-galactose allergy. *Vaccine.* 2015;33(10):1231-2.
30. Retterer MKC, Workman LJ, Bacon JR, Platts-Mills TAE. Specific IgE to gelatin as a cause of anaphylaxis to zoster vaccine. *J Allergy Clin Immunol.* 2018;141(5):1956-7.
31. Fischer J, Eberlein B, Hilger C, Eyer F, Eyerich S, Ollert M, et al. Alpha-gal is a possible target of IgE-mediated reactivity to antivenom. *Allergy.* 2017;72(5):764-71.
32. Rizer J, Brill K, Charlton N, King J. Acute hypersensitivity reaction to Crotalidae polyvalent immune Fab (CroFab) as initial presentation of galactose-alpha-1,3-galactose (alpha-gal) allergy. *Clin Toxicol (Phila).* 2017;55(7):668-9.
33. Sell-Dottin K, Sola M, Caranasos T. Impact of Newly Emerging Alpha-Gal Allergies on Cardiac Surgery: A Case Series. *Clinics in Surgery.* 2017;2.
34. Muglia C, Kar I, Gong M, Hermes-DeSantis ER, Monteleone C. Anaphylaxis to medications containing meat byproducts in an alpha-gal sensitized individual. *J Allergy Clin Immunol Pract.* 2015;3(5):796-7.
35. Swiontek K, Morisset M, Codreanu-Morel F, Fischer J, Mehlich J, Darsow U, et al. Drugs of porcine origin-A risk for patients with alpha-gal syndrome? *J Allergy Clin Immunol Pract.* 2019;7(5):1687-90 e3.
36. Vidal C, Mendez-Brea P, Lopez-Freire S, Gonzalez-Vidal T. Vaginal Capsules: An Unsuspected Probable Source of Exposure to alpha-Gal. *J Investig Allergol Clin Immunol.* 2016;26(6):388-9.
37. Mozzicato SM, Tripathi A, Posthumus JB, Platts-Mills TAE, Commings SP. Porcine or bovine valve replacement in 3 patients with IgE antibodies to the mammalian oligosaccharide galactose-alpha-1,3-galactose. *J Allergy Clin Immunol Pract.* 2014;2(5):637-8.
38. Hawkins RB, Frischtak HL, Kron IL, Ghanta RK. Premature Bioprosthetic Aortic Valve Degeneration Associated with Allergy to Galactose-Alpha-1,3-Galactose. *J Card Surg.* 2016;31(7):446-8.
39. Wilson JM, Schuyler AJ, Workman L, Gupta M, James HR, Posthumus J, et al. Investigation into the alpha-Gal Syndrome: Characteristics of 261 Children and Adults Reporting Red Meat Allergy. *J Allergy Clin Immunol Pract.* 2019;7(7):2348-58 e4.
40. Hamsten C, Tran TA, Starkhammar M, Brauner A, Commings SP, Platts-Mills TA, et al. Red meat allergy in Sweden: association with tick sensitization and B-negative blood groups. *J Allergy Clin Immunol.* 2013;132(6):1431-4.
41. Commings SP, James HR, Stevens W, Pochan SL, Land MH, King C, et al. Delayed clinical and ex vivo response to mammalian meat in patients with IgE to galactose-alpha-1,3-galactose. *J Allergy Clin Immunol.* 2014;134(1):108-+.
42. Mabelane T, Basera W, Botha M, Thomas HF, Ramjith J, Levin ME. Predictive values of alpha-gal IgE levels and alpha-gal IgE: Total IgE ratio and oral food challenge-proven meat allergy in a population with a high prevalence of reported red meat allergy. *Pediatr Allergy Immunol.* 2018;29(8):841-9.
43. Kennedy JL, Stallings AP, Platts-Mills TA, Oliveira WM, Workman L, James HR, et al. Galactose-alpha-1,3-galactose and delayed anaphylaxis, angioedema, and urticaria in children. *Pediatrics.* 2013;131(5):e1545-52.
44. Levin M, Apostolovic D, Biedermann T, Commings SP, Iweala OI, Platts-Mills TAE, et al. Alpha-gal phenotypes-lessons from various patient populations. *Ann Allergy Asthma Immunol.* 2019.

