Published in final edited form as:

Curr Allergy Asthma Rep. 2017 January ; 17(1): 8. doi:10.1007/s11882-017-0672-7.

Galactose- α -1,3-Galactose: Atypical Food Allergen or Model IgE Hypersensitivity?

Jeffrey M. Wilson¹, Alexander J. Schuyler¹, Nikhila Schroeder¹, and Thomas A. E. Platts-Mills¹

¹Division of Allergy, Asthma, and Immunology, University of Virginia Health System, PO Box 801355, Charlottesville, VA 22908, USA

Abstract

Purpose of Review—Galactose- α -1,3-galactose (α -gal) is a carbohydrate allergen with several unique characteristics. In this article, we discuss some recent advances in our understanding of the ‘alpha-gal syndrome,’ highlight data supporting the role of ticks in pathogenesis, and speculate on immune mechanisms that lead to sensitization.

Recent Findings—First described as the target of IgE in individuals suffering immediate hypersensitivity reactions to the novel anti-EGF monoclonal antibody cetuximab, it is now clear that α -gal sensitization is associated with mammalian meat allergy as well as reactions to other mammalian products. Unlike traditional IgE-mediated food allergies, reactions to α -gal often do not manifest until several hours following an exposure, although co-factors can influence the presentation. Multiple pieces of evidence, including recent work with a mouse model, point to the fact that sensitization is mediated by exposure to certain hard ticks and increasingly we are aware of its globally widespread impact.

Summary—The oligosaccharide α -gal represents a novel allergen with several unusual clinical features. It has been recognized now on multiple continents and its clinical presentation can be quite variable. Moreover, efforts to delineate the mechanisms leading to α -gal sensitization may have ramifications for our broader understanding of type 2 immunity.

Keywords

Alpha-gal (α -gal); Food allergy; Red meat allergy; Th2; Type 2 immunity

Correspondence to: Jeffrey M. Wilson.

Compliance with Ethical Standards

Conflict of Interest Dr. Platts-Mills reports grants from NIAID AI-20565 and support for IgE assays from Thermo-Fisher/Phadia. Drs. Wilson, Schuyler, and Schroeder declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki Declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

Introduction

Until recently, the oligosaccharide galactose- α -1,3-galactose (α -gal) was best known for its role as a major antigenic barrier in xenotransplantation [1]. Owing to a loss of functional 1,3 galactosyltransferase, humans and other members of the catarrhine group of primates lack the synthetic machinery to generate α -gal, distinguishing them from other mammals. The emergence of the non-functional pseudogene is estimated to have happened ~28 million years ago [2], and while the selective pressures for this divergence are not certain, one hypothesis is that loss of α -gal evolved because it provided a mechanism of pathogen immuno-surveillance. Indeed, humans produce abundant natural antibodies to α -gal, likely related to the presence of α -gal in microbial flora [3]. While earlier work reported that anti- α -gal constituted 1% of total IgG, more recent work argues it is about a log order less, but nonetheless a notable fraction [1, 4]. It is these pre-existing antibodies to α -gal, predominantly IgM and IgG2, that represent the dominant mechanism of hyperacute rejection in porcine xenotransplantation [4, 5]. It has only been over the last decade that α -gal has been appreciated as an important epitope in IgE-mediated drug and food reactions.

Discovery of an Important Carbohydrate Allergen

The initial realization that α -gal was a target for IgE-mediated allergy stemmed from efforts to understand why a number of patients with carcinoma of the colon in the Southeastern U.S. were suffering immediate hypersensitivity reactions during the first infusion of the monoclonal antibody cetuximab [6]. Combining data from structural studies of cetuximab, IgE binding/inhibition studies, as well as the availability of an alternatively glycosylated variant of cetuximab, it became clear that α -gal was the relevant epitope for IgE [7–9]. As part of those investigations, it was also clear that α -gal sensitization was quite common at the population level in the Southeastern U.S., but not in other parts of the country. It was this regional prevalence that led to efforts to screen for α -gal IgE levels in individuals presenting to the University of Virginia Allergy Clinic with atopic histories including asthma, chronic urticaria, atopic dermatitis, and anaphylaxis. Based on these efforts, it quickly became apparent that α -gal levels correlated strongly with individuals who described a history of delayed urticaria or anaphylaxis to red meat [10]. A subsequent open challenge with red meat confirmed reactions in most individuals tested who had both a personal history of reactions to red meat and positive α -gal IgE [11].

