



Review

Galactose α -1,3-galactose phenotypes

Lessons from various patient populations

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Key messages

- Galactose α -1,3-galactose (α -gal) is a carbohydrate epitope found on proteins and lipids in nonprimate mammals and present in foods (particularly organ or fat-rich red meat) and medications (eg, cetuximab, certain vaccines, and antivenoms), where it causes delayed onset and immediate-onset anaphylaxis.
- Several species of ticks contain α -gal epitopes and possibly salivary adjuvants that modulate the immune response for IgE production and promote clinical reactivity to the epitope.
- Risk factors for α -gal syndrome include exposure to ticks of particular species, and age and sex differences possibly reflect the prevalence of these exposures in different cohorts and thus vary according to setting.
- Symptoms are skewed toward urticaria and a high prevalence of isolated abdominal reactions.
- The reason and mechanisms for delayed onset of food-related anaphylaxis are not clear but may involve the kinetics of allergen digestion and processing or immunologic presentation via a different mechanism from usual immediate type food allergy.

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ABSTRACT

Objective: To review published studies on galactose α -1,3-galactose (α -gal), a carbohydrate epitope found on proteins and lipids in nonprimate mammals and present in foods (particularly organ or fat-rich red meat) and medications, where it causes delayed-onset and immediate-onset anaphylaxis.

Data Sources: A literature search for the terms *galactose α -1,3-galactose* and *α -gal* using PubMed and Embase was performed.

Study Selections: Studies on α -gal were included in this review.

Results: Several species of ticks contain α -gal epitopes and possibly salivary adjuvants that promote high titer sensitization and clinical reactivity. Risk factors for α -gal syndrome include exposure to ticks of particular species. Age and sex differences seen in various cohorts possibly reflect the prevalence of these exposures that vary according to setting.

Conclusion: The reason and mechanisms for delayed onset of food-related anaphylaxis and the preponderance of abdominal reactions are not clear but may involve the kinetics of allergen digestion and processing or immunologic presentation via a different mechanism from usual immediate-type food allergy.

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Introduction

Galactose α -1,3-galactose (α -gal) is a carbohydrate epitope found on proteins and lipids in nonprimate mammals, synthesized by the α -1,3-galactosyltransferase enzyme. The enzyme responsible for the production of α -gal is coded by the α -1,3-galactosyltransferase gene, which is inactivated in ancestral old world monkeys and apes because of the presence of premature stop codons.¹ Thus, humans, apes, and old world monkeys lack the α -gal epitope, and almost all immunocompetent individuals produce IgG and IgM antibodies to α -gal.² The presence of these α -gal antibodies and their efficacy in enhancing immunity to various α -gal-containing pathogens could explain the evolutionary pressure for selection of primate populations with inactivation of the α -1,3-galactosyltransferase gene.³ We performed a literature search of PubMed and Embase for studies on α -gal using the search terms *galactose α -1,3-galactose* and *α -gal*. All studies on α -gal were included in this review.

Story of α -gal

Anaphylactic or urticarial reactions after ingestion of red meat in individuals who had been bitten by ticks were first reported in Australia by Van Nunen et al⁴ in 2007 and a few years later in Virginia by Commins et al⁵ in 2009. At the same time it was confirmed that individuals receiving cetuximab, a chimeric mouse-human monoclonal antibody against the epidermal growth factor receptor, in North Carolina and Tennessee experienced hypersensitivity reactions at a much higher rate than were reported nationally and internationally.⁶ This finding was shown to be linked with a high prevalence of IgE antibodies to cetuximab in the blood of control patients in the same areas of the Southeastern United States.⁷ These IgE antibodies were specific for α -gal,⁷ which is present on 2 N-linked oligosaccharide domains on the Fab portion of the cetuximab heavy chain.⁸ In 2009, a case series described the syndrome of delayed reactions to the ingestion of red meat in individuals from Virginia and Missouri, linking this syndrome with the presence of antibodies to α -gal and to cetuximab.^{5,9} Since then the α -gal syndrome of delayed reactions to the ingestion of red meat in individuals with antibodies to α -gal has been reported from various parts of the world.^{10–12}

The Effect of Tick Bites?

