

Review Article

Tick–human interactions: from allergic klendusity to the α -Gal syndrome

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Ticks and the pathogens they transmit, including bacteria, viruses, protozoa, and helminths, constitute a growing burden for human and animal health worldwide. The ability of some animal species to acquire resistance to blood-feeding by ticks after a single or repeated infestation is known as acquired tick resistance (ATR). This resistance has been associated to tick-specific IgE response, the generation of skin-resident memory CD4⁺ T cells, basophil recruitment, histamine release, and epidermal hyperplasia. ATR has also been associated with protection to tick-borne tularemia through allergic klendusity, a disease-escaping ability produced by the development of hypersensitivity to an allergen. In addition to pathogen transmission, tick infestation in humans is associated with the α -Gal syndrome (AGS), a type of allergy characterized by an IgE response against the carbohydrate Gal α 1-3Gal (α -Gal). This glycan is present in tick salivary proteins and on the surface of tick-borne pathogens such as *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, the causative agents of Lyme disease and granulocytic anaplasmosis. Most α -Gal-sensitized individuals develop IgE specific against this glycan, but only a small fraction develop the AGS. This review summarizes our current understanding of ATR and its impact on the continuum α -Gal sensitization, allergy, and the AGS. We propose that the α -Gal-specific IgE response in humans is an evolutionary adaptation associated with ATR and allergic klendusity with the trade-off of developing AGS.

Introduction

Ticks are hematophagous ectoparasites of vertebrates. Besides causing direct damage associated with blood feeding and in some cases through the excretion of toxins within their saliva, the main relevance of ticks lies in the wide variety of pathogens they can transmit, including bacteria, viruses, protozoa, and helminths [1]. For example, Lyme disease or borreliosis, caused by the spirochete *Borrelia burgdorferi*, is the most common tick-borne disease in temperate regions of North America, Europe, and Asia, and the number of reported cases has increased sharply in the last years [2]. *Anaplasma phagocytophilum* is a pathogen causing granulocytic anaplasmosis in humans and tick-borne fever in animals [3]. Recent evidence incriminates ticks in a novel form of delayed anaphylaxis in humans [4]. In particular, the immunoglobulin (Ig) E antibody response to the carbohydrate Gal α 1-3Gal (α -Gal) following a tick bite is associated with delayed anaphylaxis to mammalian meat (named 'alpha-Gal syndrome', hereafter AGS) [4–9]. This was the first proof that glycans play a major role in allergy development with the potential to cause fatal anaphylaxis. AGS has been reported in the USA, Australia, Sweden, Germany, Spain, and France, and has been

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associated with several tick species including *Amblyomma americanum* (USA), *Amblyomma sculptum* (Brazil), *Haemaphysalis longicornis* (Japan), *Ixodes holocyclus* (Australia) and the principal vector of Lyme borreliosis in Europe, *Ixodes ricinus* [5].

Inactivation of the α -1,3-galactosyltransferase gene in old world monkeys, apes, and humans resulted in an almost unique ability of this group of primates to produce high antibody titers against α -Gal [10]. Gut microbiota bacteria induce IgM and IgG anti- α -Gal antibodies (Abs) which are widely expressed in humans [11]. At high levels, these Igs protect against malaria transmission by *Anopheles* mosquitoes [12]. Furthermore, α -Gal immunization protects against Chagas disease and leishmaniasis [13,14]. All pathogens producing these diseases have the α -Gal epitope exposed on their surface [12–14]. This finding suggests that anti- α -Gal IgM and IgG protect against pathogens expressing α -Gal. Ticks produce endogenous α -Gal [15] and the glycan have been detected in tick-borne pathogens such as *B. burgdorferi* and *A. phagocytophilum* [16]. In contrast to the gut microbiota, α -Gal in tick salivary glycoproteins induces a significant increase in the levels of anti- α -Gal IgE in the human host leading, in some cases, to AGS [8]. This review summarizes our current understanding of the role of allergy in the development of resistance to tick infestation and tick-borne diseases as well as the pathological role played by α -Gal-specific IgE antibodies in AGS.