Red Meat Allergy and More

Unlike traditional IgE-mediated food hypersensitivities, the reactions involving α -gal and mammalian meat typically are delayed by at least 2 h. This was formally shown in the open food challenge study where reactions occurred after 3–6 h and has been born out with clinical experience with hundreds of such cases in our clinic in Central Virginia, as well as a number of cases reported overseas [10, 12–17, 18•]. The explanation for this delay remains enigmatic, but the prevailing suspicion is that digestion, absorption, and transit of glycoproteins and/or glycolipids are relevant. The manifestations of the syndrome most commonly involve urticaria with severe pruritis, as well as signs of systemic anaphylaxis and in some cases abdominal pain. The syndrome is also idiosyncratic in that not every

exposure leads to a clinical reaction, suggesting that allergen dose or other modifying factors are important. Concomitant alcohol consumption appears to be a significant co-factor in the experience of ourselves and others [14]. Multiple investigators have probed for the relevant source of allergen in common food triggers, which include beef, pork, and lamb, but can include other mammal-derived foods as well. An immunoproteomic approach with beef demonstrated seven different α -gal containing glycoproteins, many of which were resistant to heat [19]. Studies of individuals with a history of allergic reactions to pork kidney, where reactions frequently happen within 2 h, identified α -gal as the target allergen. Based on binding/inhibition assays, pork kidney was shown to have abundant α -gal compared to pork meat which may explain the kinetics of this phenomena [14]. More recent work has demonstrated that α -gal is carried by two transmembrane metalloproteases in pork kidney, both of which were sufficient to stimulate basophils from α -gal patients in a basophil activation study [20••]. Interestingly, there was some inter-individual variability in IgE binding to metalloproteases and α -gal-conjugated human serum albumin (HSA), suggesting that the protein backbone may be relevant to the recognition of the epitope [20••]. Consistent with the findings with beef, heat treatment did not impact α -gal glycosylation of the pork kidney metalloproteases, which collectively argues that cooking meat or innards is not an adequate strategy to prevent α -gal-related hypersensitivity reactions. There are no known interventions aside from allergen avoidance once the allergy develops, although anecdotal experience has shown that many individuals will outgrow the hypersensitivity with time, likely reflecting the absence of ongoing exposure or long-lived IgE-secreting plasma cells. In our practice, we often check α -gal IgE levels on a periodic basis, typically yearly, to help guide clinical recommendations with consideration of cautious re-introduction of mammalian foods. Because of the delayed nature of the reaction, we do not usually advocate for in-clinic challenges, but an anaphylaxis action plan is critical. An important question, especially in lieu of recent work with other food allergies, is whether maintaining exposure to low levels of mammalian products could play a role in the management of the syndrome [21]. For example, it is possible that continued consumption of dairy in a sensitized individual could favor tolerance. The low-level α -gal exposure could mitigate the risk of a severe systemic reaction on future exposure to a larger allergen dose, such as a rare steak, by multiple potential mechanisms. These include induction of allergen-specific regulatory Tcells or low level tonic stimulation of IgE coated mast cells. Another possibility would be induction of blocking IgG4 antibodies, but preliminary data in our group has not supported this. To date, this premise that continuous low-level exposure of α -gal-containing foods is protective has not been formally studied and as such is not recommended outside of research protocols. That said, while we do not routinely discuss the concept of tolerance in our practice, we do not advise strict avoidance of all mammalian products (such as dairy) if they are clinically tolerated.