The hypothesis that tick bites are an important cause of sensitization to α -gal is supported by epidemiologic evidence showing a colocation of cases of cetuximab reactions and α -gal-related meat allergy in areas of high prevalence of the lone star tick, *Amblyomma americanum*,¹³ and in individuals at risk of or with a history of tick bites.^{14,15} In addition, some patients experience urticaria at the site of previous tick bites after exposure to α -gal rich meat (recall urticaria), implying that a memory response is present at the site of percutaneous exposure to α -gal-containing protein.¹⁶

Increased titers of α -gal specific IgE have been reported in patients with α -gal syndrome after tick bites.^{13,17} In addition, immunohistochemistry on cryostat cut lateral sections of the European tick *Ixodus ricinus* revealed the presence of the α -gal epitope in the gastrointestinal tract of the tick with the use of serum from red meat allergic patients and monoclonal and polyclonal antibodies against α -gal.¹⁸ More recently, α -gal epitopes were identified in saliva of *Amblyomma sculptum*,¹⁹ *Haemaphysalis longicornis*,²⁰ and *Ixodes scapularis*.²¹ Moreover, the saliva from *A sculptum* was sufficient to induce IgE to α -gal in a mouse model¹⁹ and in human by *Amblyomma testudinarium*.¹⁷ In a murine model of α -gal red meat allergy, intradermal injection of tick salivary gland extract from *A americanum* resulted in

sensitization to α -gal and systemic allergic responses after ingestion of pork sausage.²² These reports suggest exposure to α -gal during a tick bite.

Butchers and abattoir workers are exposed to large amounts of α -gal-containing products through skin and possibly mucous membranes but lack high-titer α -gal specific IgE in the absence of tick bites. This finding suggests that additional factors are required to initiate α -gal sensitization or to boost titers of α -gal specific IgE. Furthermore, the route of sensitization through the skin via tick bites seems to be of major importance for the induction of IgE antibodies against α -gal because these antibodies are not found in the serum of individuals living in the northern arctic part of Sweden, where ticks are not present.¹³ In Japan, no α -gal specific IgE was present in a group of 21 healthy individuals who were not bitten by ticks. Individuals who had more than 2 tick bites had higher levels of α -gal specific IgE than those with 0 or 1 tick bite.¹⁷ To date, the ability of tick bites to promote sensitization to α -gal also appears to be species specific because IgE reactivity to α -gal has not been found to have any association with the presence of previous Lyme disease, a disease transmitted by *I scapularis*.²³ Perhaps tick bites transfer both α -gal epitopes and species-specific salivary adjuvants, initiating the percutaneous immune reaction toward α -gal epitopes.²⁴ Adjuvants may differ between species and explain the reason why some tick species induce high-titer α -gal sensitization and clinical reactivity, whereas others do not. In addition to possibly introducing α -gal epitopes to the skin, tick bites may also compromise the skin epithelial barrier through direct trauma or alteration of the skin microbiota, promoting the generation of allergic and IgE antibody responses to α -gal.

Studies in humans and mice have confirmed that basophils are recruited to sites of tick bites, and the animal models suggest that the basophil recruitment, and possibly the associated IgE production, is dependent on CD4⁺ memory T cells.²⁵ Thus, the sequential exposure to tick bites that is often reported by patients developing the α -gal syndrome may actually be dependent on a cascade of events within the immune system. When the epithelial barrier is broken, alarmins such as thymic stromal lymphopoietin, interleukin 33, and interleukin 25, are generated. They can attract innate immune cells to the exposure site and uptake of α -gal antigens, which are further presented to CD4⁺ T cells or other unconventional T-cell subsets, inducing the production of T_H2-like cytokines, including interleukins 3, 4, 5, and 13. Thus, ticks may generate a microclimate that supports the production of IgE antibodies reactive to α -gal.^{24,25}