45. Commins SP. Invited Commentary: Alpha-Gal Allergy: Tip of the Iceberg to a Pivotal Immune Response. *Curr Allergy Asthma Rep.* 2016;16(9):61.
46. Wolbing F, Fischer J, Koberle M, Kaesler S, Biedermann T. About the role and underlying mechanisms of cofactors in anaphylaxis. *Allergy.* 2013;68(9):1085-92.
47. Commins SP, James HR, Stevens W, Pochan SL, Land MH, King C, et al. Delayed clinical and ex vivo response to mammalian meat in patients with IgE to galactose-alpha-1,3-galactose. *J Allergy Clin Immunol.* 2014;134(1):108-15.
48. Wilson JM, Platts-Mills TAE. The oligosaccharide galactose- α -1,3-galactose and the α -Gal syndrome: insights from an epitope that is causal in IgE-mediated immediate and delayed anaphylaxis. *EMJ Allergy Immunol.* 2018;3(1):89-98.
49. Morisset M, Richard C, Astier C, Jacquenet S, Croizier A, Beaudouin E, et al. Anaphylaxis to pork kidney is related to IgE antibodies specific for galactose-alpha-1,3-galactose. *Allergy.* 2012;67(5):699-704.
50. Steinke JW, Pochan SL, James HR, Platts-Mills TAE, Commins SP. Altered metabolic profile in patients with IgE to galactose-alpha-1,3-galactose following in vivo food challenge. *J Allergy Clin Immunol.* 2016;138(5):1465-7 e8.
51. Mullins RJ, James H, Platts-Mills TA, Commins S. Relationship between red meat allergy and sensitization to gelatin and galactose-alpha-1,3-galactose. *J Allergy Clin Immunol.* 2012;129(5):1334-42 e1.
52. Caponetto P, Fischer J, Biedermann T. Gelatin-containing sweets can elicit anaphylaxis in a patient with sensitization to galactose-alpha-1,3-galactose. *J Allergy Clin Immunol Pract.* 2013;1(3):302-3.
53. Roenneberg S, Bohner A, Brockow K, Arnold A, Darsow U, Eberlein B, et al. alpha-Gal-a new clue for anaphylaxis in mastocytosis. *J Allergy Clin Immunol Pract.* 2016;4(3):531-2.
54. Galili U, Basbaum CB, Shohet SB, Buehler J, Macher BA. Identification of erythrocyte Gal alpha 1-3Gal glycosphingolipids with a mouse monoclonal antibody, Gal-13. *J Biol Chem.* 1987;262(10):4683-8.
55. Hendricks SP, He P, Stults CL, Macher BA. Regulation of the expression of Gal alpha 1-3Gal beta 1-4GlcNAc glycosphingolipids in kidney. *J Biol Chem.* 1990;265(29):17621-6.
56. Diswall M, Angstrom J, Karlsson H, Phelps CJ, Ayares D, Teneberg S, et al. Structural characterization of alpha1,3-galactosyltransferase knockout pig heart and kidney glycolipids and their reactivity with human and baboon antibodies. *Xenotransplantation.* 2010;17(1):48-60.