Aside from its involvement in red meat allergy, α -gal sensitization can also play a role in reactions to many other sources of mammalian antigens. As recently reviewed in this journal, dairy products, gelatin, and certain vaccines and medications warrant consideration as triggers in highly sensitive individuals [22]. In our experience, magnesium stearate, an excipient that can be derived from mammalian sources and is a common inactive ingredient in medications, has not been problematic, but based on a published case report, it should also

be considered [23]. Other occult sources must also be considered. For example, while poultry is not a source of α -gal, poultry sausage prepared in pork casings should be avoided. While unproven, we suspect pork casings express abundant α -gal based on the severity of reported reactions. Aside from ingestion, recent work has highlighted a role for α -gal IgE in premature degeneration of porcine aortic valves [24•] and we have previously reported peri-operative reactions in individuals receiving new porcine or bovine valves [25]. Taken together the name ' α -gal syndrome' seems an appropriate descriptor. Although anecdotally we have seen a few cases of chronic urticaria in α -gal patients on full avoidance diets, a recent report from Sweden did not substantiate a link [26].

The Tick Connection

The initial suggestion of a possible association with ticks and α -gal stemmed from the observation that the prevalence of α -gal IgE sensitization in the USA overlapped closely with the incidence of Rocky Mountain Spotted Fever and its associated tick vectors *Dermacentor variabilis* (the brown dog tick) and *Amblyomma americanum* (the lone star tick) [10]. This dovetailed with observations by Dr. Sheryl van Nunen who had reported an association between large local reactions to tick bites and delayed reactions to red meat in a series of patients she followed in the New South Wales Region of Australia [12, 27]. Supporting a role for the lone star tick, we found an excellent correlation between IgE to α -gal and IgE targeting lone star whole tick extract (but not the brown dog tick) in individuals with a history of red meat allergy [28]. There was a strong correlation between reported history of tick bites and levels of IgE to α -gal [28]. Importantly, we have also documented prospective increase in IgE titer to α -gal in individuals receiving multiple tick bites [28] (Fig. 1). Similarly important, we are unaware of any connection between *Ixodes scapularis*, which harbors the pathogen responsible for Lyme disease, and α -gal sensitization. This is strongly supported by the fact that α -gal reactions are essentially unheard of in the Northeastern U.S. in areas where *I. scapularis* is endemic, but *A. americanum* is absent (manuscript in preparation). Work from Sweden also directly assessed α -gal IgE titers in patients with Lyme disease and while rates of sensitization were minimally increased from the baseline population, the relative titers were modest [29]. While the connection between ticks and α -gal was initially described in the USA with *A. americanum*, α -gal-related reactions have now been reported in Australia, Europe, Japan, South Korea, and Central America [12–17, 18•]. Notably, ticks are endemic in all of these locales, albeit with species variation by geography. For example in southern Sweden, α -gal IgE sensitization is common and has been related to bites of *Ixodes ricinus*. Tellingly, much of northern Sweden is devoid of *I. ricinus* or other ticks, and in keeping with that, there have been no reports of the syndrome in that area [30]. Work from Swedish colleagues has also shown a strong correlation between IgE to α -gal and IgE targeting *I. ricinus* whole tick extract, and demonstrated the presence of α -gal in *I. ricinus* midgut [15, 31•]. More recent work has demonstrated the presence of α -gal in saliva from *Amblyomma sculptum* [32••], a tick species that is part of the *Amblyomma cajennense* complex that also includes tick species prevalent near the Panama Canal where α -gal reactions have been reported [18•]. An extension of that work, and consistent with the premise that tick saliva is critical for sensitization, it was also demonstrated that s.c. injection of tick saliva was sufficient to

induce α -gal-specific IgE in an α -gal knockout mouse model [32••]. Taken together, there is strong evidence that hard ticks including *A. americanum*, *I. ricinus*, *Ixodes holocyclus*, and members of the *A. cajennense* complex are important agents of α -gal sensitization, but many questions remain unanswered (see Table 1). The fact that α -gal IgE levels (but not delayed red meat allergy) are prevalent in cohorts from Kenya and Zimbabwe with chronic parasite infections certainly raises the possibility that ticks are not the complete story [30, 33]. We are not aware of reports of anaphylaxis to red meat in these countries, but admittedly this has not been well studied. An interesting possibility is that non-tick parasites, while capable of promoting IgE responses to α -gal in some cases, are particularly effective at inducing regulatory T cells which would help to suppress allergic responses. This premise, while unproven, is attractive in that it fits with current concepts of the role that parasites play in the 'hygiene hypothesis' [34].