In addition, tick exposure may not be the sole trigger for α -gal sensitization. Other endoparasites and ectoparasites may also cause low levels of α -gal sensitization but may lack the adjuvant activity required to cause high-level sensitization and/or clinical α -gal allergy. This theory would be consistent with reports that have found a high prevalence of α -gal sensitization in areas where chronic parasitism is common.^{11,13,26}

Clinical Manifestations of α -gal Hypersensitivity: Medication and Transplantation Reactions

IgG- and IgM-mediated antibody responses to α -gal have been identified as an important cause of acute, severe hypersensitivity reactions after xenotransplantation.²⁷ Immediate IgE-mediated reactions occur when α -gal-containing epitopes are administered systemically into sensitized individuals, as was observed in α -gal sensitized individuals receiving intravenous cetuximab.⁷ Others have reported immediate hypersensitivity responses to intravenous α -gal-containing gelatin-colloid followed by separate incidents of delayed hypersensitivity reactions after the ingestion of

red meat in the gelatin allergic individuals.²⁸ α -Gal is present in certain vaccines²⁹ and antivenoms,³⁰ and although α -gal IgE-mediated hypersensitivity reactions to these products have been described, they are probably uncommon.

Ingestion

Delayed-Onset Food Allergy

The α -gal epitope is commonly present in beef proteins recognized by IgE from meat-allergic patients and is stable to heat treatment, perhaps explaining why allergenicity of red meat proteins persists even on different thermal cooking.³¹ Reactions to the ingestion of α -gal-containing foods results in an unusual form of food allergy that is delayed in onset, in stark contrast to other food allergies. Studies that use formal food challenge tests for the diagnosis of α -gal allergy are rarely performed but provide the opportunity to assess the time course of a reaction in detail, avoiding the problems of patient recall. Commins et al¹² challenged 12 red meat allergic individuals and described reactions that occurred 3 to 7 hours after the initial ingestion of mammalian meat associated with time-dependent increases in basophil activation, corresponding with the appearance of clinical symptoms. Mabe-lane et al,¹¹ however, reported a more rapid onset of symptoms (median, 100 minutes; interquartile range, 75–135 minutes) than previously described. Nonetheless, the delay reported by both these groups is unusual for an IgE-mediated reaction, classically understood as a type I Gel and Coombs immediate hypersensitivity reaction. The mechanisms for the delay remain an open question. Possible explanations may involve the kinetics of allergen digestion and processing or immunologic presentation via a different mechanism.³²

Spectrum of Abdominal vs Skin Symptoms

Most reactions reported in the literature focus on the severe spectrum of reaction that presents with delayed onset of urticaria, angioedema, and even anaphylaxis.⁵ Authors have also described reactions that manifest with abdominal pain, in addition to skin reactions or as isolated reactions. Articles with large case series of challenge-proven α -gal allergy report a high prevalence of isolated abdominal reactions (approximately 20%),¹¹ raising the possibility that these reactions are underreported and underdiagnosed in clinical practice. Surprisingly, the onset of symptoms did not vary between the group of participants with isolated abdominal symptoms and those with skin symptoms, and there were no differences in clinical or laboratory parameters between the 2 groups, except for a trend toward a higher proportion of females having isolated abdominal symptoms. This finding is unlikely to be attributable to uterine cramps because most female participants with isolated abdominal reactions were prepubescent.¹¹

Dose Dependence, Cofactors, and Type of Food Ingested

Unlike (most) classic IgE-mediated food allergies, there is marked intraindividual variability in the dose required to cause an α -gal reaction, with many individuals reporting mild or no reactions with some exposures but severe reactions with other exposures. Others still react to microgram quantities of α -gal present in milk and gelatin-containing medications²⁸ and sweets.³³

The concentration of α -gal varies in different foods, being particularly high in foods derived from internal organs.³⁴ In some areas of Germany, the consumption of meat products from organs such as pork kidney is not uncommon,^{35,36} partially accounting for the reported reactions in this setting. Ingestion of these high-risk foods was found to produce symptoms of α -gal syndrome much more rapidly than ingestion of mammalian muscle meat.^{34,35}