Allergic responses and acquired tick resistance

The tick-host interface is characterized by complex interactions between the host and the arthropod saliva and cement [17–19]. The intrusion of tick mouthparts disrupts the epidermis, enters the dermis, and triggers host hemostatic responses, such as coagulation, vasoconstriction, and platelet aggregation [20,21]. Host keratinocytes, endothelial cells, and different leukocytes engage in the immediate response to a tick bite by releasing anti-microbial peptides and pro-inflammatory cytokines and chemokines that lead to the recruitment of neutrophils and other pro-inflammatory cells [17]. Subsequently, adaptive immune cells, such as T and B cells, unfold antigen-specific inflammatory and humoral responses against tick antigens [18]. In response, ticks secrete pharmacologically active saliva containing vasodilator, anti-hemostatic, anti-inflammatory, and immunosuppressive compounds that together result in a molecular counterattack that hampers the host response allowing for a successful blood feeding and completion of the so-called first infestation [17–19]. Interestingly, while basophils are not recruited during the first tick infestation, intensive basophil infiltration, histamine release, epidermal hyperplasia and thickening of epidermis hinder tick attachment and/or blood-feeding during a second infestation [20]. Considering that keratinocytes express functional H1 receptors [22], it is reasonable to assume a positive feedback loop by which histamine released from basophils recruited at the tick feeding site promotes keratinocyte proliferation [23,24], which in turn induce further basophil activation and recruitment through epidermal keratinocyte-derived basophil promoting activity [25].

Infiltration of basophils, accounting for up to 70% of the skin-infiltrating cells [26], in the tick re-infestation site leads to acquired tick resistance (ATR) [20]. The non-redundant role of basophils in ATR has been demonstrated using different experimental approaches [27]. Firstly, early studies by Brown et al. [27] in guinea pigs demonstrated that anti-basophil serum depleted basophils and abolished ATR. Secondly, using RT-PCR analysis, Wada et al. [28] detected transcripts of the *Mcpt8* gene encoding the basophil-specific protease mMCP-8 in tick-feeding sites during the second but not first infestation, a finding confirmed by Ohta et al. [29] by using intravital imaging of *Mcpt8*^{GFP} mice. Thirdly, basophil ablation by either basophil-depleting monoclonal antibodies or diphtheria toxin-mediated basophil depletion before the second infestation completely abolished ATR [28]. The role of basophils in ATR remains to be demonstrated in humans, but basophil infiltration at the tick-feeding sites has been observed in humans [30]. Importantly, ATR is not confined to tick feeding sites, but it can be induced in portions of the skin distant from the first infestation site [20]. This suggests that ATR is a systemic rather than a localized immune response to tick infestation. Despite mast cells also play a role in the manifestation of ATR [20], only basophils and not mast cells appear to play an important role in IgE-dependent ATR via Fc ϵ RI-mediated activation in mice (Figure 1).

Tick-host coevolution and ATR

The absence of ATR in reservoir hosts (i.e. natural hosts of a specific tick species that is frequently parasitized by the tick to complete its natural life cycle) and the strong ATR of non-reservoir hosts (i.e. hosts that could not be encountered naturally by a specific tick species and are not parasitized by the tick to