57. Michel CC, Nanjee MN, Olszewski WL, Miller NE. LDL and HDL transfer rates across peripheral microvascular endothelium agree with those predicted for passive ultrafiltration in humans. *J Lipid Res.* 2015;56(1):122-8.
58. Labbe SM, Grenier-Larouche T, Croteau E, Normand-Lauziere F, Frisch F, Ouellet R, et al. Organ-specific dietary fatty acid uptake in humans using positron emission tomography coupled to computed tomography. *Am J Physiol Endocrinol Metab.* 2011;300(3):E445-53.
59. Roman-Carrasco P, Lieder B, Somoza V, Ponce M, Szepefalusi Z, Martin D, et al. Only aGal bound to lipids, but not to proteins, is transported across enterocytes as an IgE reactive molecule that can induce effector cell activation. *Allergy.* 2019.
60. Wilson JM, Nguyen AT, Schuyler AJ, Commins SP, Taylor AM, Platts-Mills TAE, et al. IgE to the Mammalian Oligosaccharide Galactose-alpha-1,3-Galactose Is Associated With Increased Atheroma Volume and Plaques With Unstable Characteristics-Brief Report. *Arterioscler Thromb Vasc Biol.* 2018;38(7):1665-9.
61. Sun J, Sukhova GK, Wolters PJ, Yang M, Kitamoto S, Libby P, et al. Mast cells promote atherosclerosis by releasing proinflammatory cytokines. *Nat Med.* 2007;13(6):719-24.
62. Kounis NG, Hahalis G. Serum IgE levels in coronary artery disease. *Atherosclerosis.* 2016;251:498-500.
63. Kounis NG. Coronary hypersensitivity disorder: the Kounis syndrome. *Clin Ther.* 2013;35(5):563-71.
64. Abdelghany M, Subedi R, Shah S, Kozman H. Kounis syndrome: A review article on epidemiology, diagnostic findings, management and complications of allergic acute coronary syndrome. *Int J Cardiol.* 2017;232:1-4.
65. Wilson JM, McNamara CA, Platts-Mills TAE. IgE, alpha-Gal and atherosclerosis. *Aging (Albany NY).* 2019;11(7):1900-2.
66. Dahlgren FS, Paddock CD, Springer YP, Eisen RJ, Behravesh CB. Expanding Range of *Amblyomma americanum* and Simultaneous Changes in the Epidemiology of Spotted Fever Group Rickettsiosis in the United States. *Am J Trop Med Hyg.* 2016;94(1):35-42.
67. Gaines DN, Operario DJ, Stroup S, Stromdahl E, Wright C, Gaff H, et al. Ehrlichia and spotted fever group Rickettsiae surveillance in *Amblyomma americanum* in Virginia through use of a novel six-plex real-time PCR assay. *Vector Borne Zoonotic Dis.* 2014;14(5):307-16.