Consumption of lipid-laden mammalian meat has also been associated with more consistent severe hypersensitivity responses in α -gal sensitized and allergic individuals.³⁷

Individual susceptibility to elicitation of symptoms and the severity of the reaction may be influenced by other factors apart from merely the degree of sensitization. In patients with mastocytosis, as little as 3 to 5 g of pork kidney may be sufficient to elicit anaphylactic reactions.³⁸ This individual susceptibility can in addition be modulated by so-called cofactors or augmentation factors.³⁹ In keeping with many other forms of allergy, particularly exercise-induced anaphylaxis, the most prominent cofactors are exercise, nonsteroidal analgesics, and alcohol. Exposure to these cofactors may enhance absorption of α -gal-containing epitopes, or cofactors may increase susceptibility to react to even low doses of α -gal antigen, contributing to severe allergic responses to α -gal.³⁰

Age of Affected Individuals

The age of individuals experiencing reactions to red meats varies in cohorts across the world. Most reactions are reported in adult populations, often specifically related to a risk factor for acquisition of sensitization through tick bites,⁴⁰ such as being a hunter, hiker,⁴¹ or forest worker.^{14,15} However, the syndrome also occurs in children. In the high-risk area of Virginia, 45 of 51 screened pediatric patients with a clinical history of delayed reactions to mammalian meat were sensitized to α -gal.⁴² In the South African cohort, participants with α -gal allergy were younger (median, 12 years; interquartile range, 8–25.5 years; almost two-thirds being 13 years or younger) than described in other cohorts.¹¹ This likely results from the ubiquitous exposure to causative ectoparasites or endoparasites in the region in which the study was performed. Allergic reactions have not differed significantly by age, and children are likely to report urticaria, gastrointestinal distress, and anaphylaxis just as demonstrated in adults with an equally delayed response.

Sex Differences in Sensitization or Symptoms

Questions about the ratio of male and female patients with α -gal syndrome have been reported in relation to the prevalence and symptoms. In this regard, it is important to recognize that the patterns of patient presentation or referrals can be strikingly different in different communities.

In the earliest reports, there appeared to more male than female patients,^{5,13} but these were small cohorts. At the time it was assumed that this reflected more men hunting and/or working outside. However, more recent, larger cohorts have found no significant difference between male and female cases. In the South African cohort, there were more females than males diagnosed with α -gal allergy.¹¹ This finding likely reflects the widespread exposure to ticks in traditional rural African settings and the local gender roles where women spend much of their day working outside.

Some data have suggested that more women get symptoms early (ie, in <3 hours).¹¹ However, most of that data come from challenge studies in younger patients, and it is not clear what element of the challenge is responsible for early reactions. Currently, the worldwide data do not suggest evidence of a significant sex difference in either the IgE response to α -gal or the presenting signs and symptoms during a reaction. However, more studies are needed to address this definitively, particularly because one murine model of α -gal food allergy appears to have a more severe phenotype in male mice (Choudhary and Commins, oral personal communication, February 2019), possibly hinting that sex differences (even subtle) in clinical presentation of α -gal syndrome in humans may emerge with further study.

Interactions With B Antigen

The α -gal epitope is structurally similar to the blood group B-antigen, which has an extra fucose residue on the glycan core compared with the α -gal epitope. The homology between α -gal and the B-antigen likely explains why individuals with blood group B (B and AB) have nearly nonexistent IgE and low IgG responses to α -gal.^{43,44}

This finding suggests that IgE response to α -gal may result from class switch of preexisting B cells.^{41,45,46} However, several questions remain regarding the mechanisms of α -gal specific IgE production from the B-cell subsets in meat allergic individuals. Although IgG4 is often considered part of a traditional type 2 immune response, high-titer IgG4 to α -gal has not consistently been reported in individuals with α -gal syndrome.^{44,45,47}

Interestingly, the effect of blood type on anti- α -gal immunity and incidence of infectious diseases was lately reported by Cabezas-Cruz et al,⁴⁸ indicating that susceptibility to malaria and tuberculosis correlated positively with the frequency of blood type B in endemic regions. These diseases are caused by pathogens with α -gal on their surface. However, this positive correlation was not found in the case of dengue fever, which is caused by a pathogen without α -gal.