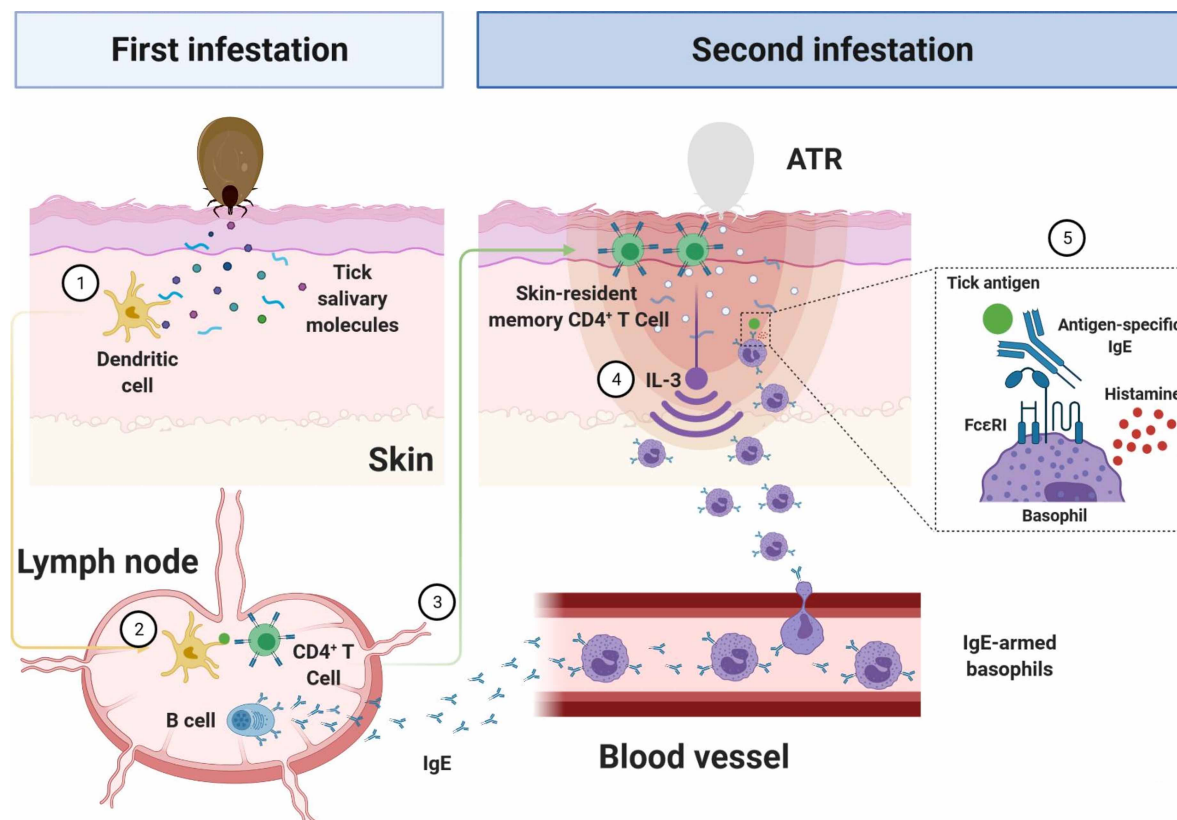


Figure 1. Immune mechanism of ATR.

The manifestation of ATR can be separated in two phases. The sensitization phase in which the first tick infestation exposes dendritic cells to tick salivary molecules (1) and activated dendritic cells migrate to draining lymph nodes activating tick antigen-specific B cells and CD4⁺ T cells (2). Activated plasma B cells release anti-tick specific IgE to the circulation and these antibodies bind high-affinity receptors on the surface of blood-circulating basophils. A portion of activated CD4⁺ T cells differentiate to memory cells, migrate and remain in the skin (3). In a second tick infestation, the effector phase of ATR is triggered when skin-resident memory CD4⁺ T cells are activated by tick salivary molecules and secrete IL-3, which in turns recruits IgE-armed basophils (4). Skin-infiltrating basophils cluster around the tick mouthpart and release histamine after being stimulated by tick salivary antigens and cross-linking of tick antigen-specific IgE and FcεRI complex on the cell surface (5) [20].

complete its natural life cycle) suggest tick-host adaptive coevolution [20,31]. For example, *Peromyscus leucopus* (white-footed mouse), the natural host of *Ixodes scapularis*, does not show ATR even after repeated infestations with *I. scapularis* nymphs [32]. Likewise, two strains of laboratory mice *Mus musculus* (i.e. BALB/c and C3H/HeN), used as models of natural hosts, could not develop ATR to *I. scapularis* or *Ixodes ricinus* nymphs upon repeated infestations [33–35]. In contrast, *I. scapularis* ticks attached to sensitized guinea pigs, *Cavia porcellus*, fed poorly or were dislodged [32]. Furthermore, when repeatedly infested with non-native ticks for mice, *Dermacentor variabilis* and *Haemaphysalis longicornis*, BALB/c and C3H/HeN mice showed strong ATR [20]. The mechanism underlying the conditional manifestation of ATR remains poorly understood. Notably, transcriptome and proteome analyses demonstrated that ticks of the same species generate host-specific profiles of tick salivary proteins [35,36]. In addition, tick salivary proteins are usually encoded by multigenic families [37]. In an attempt to explain the expansion of salivary gene families in ticks, Chmelař et al. [37] proposed that multigenic families of tick salivary secreted proteins evolved to reduce the immunogenicity of immunomodulatory tick effectors by continuous antigenic shift. Antigenic matches in tick-natural host entanglements may render some of the steps of ATR development (Figure 1) as non-operative. In contrast, antigenic mismatches in tick-non-natural host entanglements may produce a strong host response resulting in efficient ATR.