68. Apperson CS, Engber B, Nicholson WL, Mead DG, Engel J, Yabsley MJ, et al. Tick-borne diseases in North Carolina: is "*Rickettsia amblyommii*" a possible cause of rickettsiosis reported as Rocky Mountain spotted fever? Vector Borne Zoonotic Dis. 2008;8(5):597-606.
69. Trout Fryxell RT, Steelman CD, Szalanski AL, Billingsley PM, Williamson PC. Molecular Detection of *Rickettsia* Species Within Ticks (Acari: Ixodidae) Collected from Arkansas United States. J Med Entomol. 2015;52(3):500-8.
70. Karpathy SE, Slater KS, Goldsmith CS, Nicholson WL, Paddock CD. *Rickettsia amblyommatis* sp. nov., a spotted fever group *Rickettsia* associated with multiple species of *Amblyomma* ticks in North, Central and South America. Int J Syst Evol Microbiol. 2016;66(12):5236-43.
71. Monzon JD, Atkinson EG, Henn BM, Benach JL. Population and Evolutionary Genomics of *Amblyomma americanum*, an Expanding Arthropod Disease Vector. Genome Biol Evol. 2016;8(5):1351-60.
72. Springer YP, Eisen L, Beati L, James AM, Eisen RJ. Spatial distribution of counties in the continental United States with records of occurrence of *Amblyomma americanum* (Ixodida: Ixodidae). J Med Entomol. 2014;51(2):342-51.
73. Hamsten C, Starkhammar M, Tran TA, Johansson M, Bengtsson U, Ahlen G, et al. Identification of galactose- α -1,3-galactose in the gastrointestinal tract of the tick *Ixodes ricinus*; possible relationship with red meat allergy. Allergy. 2013;68(4):549-52.
74. Araujo RN, Franco PF, Rodrigues H, Santos LC, McKay CS, Sanhueza CA, et al. *Amblyomma sculptum* tick saliva: α -Gal identification, antibody response and possible association with red meat allergy in Brazil. Int J Parasitol. 2016;46(3):213-20.
75. Kim M, Straesser MD, Keshavarz B, Workman L, McGowan EC, Platts-Mills TAE, et al. IgE to Galactose- α -1, 3-Galactose Wanes Over Time in Patients Who Avoid Tick Bites. J Allergy Clin Immunol Pract. 2019.
76. van Nunen SA. Tick-induced allergies: mammalian meat allergy and tick anaphylaxis. Med J Aust. 2018;208(7):316-21.
77. Gonzalez-Quintela A, Dam Laursen AS, Vidal C, Skaaby T, Gude F, Linneberg A. IgE antibodies to α -gal in the general adult population: relationship with tick bites, atopy, and cat ownership. Clin Exp Allergy. 2014;44(8):1061-8.
78. Diuk-Wasser MA, Gatewood AG, Cortinas MR, Yaremych-Hamer S, Tsao J, Kitron U, et al. Spatiotemporal patterns of host-seeking *Ixodes scapularis* nymphs (Acari: Ixodidae) in the United States. J Med Entomol. 2006;43(2):166-76.
79. Springer YP, Jarnevich CS, Barnett DT, Monaghan AJ, Eisen RJ. Modeling the Present and Future Geographic Distribution of the Lone Star Tick, *Amblyomma americanum* (Ixodida: Ixodidae), in the Continental United States. Am J Trop Med Hyg. 2015;93(4):875-90.
80. Raghavan RK, Peterson AT, Cobos ME, Ganta R, Foley D. Current and Future Distribution of the Lone Star Tick, *Amblyomma americanum* (L.) (Acari: Ixodidae) in North America. PLoS One. 2019;14(1):e0209082.
81. Stoltz LP, Cristiano LM, Dowling APG, Wilson JM, Platts-Mills TAE, Traister RS. Could chiggers be contributing to the prevalence of galactose- α -1,3-galactose sensitization and mammalian meat allergy? J Allergy Clin Immunol Pract. 2019;7(2):664-6.
82. Burns EC, Melancon DG. Effect of imported fire ant (Hymenoptera: Formicidae) invasion on lone star tick (Acarina: Ixodidae) populations. J Med Entomol. 1977;14(2):247-9.
83. Paddock CD, Yabsley MJ. Ecological havoc, the rise of white-tailed deer, and the emergence of *Amblyomma americanum*-associated zoonoses in the United States. Curr Top Microbiol Immunol. 2007;315:289-324.
84. Chinuki Y, Ishiwata K, Yamaji K, Takahashi H, Morita E. *Haemaphysalis longicornis* tick bites are a possible cause of red meat allergy in Japan. Allergy. 2016;71(3):421-5.
85. Hashizume H, Fujiyama T, Umayahara T, Kageyama R, Walls AF, Satoh T. Repeated *Amblyomma testudinarium* tick bites are associated with increased galactose- α -1,3-galactose carbohydrate IgE antibody levels: A retrospective cohort study in a single institution. J Am Acad Dermatol. 2018;78(6):1135-41 e3.
86. Karim S, Ribeiro JM. An Insight into the Sialome of the Lone Star Tick, *Amblyomma americanum*, with a Glimpse on Its Time Dependent Gene Expression. PLoS One. 2015;10(7):e0131292.
87. Cabezas-Cruz A, Valdes JJ. Are ticks venomous animals? Front Zool. 2014;11:47.
88. Nuttall PA. Wonders of tick saliva. Ticks Tick Borne Dis. 2019;10(2):470-81.
89. Cabezas-Cruz A, Espinosa PJ, Alberdi P, Simo L, Valdes JJ, Mateos-Hernandez L, et al. Tick galactosyltransferases are involved in α -Gal synthesis and play a role during *Anaplasma phagocytophilum* infection and *Ixodes scapularis* tick vector development. Sci Rep. 2018;8(1):14224.