Putative Mechanisms

One of the interesting aspects of α -gal hypersensitivity is that it appears to develop equally in those with and without atopic histories (manuscript in preparation). This is intriguing given that it is well accepted that allergic disease has an important heritable component and argues that tick-mediated sensitization represents a robust form of type 2 immune induction [35]. It is important to emphasize that we are referring to the same response that has been historically referred to as a type I hypersensitivity by Gell-Coombs classification, a nomenclature shift that reflects the fundamental importance of Th2 cells and related cytokine mediators in the response pathway. To date, the immunologic pathways that link tick exposure to the production of α -gal-specific IgE antibody remain poorly understood. There is, however, a large body of evidence showing that tick bites generally favor Th2 responses, although the governing mechanisms are not clear [36, 37]. Preliminary work in our lab has shown that there is abundant recruitment of T cells and neutrophils to the site of a tick lesion in a naïve human host by 48 h, though interestingly basophils were not predominant [38] (Fig. 2). Based on emerging concepts in type 2 immunity, which includes the innate and adaptive immune factors that contribute to allergic responses in general, and specifically CD4⁺ Th2 responses and IgE production, an initial cascade of cytokines such as TSLP, IL-33, and IL-25 are released from epithelial cells as an early signal to hematopoietic immune cells. The epithelial response may involve recognition of pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) by pathogen-recognition receptors (PRR) expressed on the epithelia, or alternatively may be triggered by damage to the epithelia itself [39]. Other mediators such as prostaglandins or leukotrienes may also play an early role [40, 41]. A number of putative candidates within the saliva have been described, including prostaglandins, lipocalins, phospholipases, and adenosine, all of which could act on epithelia or on sub-epithelial immune cells [42–44]. An interesting possibility is that α -gal has intrinsic PAMP activity that helps trigger the type 2 immune cascade. Lectins represent a family of PRRs that recognize carbohydrate epitopes and include C-type lectins, galectins, and siglecs (Fig. 3). Prior work has shown that lectin recognition of allergen-associated carbohydrate promotes type 2 immune responses [41]. Moreover, human monocyte activation by porcine endothelial cells involves α -gal recognition by galectin-3 [45, 46], a lectin also implicated in a murine model of allergen-

induced airway remodeling and mast cell function [47, 48]. Thus, differential expression of α -gal is a possible but unproven reason that *A. americanum*, *I. ricinus*, *I. holocyclus*, and members of *A. cajennense* lead to clinically important sensitization but other ticks do not. The explanation for why ingestion of α -gal-laden food is inadequate for sensitization could involve a dependence on an immunomodulatory tick co-factor, or could just reflect differences between exposure via skin or the oral route, consistent with our current understanding of other model food allergens such as peanut [49, 50].

The role of T cell help in governing B cell switch to IgE also remains an open question. While classically carbohydrates are not considered to be T cell antigens, multiple lines of evidence suggest that anti- α -gal responses could involve T cell help. Direct evidence comes from studies using an α -gal knockout mouse where minimal anti- α -gal responses were induced in the absence of T cells or CD40L blockade [51]. The role of T cell help is indirectly supported by work showing that T cell receptors can specifically recognize carbohydrate epitopes independent of their protein conjugate, though MHC-II can still have a role as scaffold during presentation [52]. Not incongruent with this premise, the differential IgE recognition observed for metallopeptidase-laden α -gal versus HSA-conjugated α -gal by Hilger et al. could be explained by variation in the peptide backbone available for MHC-II binding [20••]. A counter-argument for a T cell-independent mechanism of IgE switch is also plausible. One aspect that distinguishes α -gal from other allergens is the presumed presence of pre-existing memory B cells that could possibly facilitate class switch recombination in the absence of T cell help.