Hidden α -gal Ingestion: Idiopathic Anaphylaxis

In an area of high prevalence of α -gal allergy, 9% of participants referred with a diagnosis of idiopathic anaphylaxis were found to have α -gal allergy.⁴⁹ Similarly, a report from Memphis, Tennessee, stated that α -gal syndrome was the major cause of adult-onset anaphylaxis in their clinics and that with the recognition of α -gal the number of cases of idiopathic anaphylaxis has significantly decreased.⁵⁰ Taken together, these studies support a role for systematic assessment of possible α -gal allergy in patients with idiopathic anaphylaxis who live in high-prevalence areas. This assessment should involve a detailed history of any meat associated reactions and tick bite exposure in addition to measurement of specific IgE in the serum.

Anaphylaxis From Tick Bites

Acute anaphylactic reactions to tick bites are not mediated by α -gal sensitization but through sensitization to other tick proteins.⁵¹ This type of tick anaphylaxis is uncommon in most countries apart from Australia where bites from *Ixodes holocyclus* cause these severe reactions.⁵² Anaphylactic reactions caused by bites from *Ixodes pacificus* have also been reported.⁵³ However, generalized urticaria after several tick bites might be attributable to α -gal related symptoms rather than other tick proteins. One such possibility is southern tick associated rash illness (STARI), which has been associated with bites from *A. americanum* but without a known causative organism or infectious origin.⁵⁴ Although STARI has many atypical features of a classic allergic reaction, including fatigue, fever, headache, and muscle or joint pains, and the rash is atypical of urticaria, it is possible that some cases diagnosed as STARI are α -gal-related urticaria appearing several hours after consumption of red meat.

Beyond Allergy?

A link between the immune response to α -gal and nonallergic diseases has been suggested by reports of elevated antibody titers to α -gal in conditions such as Crohn disease and thyroid disease.^{55–57} A recent report from an area where ticks are endemic found an association between α -gal IgE sensitization and the severity of coronary artery disease.⁵⁸ A working model to explain the connection posits that long-term ingestion of α -gal epitopes could stimulate cells present in atherosclerotic lesions, an idea that is supported by the known role of mast cells in atherosclerotic

lesions.⁵⁹ It remains to be seen whether this observation will be borne out in prospective investigations, but it raises the possibility that IgE specific to α -gal could be related to disease phenotypes distinct from traditional allergic disease.

It is unclear whether the α -gal syndrome is a newly emergent food allergy or whether it has merely been unrecognized in the past. Increasing human populations and change in lifestyle may account for increased contact between humans and ticks. Other effects of human modification of the environment may also account for increased contact between humans and ticks. For example, in the rural South African population, it is possible that the reduction in the bird population that survives on ticks has led to an increase in the tick burden in the local cattle. In the United States, it has been suggested that reduction in hunting and resultant increase in the deer population has led to an increased possibility of tickborne diseases.

Conclusion

The immune response to α -gal can include innate and dominantly adaptive immunity with high antibody production. This finding may explain the varied clinical syndromes associated with the anti-gal response: rejection of xenotransplantation, IgE-mediated allergic disease, autoimmune disease, and coronary heart disease. α -Gal-directed IgE mediates adverse hypersensitivity responses to drugs and foods, affecting multiple organ systems. It remains unclear what immunologic insult pushes the natural anti- α -gal response to severe and delayed IgE responses. It has become clear that the development of α -gal specific IgE is associated with species-specific tick bites, which could promote anti-gal antibody class switching to IgE, leading to loss of tolerance and clinical manifestations of the α -gal syndrome. This unusual food allergy has generated a renewed attention to the biological importance of the α -gal not just in immune-mediated hypersensitivity responses but also in endocrine, cardiac, and other nonallergic conditions.

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