90. Apostolovic D, Mihailovic J, Commins SP, Wijnveld M, Kazimirova M, Starkhammar M, et al. Allergenomics of the tick *Ixodes ricinus* reveal important alpha-Gal-carrying IgE-binding proteins in red meat allergy. *Allergy*. 2019.
91. Crispell G, Commins SP, Archer-Hartman SA, Choudhary S, Dharmarajan G, Azadi P, et al. Discovery of Alpha-Gal-Containing Antigens in North American Tick Species Believed to Induce Red Meat Allergy. *Front Immunol*. 2019;10:1056.
92. Park Y, Kim D, Boorgula GD, De Schutter K, Smagghe G, Simo L, et al. Alpha-Gal and Cross-Reactive Carbohydrate Determinants in the N-Glycans of Salivary Glands in the Lone Star Tick, *Amblyomma americanum*. *Vaccines (Basel)*. 2020;8(1).
93. Lack G. Epidemiologic risks for food allergy. *J Allergy Clin Immunol*. 2008;121(6):1331-6.
94. Tordesillas L, Goswami R, Benede S, Grishina G, Dunkin D, Jarvinen KM, et al. Skin exposure promotes a Th2-dependent sensitization to peanut allergens. *J Clin Invest*. 2014;124(11):4965-75.
95. Woodfolk JA, Commins SP, Schuyler AJ, Erwin EA, Platts-Mills TA. Allergens, sources, particles, and molecules: Why do we make IgE responses? *Allergol Int*. 2015;64(4):295-303.
96. Beck LA, Leung DY. Allergen sensitization through the skin induces systemic allergic responses. *J Allergy Clin Immunol*. 2000;106(5 Suppl):S258-63.
97. Brown SJ, Knapp FW. *Amblyomma americanum*: sequential histological analysis of adult feeding sites on guinea pigs. *Exp Parasitol*. 1980;49(3):303-18.
98. Harrison OJ, Linehan JL, Shih HY, Bouladoux N, Han SJ, Smelkinson M, et al. Commensal-specific T cell plasticity promotes rapid tissue adaptation to injury. *Science*. 2019;363(6422).
99. Chandrasekhar JL, Cox KM, Loo WM, Qiao H, Tung KS, Erickson LD. Cutaneous Exposure to Clinically Relevant Lone Star Ticks Promotes IgE Production and Hypersensitivity through CD4(+) T Cell- and MyD88-Dependent Pathways in Mice. *J Immunol*. 2019.
100. Cabezas-Cruz A, de la Fuente J, Fischer J, Hebsaker J, Lupberger E, Blumenstock G, et al. Prevalence of type I sensitization to alpha-gal in forest service employees and hunters: Is the blood type an overlooked risk factor in epidemiological studies of the alpha-Gal syndrome? *Allergy*. 2017;72(12):2044-7.
101. Venturini M, Lobera T, Sebastian A, Portillo A, Oteo JA. IgE to alpha-Gal in Foresters and Forest Workers From La Rioja, North of Spain. *J Investig Allergol Clin Immunol*. 2018;28(2):106-12.
102. Commins SP, Karim S. Development of a novel murine model of alpha-gal meat allergy. *J Allergy Clin Immunol*. 2017;139(2):Ab193-Ab.
103. Choudhary S, Iweala OI, Addison CT, Commins SP. Tick Salivary Extract Induces Alpha-Gal Allergy in Alpha-Gal Deficient Mice. *J Allergy Clin Immunol*. 2019;143(2):Ab252-Ab.
104. Bowman AS, Gengler CL, Surdick MR, Zhu K, Essenberg RC, Sauer JR, et al. A novel phospholipase A2 activity in saliva of the lone star tick, *Amblyomma americanum* (L.). *Exp Parasitol*. 1997;87(2):121-32.
105. Cabezas-Cruz A, Mateos-Hernandez L, Chmelar J, Villar M, de la Fuente J. Salivary Prostaglandin E2: Role in Tick-Induced Allergy to Red Meat. *Trends Parasitol*. 2017;33(7):495-8.
106. Trager W. Acquired immunity to ticks. *Journal of Parasitology Urbana*. 1939;25(1):pp. 57-78.
107. Wada T, Ishiwata K, Koseki H, Ishikura T, Ugajin T, Ohnuma N, et al. Selective ablation of basophils in mice reveals their nonredundant role in acquired immunity against ticks. *J Clin Invest*. 2010;120(8):2867-75.
108. Karasuyama H, Tabakawa Y, Ohta T, Wada T, Yoshikawa S. Crucial Role for Basophils in Acquired Protective Immunity to Tick Infestation. *Front Physiol*. 2018;9:1769.
109. Schmidle P, Reidenbach K, Kugler C, Eberlein B, Biedermann T, Darsow U. Recall urticaria-A new clinical sign in the diagnosis of alpha-gal syndrome. *J Allergy Clin Immunol Pract*. 2019;7(2):685-6.
110. Galili U, Rachmilewitz EA, Peleg A, Flechner I. A unique natural human IgG antibody with anti-alpha-galactosyl specificity. *J Exp Med*. 1984;160(5):1519-31.
111. Hamadeh RM, Galili U, Zhou P, Griffiss JM. Anti-alpha-galactosyl immunoglobulin A (IgA), IgG, and IgM in human secretions. *Clin Diagn Lab Immunol*. 1995;2(2):125-31.
112. Hamanova M, Chmelikova M, Nentwich I, Thon V, Lokaj J. Anti-Gal IgM, IgA and IgG natural antibodies in childhood. *Immunol Lett*. 2015;164(1):40-3.
113. Aguilar R, Ubillos I, Vidal M, Balanza N, Crespo N, Jimenez A, et al. Antibody responses to alpha-Gal in African children vary with age and site and are associated with malaria protection. *Sci Rep*. 2018;8(1):9999.