Conclusion

The α -gal syndrome represents a unique form of allergy on account of several features including the non-protein epitope, the delayed nature of the reaction, sensitization by an agent seemingly unrelated to the ultimate trigger, and the lack of relationship with other atopic diseases. While atypical in many regards, the commonly observed robust allergen-specific IgE response is shared with traditional food allergies, as is the IgE/IgG4 ratio [53]. As an extension of the recent paradigm shift in the way we approach other food allergens, namely peanut, the idea that persistent low-level exposure to α -gal could have a protective effect is an intriguing but unproven concept. In regards to sensitization, the most logical conclusion is that events during tick exposure, presumably involving elements within the saliva, induce a vigorous immune cascade that results in IgE class switch. As such, the α -gal syndrome represents a potentially fertile area for discovery of mediators and pathways that govern type 2 immunity, an area of immunology that remains enigmatic. This premise would also be compatible with an emerging literature describing a protective role for type 2 immunity against venomous organisms, which arguably ticks represent [54–56].

Acknowledgments

Funding NIAID AI-20565

References

Papers of particular interest, published recently, have been highlighted as:

Curr Allergy Asthma Rep. Author manuscript; available in PMC 2018 July 03.

- Of importance
- Of major importance

1. Galili U. The alpha-gal epitope and the anti-Gal antibody in xeno-transplantation and in cancer immunotherapy. *Immunol Cell Biol.* 2005; 83(6):674–86. [PubMed: 16266320]
2. Koike C, Uddin M, Wildman DE, Gray EA, Trucco M, Starzl TE, et al. Functionally important glycosyltransferase gain and loss during catarrhine primate emergence. *Proc Natl Acad Sci U S A.* 2007; 104(2):559–64. [PubMed: 17194757]
3. Galili U, Mandrell RE, Hamadeh RM, Shohet SB, Griffiss JM. Interaction between human natural anti-alpha-galactosyl immunoglobulin G and bacteria of the human flora. *Infect Immun.* 1988; 56(7):1730–7. [PubMed: 3290105]
4. Rispen T, Derksen NI, Commens 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. [PubMed: 23390540]
5. Sandrin MS, McKenzie IF. Gal alpha (1,3)Gal, the major xenoantigen(s) recognised in pigs by human natural antibodies. *Immunol Rev.* 1994; 141:169–90. [PubMed: 7532618]
6. O'Neil BH, Allen R, Spigel DR, Stinchcombe TE, Moore DT, Berlin JD, et al. High incidence of cetuximab-related infusion reactions in Tennessee and North Carolina and the association with atopic history. *J Clin Oncol.* 2007; 25(24):3644–8. [PubMed: 17704414]
7. Chung CH, Mirakhor 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. [PubMed: 18337601]
8. 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. [PubMed: 17362871]
9. Platts-Mills TA, Schuyler AJ, Tripathi A, Commens SP. Anaphylaxis to the carbohydrate side chain alpha-gal. *Immunol Allergy Clin N Am.* 2015; 35(2):247–60.
10. Commens 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. [PubMed: 19070355]
11. Commens 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. [PubMed: 24656556]
12. 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. [PubMed: 19413526]
13. Jacquenet S, Moneret-Vautrin DA, Bihain BE. Mammalian meat-induced anaphylaxis: clinical relevance of anti-galactose-alpha-1,3-galactose IgE confirmed by means of skin tests to cetuximab. *J Allergy Clin Immunol.* 2009; 124(3):603–5. [PubMed: 19733301]
14. 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. [PubMed: 22494361]
15. Hamsten C, Tran TA, Starkhammar M, Brauner A, Commens 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. [PubMed: 24094548]
16. Sekiya K, Fukutomi Y, Nakazawa T, Taniguchi M, Akiyama K. Delayed anaphylactic reaction to mammalian meat. *J Invest Allergol Clin Immunol.* 2012; 22(6):446–7.
17. Lee JH, Kim JH, Kim TH, Kim SC. Delayed mammalian meat-induced anaphylaxis confirmed by skin test to cetuximab. *J Dermatol.* 2013; 40(7):577–8. [PubMed: 23663076]