Evolution of α -Gal synthesis and the emergence of allergic klendusity in humans

Bell et al. [38] proposed allergic klendusity as the immune property by which tick-sensitized rabbits developed resistance to tick-borne *Francisella tularensis* infection. Allergic klendusity refers to a disease-escaping ability produced by the development of hypersensitivity to an allergen. To date, the klendusity phenomenon has not been associated with specific allergens present in ticks and tick-borne pathogens. Despite that the manifestation of ATR has not been tested in humans, indirect evidence suggests that the individual response to tick salivary allergens plays an important role in the development of allergic sensitization to tick antigens. For example, a comparative proteomics approach used to characterize tick proteins inducing IgE response in individuals with records of tick bites revealed an individual-specific IgE antibody response to tick proteins in both healthy (i.e. individuals exposed to tick bites, but without allergic reactions to tick bites) and patients that developed anaphylactic reactions to tick bites [39]. A high proportion of tick glycoproteins recognized by patient sera carried the carbohydrate α -Gal [39]. Allergic responses to tick bites have also been associated with protection against tick-borne pathogen infection in laboratory animals [38,40] and humans [41]. For example, fewer episodes of Lyme disease were reported among people that experienced itching related to tick attachment compared to those that did not develop such reactions [41]. One possible explanation for this observation is that some tick allergens, also expressed on tick-borne pathogens, trigger the manifestation of allergic klendusity. Recent evidence suggests that the carbohydrate α -Gal can be one of such klendusity-inducing allergens.

The evolution of α -Gal synthesis in metazoan is very peculiar. Most extant mammals express the antigen α -Gal due to the activity of a functional α -1,3-galactosyltransferase (α 1,3GT) enzyme encoded by the gene *GGTA1* (Figure 2A). However, up to date, no *GGTA1* orthologous gene has been identified in non-mammalian craniates. Nor the ability to synthesize α -Gal has been reported in any species of these animals. Of note, the presence of α -Gal in jawless fishes has not been tested yet. Therefore, although there is no reason to believe that jawless fishes (e.g. Lampreys) produce endogenous α -Gal, we cannot rule out the possibility of the glycan synthesis in this lineage of freshwater fish. However, the evolutionary history of *GGTA1* displayed in Figure 2A suggests that the common ancestor of craniates could not synthesize the α -Gal. In addition, the most parsimonious explanation for the *GGTA1* distribution in Craniata is that this gene appeared for the first time in the common ancestor of mammals, as an evolutionary innovation (Figure 2A). Most mammalian tick hosts such as those in the Orders Artiodactyla, Rodentia and Carnivora [42,43] express a functional copy of *GGTA1*, synthesize endogenous α -Gal [44], and therefore are not able to develop an effective immune response against this self-antigen. It is then reasonable to assume that coating the salivary antigens with α -Gal may disguise tick molecules during feeding on non-catarrhine mammalian hosts.

Of note, while α -Gal is not expressed in Mesostigmata, the sister taxa of Ixodida, or other chelicerates (i.e. Scorpions), this glycan has been reported to be present in several tick species of the genera *Ixodes* [15,45,46], *Amblyomma* [45,47] *Rhipicephalus* [19,39], *Hyalomma* [39], and *Haemaphysalis* [48] (Figure 2B). The endogenous synthesis of α -Gal by ticks is supported by the presence of three genes in the *Ixodes scapularis* genome, *b4galt7*, *a4galt-1*, and *a4galt-2*, encoding for enzymes with α 1,3GT activity [15]. Furthermore, bacterial α 1,3GT genes including *gspA*, *waaL*, *waaO*, *waaJ*, *rfaJ*, and *waaR*, were identified in the microbiome of *I. scapularis* and *I. ricinus* [49]. On the other hand, inactivation of the gene *GGTA1* in the common ancestor of old-world monkeys, apes, and humans (i.e. a group of primates known as the Catarrhines) resulted in the absence of α -Gal in the cells of these animals, and in consequence, the ability of this group of primates to produce high antibody titers against α -Gal [10]. The production of high levels of anti- α -Gal antibodies was also confirmed in birds and fish, which as humans do not synthesize endogenous α -Gal [50–52]. Thus, the anti- α -Gal response of catarrhine animals might induce resistance to blood-feeding by ticks. Recently, we tested this hypothesis in α 1,3GT-deficient mice (α 1,3GT KO), the only available non-primate mammal that can produce natural anti- α -Gal IgM and IgG at levels similar to those in humans. The results showed that anti- α -Gal IgM and IgG produced high mortality in ticks feeding on α 1,3GT KO mice immunized with an α -Gal-containing *Escherichia coli* [49]. Interestingly, α -Gal has also been detected on the surface of tick-borne pathogens such as *B. burgdorferi* and *A. phagocytophilum* [16], although the ability of tick-borne pathogens to produce endogenous α -Gal remains to be tested. Of note, among individuals exposed to tick bites, those with Lyme disease did not develop high anti- α -Gal IgE levels when compared to those that develop anaphylactic reactions to α -Gal or AGS (a disease associated with hypersensitivity reactions to α -Gal) but did not develop Lyme disease [46].