114. Ohdan H, Swenson KG, Kruger Gray HS, Yang YG, Xu Y, Thall AD, et al. Mac-1-negative B-1b phenotype of natural antibody-producing cells, including those responding to Gal alpha 1,3Gal epitopes in alpha 1,3-galactosyltransferase-deficient mice. *J Immunol.* 2000;165(10):5518-29.
115. Parker W, Yu PB, Holzknicht ZE, Lundberg K, Buckley RH, Platt JL. Specificity and function of "natural" antibodies in immunodeficient subjects: clues to B cell lineage and development. *J Clin Immunol.* 1997;17(4):311-21.
116. Wilson JM, Schuyler AJ, Schroeder N, Platts-Mills TA. Galactose-alpha-1,3-Galactose: Atypical Food Allergen or Model IgE Hypersensitivity? *Curr Allergy Asthma Rep.* 2017;17(1):8.
117. Yilmaz B, Portugal S, Tran TM, Gozzelino R, Ramos S, Gomes J, et al. Gut microbiota elicits a protective immune response against malaria transmission. *Cell.* 2014;159(6):1277-89.
118. Cretin N, Bracy J, Hanson K, Iacomini J. The role of T cell help in the production of antibodies specific for Gal alpha 1-3Gal. *J Immunol.* 2002;168(3):1479-83.
119. Rispens T, Derksen NI, Commins SP, Platts-Mills TA, Aalberse RC. IgE production to alpha-gal is accompanied by elevated levels of specific IgG1 antibodies and low amounts of IgE to blood group B. *PLoS One.* 2013;8(2):e55566.
120. Kollmann D, Nagl B, Ebner C, Emminger W, Wohrl S, Kitzmuller C, et al. The quantity and quality of alpha-gal-specific antibodies differ in individuals with and without delayed red meat allergy. *Allergy.* 2017;72(2):266-73.
121. Apostolovic D, Rodrigues R, Thomas P, Starkhammar M, Hamsten C, van Hage M. Immunoprofile of alpha-Gal- and B-antigen-specific responses differentiates red meat-allergic patients from healthy individuals. *Allergy.* 2018;73(7):1525-31.
122. McGowan EC, Platts-Mills TAE, Wilson JM. Food Allergy, Eosinophilic Esophagitis and the Enigma of IgG4. *Ann Allergy Asthma Immunol.* 2019.
123. Ristivojevic MK, Grundstrom J, Tran TAT, Apostolovic D, Radoi V, Starkhammar M, et al. alpha-Gal on the protein surface affects uptake and degradation in immature monocyte derived dendritic cells. *Sci Rep.* 2018;8(1):12684.
124. Iweala O, Brennan PJ, Commins SP. Serum IgE Specific for Alpha-Gal Sugar Moiety Can Bind Glycolipid. *J Allergy Clin Immun.* 2017;139(2):Ab88-Ab.
125. Liu S, Kandevara T, Tchervenkova J. CD1d-mediated interaction between activated T cells and B cells is essential to B-cell proliferation and anti-alpha-Gal antibody production. *Transplant Proc.* 2009;41(1):398-402.
126. Iweala OI, Savage PB, Commins SP. A Role for CD1d-restricted Invariant Natural Killer T Cells and Glycolipids in Alpha-Gal Allergy. *J Allergy Clin Immun.* 2018;141(2):Ab288-Ab.
127. Brestoff JR, Tesfazghi MT, Zaydman MA, Jackups R, Jr., Kim BS, Scott MG, et al. The B antigen protects against the development of red meat allergy. *J Allergy Clin Immunol Pract.* 2018;6(5):1790-1 e3.
128. Galili U, Buehler J, Shohet SB, Macher BA. The human natural anti-Gal IgG. III. The subtlety of immune tolerance in man as demonstrated by crossreactivity between natural anti-Gal and anti-B antibodies. *J Exp Med.* 1987;165(3):693-704.
129. Hilger C, Fischer J, Wolbing F, Biedermann T. Role and Mechanism of Galactose-Alpha-1,3-Galactose in the Elicitation of Delayed Anaphylactic Reactions to Red Meat. *Curr Allergy Asthma Rep.* 2019;19(1):3.