- 18•. Wickner PG, Commins SP. The first 4 central american cases of delayed meat allergy with galactose-alpha-1,3-galactose positivity clustered among field biologists in panama. *J Allergy Clin Immunol*. 2014; 133(2):Ab212-Ab. This is the first report of α -gal syndrome in Latin America.
19. Apostolovic D, Tran TA, Hamsten C, Starkhammar M, Cirkovic Velickovic T, van Hage M. Immunoproteomics of processed beef proteins reveal novel galactose-alpha-1,3-galactose-containing allergens. *Allergy*. 2014; 69(10):1308–15. [PubMed: 24942937]
- 20••. Hilger C, Fischer J, Swiontek K, Hentges F, Lehnert C, Eberlein B, et al. Two galactose-alpha-1,3-galactose carrying peptidases from pork kidney mediate anaphylactogenic responses in delayed meat allergy. *Allergy*. 2016; 71(5):711–9. These authors demonstrated inter-individual variability in IgE reactivity to α -gal laden metallopeptidases versus α -gal HSA. This finding could offer an explanation for variation in responses observed clinically. [PubMed: 26728983]
21. Du Toit G, Sayre PH, Roberts G, Sever ML, Lawson K, Bahnson HT, et al. Effect of avoidance on peanut allergy after early peanut consumption. *N Engl J Med*. 2016; 374(15):1435–43. [PubMed: 26942922]
22. Commins SP. Invited commentary: alpha-gal allergy: tip of the iceberg to a pivotal immune response. *Curr Allergy Asthma Rep*. 2016; 16(9):61. [PubMed: 27520937]
23. 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. [PubMed: 26003233]
- 24•. 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. This finding suggests that screening for α -gal sensitization may have a role when considering valve replacement options; however, clearly more work is needed in this arena. [PubMed: 27238083]
25. Mozzicato SM, Tripathi A, Posthumus JB, Platts-Mills TA, Commins 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. [PubMed: 25213067]
26. Maurer M, Church MK, Metz M, Starkhammar M, Hamsten C, van Hage M. Galactose-alpha-1,3-galactose allergy is not a hitherto unrecognized cause of chronic spontaneous urticaria. *Int Arch Allergy Immunol*. 2015; 167(4):250–2. [PubMed: 26426899]
27. van Nunen S, O'Connor K, Fernando SL, Clarke LR, Boyle RX. The association between ixodes holocyclos tick bite reactions and red meat allergy. *Intern Med J*. 2007; 39(Suppl5):A132.
28. 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. [PubMed: 21453959]
29. Apostolovic D, Tran TA, Starkhammar M, Sanchez-Vidaurre S, Hamsten C, Van Hage M. The red meat allergy syndrome in Sweden. *Allergo J Int*. 2016; 25(2):49–54. [PubMed: 27656352]
30. Commins SP, Kelly LA, Ronmark E, James HR, Pochan SL, Peters EJ, et al. Galactose-alpha-1,3-galactose-specific IgE is associated with anaphylaxis but not asthma. *Am J Respir Crit Care Med*. 2012; 185(7):723–30. [PubMed: 22281828]
- 31•. Hamsten C, Starkhammar M, Tran TA, Johansson M, Bengtsson U, Ahlen G, et al. Identification of galactose-alpha-1,3-galactose in the gastrointestinal tract of the tick *Ixodes ricinus*; possible relationship with red meat allergy. *Allergy*. 2013; 68(4):549–52. The first report identifying α -gal in a tick with relevance to the α -gal syndrome. [PubMed: 23414348]
- 32••. Araujo RN, Franco PF, Rodrigues H, Santos LC, McKay CS, Sanhueza CA, et al. *Amblyomma sculptum* tick saliva: alpha-Gal identification, antibody response and possible association with red meat allergy in Brazil. *Int J Parasitol*. 2016; 46(3):213–20. These investigators are the first to report α -gal in tick saliva. This paper also reports that s.c. injection of saliva from *A. sculptum* is sufficient to induce α -gal IgE in a mouse model. [PubMed: 26812026]
33. Arkestal K, Sibanda E, Thors C, Troye-Blomberg M, Mduluzi T, Valenta R, et al. Impaired allergy diagnostics among parasite-infected patients caused by IgE antibodies to the carbohydrate epitope galactose-alpha 1,3-galactose. *J Allergy Clin Immunol*. 2011; 127(4):1024–8. [PubMed: 21376382]