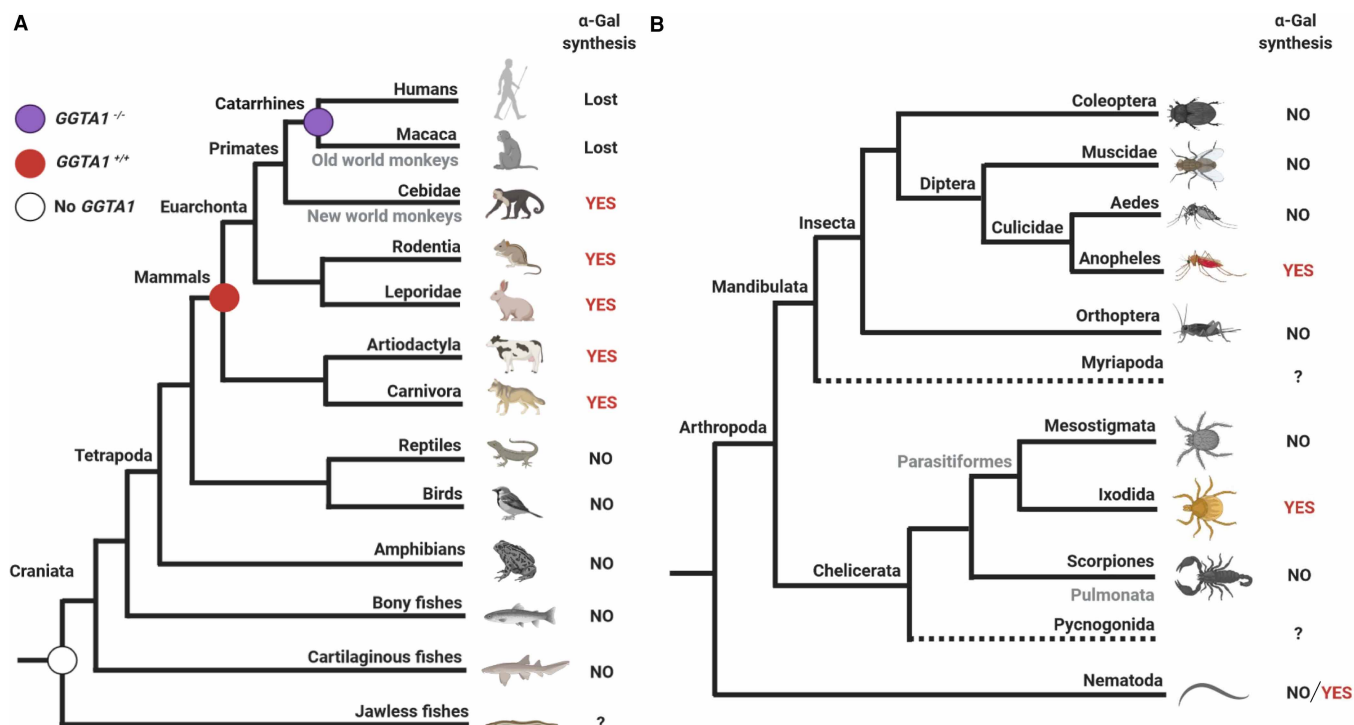


Figure 2. Evolutionary history of α-Gal synthesis in metazoans.

(A) The distribution of *GGTA1* gene among representative taxa in Craniata is displayed. The data on the *GGTA1* distribution among craniates was collected from different sources. Humans [44], Macaca [44], Cebidae [44], Rodentia [44], Leporidae [44], Artiodactyla [44], Carnivora [44], Reptiles [44], Birds [44], Amphibians [44], Bony fishes [44,51,52] and Cartilaginous fishes [99]. (B) The distribution of α-Gal synthesis ability among representative Arthropods was collected from different sources, Mesostigmata [100], Ixodida [15,19,39,45–47], Coleoptera, Muscidae, Orthoptera, and Scorpions [99]. Some members of the phylum Nematoda do not express α-Gal (e.g. *Toxocara canis* and *Ascaris suum*, [101]), while others do express the glycan (e.g. *Parelaphostrongylus tenuis* and *Haemonchus contortus* [102,103]). Similarly, some members of the family Culicidae were reported to express α-Gal (e.g. *Anopheles* sp. [12]), while in others, the carbohydrate has not been found (*Aedes* sp. [99]). Taxa for which information on the presence of α-Gal is not available were questions marked (?).