FIGURE LEGENDS

Fig 1. Depiction of the structure of the α -Gal epitope and representative α -Gal-bearing glycoproteins and glycolipids.

(A) The structure of the complete α -Gal trisaccharide epitope (galactose- α -1,3-galactose- β 1,4-GlcNAc) in comparison to the blood group B antigen. **(B)** Cetuximab is a chimeric mouse-human monoclonal IgG that expresses α -Gal glycans. Although α -Gal can be present in the Fc domain, the strongest expression of α -Gal, and that which is recognized by IgE antibodies, occurs in the Fab domain. The dominant form of α -Gal that is present in cetuximab is a bi-antennary glycan. **(C)** Lipid forms of α -Gal are less well studied than their glycoprotein counterparts but it is known that non-primate erythrocytes are an abundant source of O-linked glycosphingolipids which can bear α -Gal epitopes.

Fig 2. (A) Time course of self-reported symptom onset of allergic symptoms in 261 patients evaluated for mammalian meat allergy, of which 248 had IgE testing consistent with α -Gal syndrome, from Wilson et al³⁹. **(B)** Results of basophil activation as assessed by CD63 expression in PBMCs drawn sequentially following mammalian meat challenges in individuals with known α -Gal syndrome from Commins et al⁴⁷.

Fig 3. Representative cross-sectional imaging of coronary arteries of a subject without (left) or with (right) IgE to α -Gal⁶⁰. Images obtained by intravascular ultrasound with central black area representing the lumen of the coronary artery and surrounding colored areas indicative of atherosclerotic lesions with fibrous (dark green), fibrofatty (light green), necrotic (red) and calcified plaques (white) evident. Images courtesy of Angela Taylor, M.D., University of Virginia Health System and NHLBI.

Fig 4. (A) Cases of the α -Gal syndrome based on an ongoing survey of allergy practices in the USA (unpublished results). **(B)** Density of the most abundant tick species in eastern North America based on a sampling of 95 sites, from Diuk-Wasser et al⁷⁸. Of note, there is a striking overlap of α -Gal cases with the presence of *Amblyomma americanum* (the lone star tick), but not *Ixodes scapularis* (the black-footed deer tick) or *Dermacentor variabilis* (The American dog tick).

Box 1. Evidence
for tick bites in the
to α -Gal^{4, 6, 19, 73, 74, 90,}

supporting a role
induction of IgE
103

1. Epidemiology: Overlap in cases of the α -Gal syndrome with the distribution of the lone star tick (in North America)
2. Cases: Prospective investigation of a small number of subjects who have had tick bites
3. Many subjects who make IgE to α -Gal also make IgE directed to other elements of tick extract
4. Identification of the epitope in tick midgut and saliva
5. Models using α -Gal knock-out mice where saliva or salivary gland extract is sufficient to recapitulate the syndrome

Table 1. Description of the three main hypotheses to explain the source of the glycan that leads to an IgE response directed to the α -Gal epitope

Hypothesis	Supporting evidence	Conflicting evidence
1. Certain ticks can intrinsically produce α -Gal, either at baseline or during feeding	▪ Work from the groups of Marianne van Hage and Shahid Karim investigating tick extracts by Western Blot and Mass Spectrometry ▪ Cabezas-Cruz et al. reported that galactosyltransferase enzymes other than the α1,3-galactosyltransferase can generate α-Gal (i.e. α1,4- and β1,4 galactosyltransferases, which have been identified in ticks)	▪ No evidence that ticks express the specific enzyme that is thought to be necessary to catalyze the terminal α-1,3 bond
2. The α -Gal present in tick saliva is residual from a prior blood meal of a non-primate mammal	▪ Hard ticks have 3 life stages and their natural hosts are often mammals that would have α-Gal	▪ Does not explain how larval (i.e. seed) ticks can acquire the epitope ▪ Unclear whether α-Gal and other constituents of blood meal would persist inside tick for the duration, often many months, between different blood feeds
3. The α -Gal present in tick saliva is produced by a microbial symbiont of ticks	▪ Multiple bacteria and parasites can express the glycan	▪ Lack of any established association with known tick-borne diseases ▪ No published reports have demonstrated that microbes common to relevant ticks express α-Gal