34. McSorley HJ, Hewitson JP, Maizels RM. Immunomodulation by helminth parasites: defining mechanisms and mediators. *Int J Parasitol.* 2013; 43(3–4):301–10. [PubMed: 23291463]
35. Holloway JW, Yang IA, Holgate ST. Genetics of allergic disease. *J Allergy Clin Immunol.* 2010; 125(2 Suppl 2):S81–94. [PubMed: 20176270]
36. Ferreira BR, Silva JS. Successive tick infestations selectively promote a T-helper 2 cytokine profile in mice. *Immunology.* 1999; 96(3):434–9. [PubMed: 10233725]
37. Wikel SK, Alarcon-Chaidez FJ. Progress toward molecular characterization of ectoparasite modulation of host immunity. *Vet Parasitol.* 2001; 101(3–4):275–87. [PubMed: 11707302]
38. Schroeder N, Eccles J, Klaffky EJ, Platts-Mills TAE. Cellular infiltrate induced by bites from the tick *amblyomma americanum* in subjects with or without IgE to galactose-alpha-1,3-galactose (Alpha-gal). *J Allergy Clin Immunol.* 2014; 133(2):Ab226-Ab.
39. Pulendran B, Artis D. New paradigms in type 2 immunity. *Science.* 2012; 337(6093):431–5. [PubMed: 22837519]
40. Arima M, Fukuda T. Prostaglandin D(2) and T(H)2 inflammation in the pathogenesis of bronchial asthma. *Korean J Intern Med.* 2011; 26(1):8–18. [PubMed: 21437156]
41. Barrett NA, Rahman OM, Fernandez JM, Parsons MW, Xing W, Austen KF, et al. Dectin-2 mediates Th2 immunity through the generation of cysteinyl leukotrienes. *J Exp Med.* 2011; 208(3):593–604. [PubMed: 21357742]
42. Oliveira CJ, Sa-Nunes A, Francischetti IM, Carregaro V, Anatriello E, Silva JS, et al. Deconstructing tick saliva: non-protein molecules with potent immunomodulatory properties. *J Biol Chem.* 2011; 286(13):10960–9. [PubMed: 21270122]
43. 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. [PubMed: 9326887]
44. Preston SG, Majtan J, Kouremenou C, Rysnik O, Burger LF, Cabezas Cruz A, et al. Novel immunomodulators from hard ticks selectively reprogramme human dendritic cell responses. *PLoS Pathog.* 2013; 9(6):e1003450. [PubMed: 23825947]
45. Jin R, Greenwald A, Peterson MD, Waddell TK. Human monocytes recognize porcine endothelium via the interaction of galectin 3 and alpha-GAL. *J Immunol.* 2006; 177(2):1289–95. [PubMed: 16818789]
46. Greenwald AG, Jin R, Waddell TK. Galectin-3-mediated xenoactivation of human monocytes. *Transplantation.* 2009; 87(1):44–51. [PubMed: 19136890]
47. Ge XN, Bahaie NS, Kang BN, Hosseinkhani MR, Ha SG, Frenzel EM, et al. Allergen-induced airway remodeling is impaired in galectin-3-deficient mice. *J Immunol.* 2010; 185(2):1205–14. [PubMed: 20543100]
48. Chen HY, Sharma BB, Yu L, Zuberi R, Weng IC, Kawakami Y, et al. Role of galectin-3 in mast cell functions: galectin-3-deficient mast cells exhibit impaired mediator release and defective JNK expression. *J Immunol.* 2006; 177(8):4991–7. [PubMed: 17015681]
49. Fox AT, Sasieni P, du Toit G, Syed H, Lack G. Household peanut consumption as a risk factor for the development of peanut allergy. *J Allergy Clin Immunol.* 2009; 123(2):417–23. [PubMed: 19203660]
50. 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. [PubMed: 25295541]
51. 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. [PubMed: 11801692]
52. Avci FY, Li X, Tsuji M, Kasper DL. Carbohydrates and T cells: a sweet twosome. *Semin Immunol.* 2013; 25(2):146–51. [PubMed: 23757291]
53. Wilson JM, Schuyler AJ, Tripathi A, Erwin EA, Commings SP, Platts-Mills TAE. IgG4 component allergens are preferentially increased in eosinophilic esophagitis as compared to patients with milk anaphylaxis or galactose-alpha-1,3-galactose allergy. *J Allergy Clin Immunol.* 2016; 137(2):Ab199-Ab.
54. Galli SJ, Starkl P, Marichal T, Tsai M. Mast cells and IgE in defense against venoms: possible “good side” of allergy? *Allergol Int.* 2016; 65(1):3–15. [PubMed: 26666482]