Thus, the gain of α-Gal-synthetizing ability in Ixodida (Figure 2B) mirrors the loss of α-Gal-synthetizing ability in the Catarrhines (Figure 2A). The presence of α-Gal in Ixodida and tick-borne pathogens on one hand, and the absence of the glycan in humans, on the other hand, suggest that the loss of α-Gal expression from the cells and the capacity to produce anti-α-Gal IgE allowed humans to manifest allergic klendusity to tick infestation and tick-borne pathogen infection. Thus, α-Gal sensitization involving increased anti-α-Gal IgE and basophils activation in response to recurrent tick bites in humans may mediate allergic klendusity to tick-borne pathogens expressing the allergen α-Gal such as *B. burgdorferi* and *A. phagocytophilum*. For reasons currently unknown, in some individuals, α-Gal sensitization after tick bites becomes pathologic and produces AGS.

The continuum α-Gal sensitization, allergy, and the AGS

The vast majority of patients who develop the AGS have tolerated mammalian meat for many years before being sensitized by tick bites, which suggests that as-yet-unknown tick salivary constituents break the oral tolerance to food allergens and promote Th2-mediated immunity [53]. However, the majority of α-Gal-sensitized individuals will not develop the AGS [54]. It has become apparent that the route of sensitization through the skin via tick bites is of great importance for IgE response to α-Gal [55]. Individuals living in areas where ticks are not present, such as Northern Sweden, do not have anti-α-Gal IgE despite mammalian meat is widely represented in their diet [8]. Results of several experimental studies provide additional evidence about the importance of the skin in sensitization to both α-Gal and tick salivary proteins. Subcutaneous inoculation, but

not intraperitoneal injections of *A. americanum* larval whole-body extracts, results in a robust tick-specific IgE immune response and hypersensitivity through CD4⁺ T Cell- and MyD88-dependent pathways in wild-type mice [56]. Saliva from *Amblyomma sculptum* delivered by subcutaneous injection or directly by ticks fed on α -Gal-deficient mice induces production of IgE reactive to α -Gal [47]. The magnitude of the IgE immune response seems to be dose-dependent as individuals who are exposed to tick bites more than two times have significantly higher levels of IgE specific to α -Gal than those with only one or no tick bites [57]. Furthermore, repeated inoculation of salivary gland extracts from partially fed *A. americanum* into different transgenic murine models results in α -Gal sensitization and systemic allergic reaction after meat challenge [52,58,59]. The mechanisms whereby a tick induces sensitization to α -Gal are not known, but some species-specific components of tick sialome that have potent adjuvant activity may be required alongside the α -Gal epitope for initiation of the percutaneous immune reaction [60]. Tick bites may also compromise the skin epithelial barrier by direct tissue damage or alteration of the skin microbiome consequently inducing the IgE-mediated immune response to α -Gal [55]. Recent studies demonstrated that repeated cutaneous exposure to tick salivary antigens increase the number of basophils and mast cells at the site of tick bites [57,61,62]. The latter study also proposed that urticaria at the site of a previous tick bite could be an early sign of an allergic reaction in individuals with AGS [62]. Basophils also play a crucial role in acquired protective immunity to subsequent tick infestations [28,63]. Besides, tick bites induce the production of various alarmins (e.g. thymic stromal lymphopoietin TSLP, interleukins IL-25, IL-33) in the host's skin that can attract innate immune cells and increase uptake of α -Gal antigens, which are further presented to CD4⁺ T cells, provoking the Th2-mediated immune response [60]. In this context, tick bites may generate a microenvironment that facilitates the production of IgE antibodies to α -Gal and triggers AGS [60,61].

From mammalian meat consumption to anaphylaxis

The carbohydrate epitope α -Gal is present on glycoproteins and glycolipids in tissues of non-catarrhine mammals and saliva of various tick species, but also in some pharmaceutical products prepared in culture systems or with excipients derived from mammals, such as the monoclonal antibody cetuximab, anti-venoms, and gelatin-containing vaccines [4]. The term 'syndrome' (a group of signs and symptoms that occur together and characterize a particular condition) has been recently introduced to better describe the clinical relevance and the complexity of the allergic reactions to mammalian meat and other α -Gal-containing products [9,60]. The delayed nature of the reactions, IgE reactivity to non-protein molecules, and the strong association with tick bites are the main features that distinguish the AGS from other classical IgE-mediated food allergies [64]. The clinical manifestations that usually occur 3–6 h after mammalian meat consumption involve urticaria with severe pruritus, recurrent angioedema, and signs of systemic anaphylaxis. Affected patients may also suffer from nausea, diarrhea, indigestion, and severe abdominal pain, while arthritis and chronic pruritus are less common [6,65]. Some individuals with AGS, however, do not display clinical signs after exposure to α -Gal [54], suggesting that amount or form of accessible α -Gal determinants and other modifying factors may affect the likelihood and severity of the reaction [9,66,67]. Consumption of pork kidney and other mammalian innards, which contain quantitatively more α -Gal epitopes than muscle meat [68], frequently results in a severe and more rapid allergic reaction that appears within 2 h following the allergen exposure [68,69,70]. Apart from the allergen dose and possible individual genetic factors, concomitant alcohol consumption, physical exercise, and use of nonsteroidal anti-inflammatory drugs appear to be important modifiers that influence clinical presentation and the risk of AGS development in α -Gal sensitized individuals [71–73]. It is believed that these co-factors increase the gastrointestinal absorption and the allergen uptake by increasing blood circulation, histamine release, and gastrointestinal permeability leading to a shorter delay in symptom appearance and a more severe allergic reaction [71,74]. The AGS can also involve immediate and potentially fatal IgE-mediated reaction in patients after parenteral administration of drugs and vaccines containing mammalian products (e.g. gelatin-based colloids, collagen, albumin) [75,76]. Indeed, the reported associations between increased levels of anti- α -Gal antibodies and non-allergic disorders, such as thyroid disease [77], inflammatory bowel disease [78], and increased severity of coronary artery disease [79] highlight the relevance of α -Gal IgE beyond traditional food allergies.

Metabolic considerations

The mechanisms underlying the delay in the onset of the allergic reactions are not fully elucidated, but the time required for digestion, absorption, and presentation of α -Gal epitopes to the host immune system seems

to have an important role [60,80]. The delay in symptoms likely reflects the delayed arrival of relevant glycolipid forms of α -Gal to blood circulation and their presentation to mast cells in peripheral tissues [81]. The kinetics of lipid metabolism, which involves fat assembly into chylomicrons and transit through lymphatics and thoracic duct before entering the systemic circulation, suggests the importance of α -Gal-bearing glycolipids in the delayed allergic response [67,81]. This kinetic pattern corresponds with the results of *in vitro* basophil activation tests, which revealed a significant increase in CD63⁺ activation markers in the blood of patients with IgE to α -Gal taken three and five hours after meat challenge, while basophils collected at early time points were not considerably activated [82]. The 'lipid hypothesis' is further supported by Román-Carrasco et al. [83], who demonstrated that α -Gal bound to lipids, but not proteins, passes through a Caco-2 epithelial barrier and gets incorporated into chylomicrons. Moreover, Steinke et al. [80] reported significant and time-dependent differences in baseline expression of several metabolites in the biochemical pathways between the healthy control group and meat allergic subjects. The most dysregulated pathways were in lipid and fatty acid metabolism, where levels of multiple fatty acids, such as caprylate and stearate, were higher in the control group than in the meat allergic group following the meat challenge. These changes indicate fat hydrolysis, absorption, and/or lipid oxidation and possibly explain the delay in symptoms observed in patients with AGS. Levels of lipids conjugated to carnitine were also lower in allergic individuals and this suggests reduced fatty acid transport into mitochondria. Together, the results imply that metabolic changes, including those related to amino acid catabolism, lipid and carbohydrate metabolism, and bile acids synthesis, are all associated with AGS development and its clinical outcome. The metabolic changes may also reflect the differences in the intestinal microbiome and absorption of nutrients [80].

Risk factors associated with the α -Gal syndrome

A growing body of research indicates that α -Gal sensitization is induced by bites of certain ticks, but the molecular mechanisms involved are yet to be explored and understood. Recent studies demonstrated the presence of α -Gal-carrying proteins in the saliva of various tick species including *I. ricinus* and *A. americanum* [45,84]. These ticks are regarded as the predominant cause of AGS in Europe and the United States, respectively [8,45,70,84]. The strong connection between α -Gal sensitization and tick bites is further supported by the fact that some AGS patients who avoid recurrent bites have declined anti- α -Gal IgE antibody titers and can tolerate mammalian meat again [85]. Indeed, the IgE antibody levels decrease over the winter season when the questing activity of ticks is very low [54]. Therefore, outdoor activities and ecological components associated with exposure to ticks are the most critical risk factors for AGS [4]. Although it was initially thought that older individuals are at higher risk to develop a severe allergic reaction to α -Gal, a recent study showed no difference in the timing, clinical presentation, and IgE antibody levels to α -Gal or in tick exposure history between pediatric and adult patients [65]. The role of atopy in AGS development is still a matter of debate and while some groups report the importance of atopy as a predisposing factor influencing symptom severity [72,54,64], others showed no positive correlation between AGS and a previous atopic disposition [65,7]. The self-tolerance to the B antigen may result in a reduced risk of α -Gal sensitization and development of AGS [86]. The blood group B antigen is structurally very similar to the α -Gal epitope [87], and subjects who have blood groups B or AB seems to be partially protected from developing the AGS in contrast to those with blood groups A and O [65,85,88]. Moreover, they produce fewer anti- α -Gal IgE antibodies compared to individuals lacking antigen B [87,88]. Despite the possible protective role of blood groups B and AB, the blood type is a frequently overlooked factor in epidemiological studies of AGS [89]. As discussed above, other factors such as alcohol consumption, physical exercise, use of some medications, or cat ownership may also influence the risk of developing the AGS and disease severity [72,73].

Diagnosis and management of the α -Gal syndrome

The unspecific clinical presentations and the delay in symptoms onset make the diagnosis of AGS very complex and challenging [90]. Therefore, an extensive clinical history is fundamental for an accurate diagnosis of the syndrome. Initial diagnosis can be supported by various laboratory tests and these mainly include skin prick test (SPT), determination of serum specific IgE to α -Gal, and less often food challenges [91]. The SPT with extracts of beef or pork is a widely used diagnostic approach, but it is unreliable as it often yields poor or false-negative results [6]. Better results can be obtained by intradermal injection of fresh pork kidney preparations or 4% gelatin-derived colloid, but this method has not been used in routine allergy diagnosis [6,69,92]. Quantitative measurement of IgE antibodies specific to α -Gal still represents the most reliable diagnostic tool

[64,81], although the diagnostic performance largely depends on the antigens used [93]. Determination of IgE levels by using the abundantly α -Gal-decorated bovine thyroglobulin (bTG) proved to be the most useful test for establishing the diagnosis of the AGS as it exhibits 100% sensitivity and 92.3% specificity [93]. However, α -Gal IgE cannot discriminate between anaphylactic and non-anaphylactic patients or individuals who have AGS from those with asymptomatic α -Gal sensitization [64,91]. The basophil activation test (BAT) has been thus used as an additional *in vitro* diagnostic test to partially overcome this limitation [91]. Despite the food challenge is still the gold standard in food allergy diagnosis, this method is not recommended for diagnosis of the AGS since it may cause a severe and potentially fatal anaphylactic reaction [60]. Currently, there is no treatment to prevent or cure the AGS and complete avoidance of tick bites and mammalian meat and other α -Gal-containing foods is the only strategy proposed for preventing allergic reactions in patients [81,94]. Many patients suffering from the AGS are able to overgrow the hypersensitivity and tolerate mammalian meat again either through prolonged tick bite avoidance or through continued exposure to very low doses of α -Gal [65,81,85].

Conclusions

Our understanding of ATR and its impact on the α -Gal sensitization, allergy, and the AGS is a challenge that needs to be addressed to reduce the risk of tick-induced allergies. Current evidence supports that the specific IgE response to α -Gal in humans is an evolutionary adaptation associated with ATR and allergic clendusity with the trade-off of developing AGS. The evolutionary history of α -Gal synthesis in ticks and humans suggests that the capacity to develop an IgE-mediated immune response to α -Gal allowed humans to manifest allergic clendusity to tick bites and tick-borne pathogens. Bites by certain tick species may generate a microenvironment that facilitates the production of IgE antibodies to α -Gal and triggers AGS. Metabolic changes are associated with AGS and its clinical outcome and may also reflect the differences in the intestinal microbiota and absorption of nutrients. The study of the immune response to α -Gal suggests that it can be used to promote protection against pathogen infection [95–98]. The challenge is to identify the mechanisms and biomolecules triggering the AGS and the development of diagnostic tools and interventions to reduce its incidence while boosting protection against infectious diseases.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contributions

A.C.-C. and A.H. prepared the initial draft of this review. A.C.-C. was responsible for the preparation of the figures. L.M.-H. and M.C. contributed to the analysis of the evolution of alpha-Gal synthesis. J.F. and A.C.-C. were responsible for the initial outline of the review and for the writing and editing of the manuscript. A.H. and L.M.-H. also contributed to the preparation of the manuscript.

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Abbreviations

Ab, Antibody; AGS, alpha-Gal syndrome; ATR, acquired tick resistance; BAT, basophil activation test; bTG, bovine thyroglobulin; IL, interleukin; SPT, skin prick test; TLSP, thymic stromal lymphopoietin; α 1,3GT KO, α 1,3GT-deficient mice; α 1,3GT, α -1,3-galactosyltransferase; α -Gal, Gal α 1-3Gal β 1-4GlcNAc-R.

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