55. Palm NW, Rosenstein RK, Medzhitov R. Allergic host defences. *Nature*. 2012; 484(7395):465–72. [PubMed: 22538607]
56. Cabezas-Cruz A, Valdes JJ. Are ticks venomous animals? *Front Zool*. 2014; 11:47. [PubMed: 25006341]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1.

Serum IgE in a patient pre and post natural tick exposure. The subject was bitten in 2013 by about 30 nymphs, presumably *A. americanum*, and total and α-gal-specific IgE were followed. Interestingly, while the α-gal IgE titers rose over tenfold to a peak in about 1 month, they also decreased over a period of months in the absence of ongoing exposure

Fig. 2. Analysis of skin lesion from site of tick bite at 48 h in an α -gal naïve individual. **a** H&E staining reveals inflammatory infiltrate, **b** with pronounced neutrophils evident on further magnification. **c** Immunohistochemistry demonstrates T and B cell clusters. *Legend:* for **a** *TP* = tick 'parts,' *C* = tick 'cement'; for **c** *blue* = DAPI (nuclei stain), *red* = anti-CD3 (T cells), green = anti-CD20 (B cells)

Fig. 3.

Proposed mechanisms by which *A. americanum* and other hard ticks could promote α -gal sensitization. Our working assumption is that α -gal in the form of glycoprotein or glycolipid is present at the site of the tick lesion. There is also local damage to the epithelia and/or recognition of tick-related PAMPs. Cytokines such as IL-25, IL-33, and TSLP are released and signal to immune cells including mast cells, ILC2, and dendritic cells. Collectively, these innate cells promote differentiation of Th2 cells and/or T follicular helper 2 cells (Tfh2) and favor B cell class switch to IgE, though B cell activation independent of T cell help is also possible. Factors in tick saliva such as prostaglandin E2, phospholipase A2, lipocalins, or adenosine are putative immunomodulators that favor Th2 responses. Lastly, α -gal itself may be recognized as a PAMP by carbohydrate-binding lectin receptors and thus directly stimulate signaling pathways culminating in IgE

Table 1

Important unknowns with the α-gal syndrome

1	How does <i>A. americanum</i> lead to α-gal sensitization?
2	Why do some hard ticks lead to α-gal syndrome but others do not?
3	What explains the disparity between sensitization and clinical hypersensitivity?
4	Does the IgE-specific α-gal class switch involve T cell help?
5	What explains the IgE-mediated delayed reaction?
6	Is there a protective role for low dose α-gal exposure against severe systemic reactions?

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript