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Brief Reviews

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Where's the Beef? Understanding Allergic Responses to Red Meat in Alpha-Gal Syndrome

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Alpha-gal syndrome (AGS) describes a collection of symptoms associated with IgE-mediated hypersensitivity responses to the glycan galactose-alpha-1,3-galactose (alpha-gal). Individuals with AGS develop delayed hypersensitivity reactions, with symptoms occurring >2 h after consuming mammalian ("red") meat and other mammal-derived food products. The mechanisms of pathogenesis driving this paradigm-breaking food allergy are not fully understood. We review the role of tick bites in the development of alpha-gal–specific IgE and highlight innate and adaptive immune cells possibly involved in alpha-gal sensitization. We discuss the impact of alpha-gal glycosylation on digestion and metabolism of alpha-gal glycolipids and glycoproteins, and the implications for basophil and mast cell activation and mediator release that generate allergic symptoms in AGS. ***The Journal of Immunology*****, 2022, 208: 267–277.**

Alpha-gal syndrome (AGS) describes a collection of symptoms associated with the glycan galactose-alpha-1,3-galactose (alpha-gal). Alpha-gal is a carbohydrate found in platyrrhine ("broad-nose" or "New World") monkeys and nonprimate mammals (1). These mammals can glycosylate proteins and lipids with alpha-gal because, unlike humans and catarrhine ("down-nosed" or "Old World") monkeys, they possess a functional *GGTA1* gene that encodes for α-1,3-galactosyltransferase (2, 3).

Individuals with AGS can experience allergic reactions to food and medications. They experience delayed hypersensitivity reactions after consuming mammal-derived food products (classically mammalian or "red" meat and, in some cases, dairy products or gelatin) (4, 5) and immediate hypersensitivity responses to injected pharmaceutical products that contain alpha-gal. In fact, AGS was initially identified after the observation of regional differences within the United States in the frequency of adverse reactions to first-time infusions of the cancer drug cetuximab, a chimeric mouse-human IgG1 mAb against epidermal growth factor receptor (6). It was subsequently determined that these reactions were driven by IgE targeted against alpha-gal on the Fab of cetuximab (7). Shortly afterward, two seminal reports from the United States and Australia described a syndrome of delayed urticaria, angioedema, and/or anaphylaxis after the ingestion of red meat in patients with circulating alpha-gal–specific IgE (sIgE) (8, 9), and recognition of AGS as a food allergy emerged. For the purposes of this review, we focus on AGS as a form of food allergy.

AGS disrupts current paradigms for food allergy

AGS cases have been described on every continent except Antarctica (10, 11). In nearly every country with reported AGS cases, bites from hard-bodied ticks have been associated with the development of mammalian meat allergy (9, 11–15). In the United States, *Amblyomma americanum* (lone star tick) is the clinically relevant tick species associated with AGS (4, 11). Several other species, including *Ixodes ricinus*, *Amblyomma sculptum*, *Ixodes holocyclus*, and *Haemaphysalis longicornis*, have been linked to AGS in other countries (11) (Table I). A majority of patients with alpha-gal allergy report urticaria (≥90%) and gastrointestinal symptoms (∼70%) after eating mammalian products (16–19). Reports of anaphylaxis (i.e., allergic symptoms comprising at least two organ systems simultaneously) range from 50 to 65% in most studies (16, 17). In ∼20% of AGS cases, the clinical phenotype involves only gastrointestinal symptoms, primarily severe persistent abdominal cramping, diarrhea, and gastroesophageal reflux (20). Like conventional food allergies, AGS can develop during childhood (20, 21) but also arises in older adults who have tolerated mammalian meat for decades (19, 22).

AGS as a food allergy upsets our conventional expectations of IgE-mediated food allergies. In contrast with conventional food allergies, IgE Abs form against the alpha-gal sugar rather than a protein Ag. In addition, the development of IgE Abs to alpha-gal is associated with tick bites rather than allergen exposure through the gut or via compromised skin epithelium (12).

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Abbreviations used in this article: AGKO, alpha-galactosyltransferase deficient; AGS, alpha-gal syndrome; alpha-gal, galactose-alpha-1,3-galactose; DC, dendritic cell; iNKT, invariant NKT; MHC-II, MHC class II; MZ, marginal zone; OIT, oral immunotherapy; sIgE, specific IgE; TSGE, tick salivary gland extract; TSLP, thymic stromal lymphopoietin; TWBE, tick whole-body extract.

Table I. Global reach of AGS

Continent/Country	Associated Tick	Alpha-Gal Detected in Tick Saliva	References
Africa			
Côte d'Ivoire	*Amblyomma variegatum?*	Unknown	Kaloga et al. (101); reviewed by Cabezas-Cruz et al. (11)
South Africa			Mabelane et al. (20)
Zimbabwe			Reviewed by van Nunen (102)
Asia			
Korea	*Haemaphysalis longicornis*	Yes	Chinuki et al. (103)
	Amblyomma testudinarium	Unknown	Hashizume et al. (14)
	Ixodes nipponensis?	Unknown	Reviewed by van Nunen (45)
Japan	*Amblyomma testudinarium*	Unknown	Hashizume et al. (14)
	Haemaphysalis longicornis	Yes	Chinuki et al. (103)
Australia	*Ixodes holocyclus*	Unknown	Reviewed by van Nunen (102)
	Ixodes (Endopalpiger) australiensis	Unknown	Kwak et al. (104)
Europe			
France			Reviewed by van Nunen (102)
Germany	*Ixodes Ricinus*	Yes	Fischer et al. (50); reviewed by van Nunen (102)
Italy	*Ixodes Ricinus*	Yes	Reviewed by van Nunen (45)
The Netherlands			Reviewed by Cabezas-Cruz et al. (11)
Norway	*Ixodes Ricinus*	Yes	Reviewed by Cabezas-Cruz et al. (11)
Spain	*Rhipicephalus* spp.?	Yes (*Rhipicephalus microplus*)	Nuñez et al. (105); Villar et al. (106)
Sweden	*Ixodes Ricinus*	Yes	Hamsten et al. (107, 108)
Switzerland	*Ixodes Ricinus*	Yes	Michel et al. (90)
United Kingdom			Harper et al. (109)
North America			
Costa Rica	*Amblyomma cajennense*	Unknown	Reviewed by van Nunen (102); Cabezas-Cruz et al. (11)
Panama	*Amblyomma cajennense*	Unknown	Reviewed by van Nunen (45) and Cabezas-Cruz et al. (11)
United States	*Amblyomma americanum*	Yes	Commins et al. (12); Crispell et al. (41)
South America			
Brazil	*Amblyomma sculptum*	Yes	Araujo et al. (110)

A question mark after the name of an associated tick means the tick species listed is suggested but not definitively linked to development of alpha-gal syndrome.

Instead of an immediate hypersensitivity response characteristic of conventional food allergies, allergic symptom onset after meat consumption is often delayed, typically occurring >2 h (often 3–8 h) after the meal (8, 16, 20). This delay in symptom onset frequently makes it challenging to diagnose AGS (5).

There are conflicting reports regarding an association between AGS and other allergic diseases, such as allergic rhinitis or food protein allergy. In a study of 261 American children and adults, the frequency of aeroallergen sensitization was comparable between those with and without detectable circulating alpha-gal–sIgE > 0.35 IU/ml, but there was a higher frequency of sensitization to wheat and stinging insect venom in subjects with AGS (16). By contrast, in two independent cohorts of adult European patients, comprising >2700 people, sensitization to alpha-gal was associated with atopy, defined as a positive skin prick test to any of several inhalant allergens (23). More than half of a Swedish cohort of patients with AGS was sensitized to aeroallergens, compared with 33% of the general population, and atopy increased the risk for alpha-gal–driven anaphylaxis involving the airways (17).

The discrepancy among studies may be attributed to differences in study design, including participant age, geographic locations, methods used to assess for atopy (serum sIgE levels versus skin prick testing), and alpha-gal–sIgE levels used to define alpha-gal sensitization (>0.1 versus >0.35 IU/ml). It remains unclear whether individuals with AGS have a higher chance of developing other allergic diseases, but the aforementioned studies demonstrate that pre-existing atopy is not required to develop AGS. Individuals with AGS are not disproportionately represented among those with atopic dermatitis (24). There are no descriptions of spontaneous development of alpha-gal–sIgE or AGS in patients with pre-existing asthma, atopic dermatitis, or allergic rhinitis, despite circulating alpha-gal–specific IgG in all humans (25). Low levels of alpha-gal sIgE have been reported in association with chronic intestinal helminth infection, but even in the setting of Th2-polarizing helminth infection, AGS does not spontaneously develop (26). Thus, tick bites distinctively seem to break existing tolerance to alpha-gal, although the mechanisms for this are still unknown.

Pathogenesis of conventional food allergy: sensitization and effector phases

The drivers for the development of food allergies to conventional protein allergens have been under active investigation for more than two decades. For an individual without food allergies to generate and maintain tolerance to dietary Ags, the intestinal immune system (intestinal epithelial barrier, phagocytic innate immune cells, tolerogenic APCs, and regulatory and effector lymphocytes), integrates signals from intestinal luminal food Ags with signals from the gut microbiota and transmits these to the systemic immune system to promote oral tolerance (27). In individuals with food allergies, however, a dysregulated epithelial microenvironment causes APCs to process and present the food Ag to naive $CD4^+$ T cells in a manner that skews the T cells to a type 2/Th2-biased phenotype, characterized by production of cytokines, such as IL-4, IL-5, and IL-13. $CD4^+$ Th2 cells subsequently interact with B cells that have also taken up food Ag for presentation to these T cells. These T cells and their secreted type 2 cytokines push B cells to class-switch their Ig isotypes to Ag-sIgE. Secreted IgE eventually binds to the IgE receptor FcεRI on migrating basophils and tissue-resident mast cells, thus sensitizing the host to that food allergen (28). During the effector phase, the host is re-exposed to the food allergen, which binds to food-sIgE on the surface of mast cells and basophils, cross-links the IgE–FcεRI complexes, and drives mast cell and basophil activation and degranulation. Degranulation causes the release of preformed and newly synthesized mediators, such as histamine, leukotrienes, and PGs, which generate the symptoms associated with allergic responses (29).

Sensitization phase in AGS

Alpha-gal is immunogenic. Abs specific to alpha-gal are already present in the human body because of the presence of so-called natural Abs generated in a T cell–independent manner in response to host microbial communities (25, 30). These existing Abs are typically IgM, IgA, or IgG isotypes, with IgG isotypes being the most abundant (25, 30, 31). These alpha-gal–specific Abs help the immune system distinguish self from nonself and can drive acute organ rejection of xenogenic organ transplants (32, 33). They also increase the efficiency with which the host immune system neutralizes and resists alpha-gal–expressing pathogens (3, 34). The presence of alpha-gal on viruses improves the ability of naive human sera lacking specific viral immunity to neutralize these viruses (35). Alpha-gal–specific Abs also provide protection against parasites that express alpha-gal, such as the malaria-causing *Plasmodium* (34), and shape the quality of microbes colonizing our intestines by selecting for less pathogenic microbial colonizers (3).

Although alpha-gal–specific IgG, IgA, and IgM facilitate immune responses against pathogens, the generation of alpha-gal sIgE (allergic sensitization to alpha-gal) heralds a potentially detrimental allergic immune response (36). Moreover, the presence of alpha-gal sIgE in patients allergic to red meat has been associated with a difference in circulating alpha-gal sIgG subclasses. Investigators studying independent cohorts of patients with AGS in Europe, the United States, Kenya, and Ecuador have reported that circulating alpha-gal–sIgG4 is undetectable or significantly reduced in individuals with AGS compared with nonallergic control subjects (26, 37, 38), whereas alpha-gal–sIgG1 and IgG3 levels are higher (38). The coexistence of alpha-gal sIgE, sIgG1, and sIgG3 in individuals with AGS is intriguing because neither IgG subclass has been associated with IgE-blocking activity that might prevent the development of food allergy. By contrast, the presence or development of food-sIgG4 has been associated with a tolerance to food Ag (39).

Tick-mediated development of alpha-gal–sIgE. In the United States, significant overlap was observed between the geographic distribution of known cases of AGS and cases of Rocky Mountain spotted fever and ehrlichiosis (40, 41). The causative species of these diseases (*Rickettsia* and *Ehrlichia*, respectively) are transmitted by *A. americanum* (41). This led investigators to hypothesize that in the United States, bites from the lone star tick triggered sensitization to alpha-gal (8, 12). Additional reports have supported the association of AGS with tick bite (9, 13–15), with high-titer alpha-gal–sIgE linked to two or more bites in close temporal succession (12, 14). Titers decline in those who avoid repeat tick bites (42), implicating ticks as sensitizing agents.

Th2 and type I immediate hypersensitivity reactions, including anaphylaxis, to tick bites have been described (43–45), making ticks and tick saliva plausible sensitizing agents. Basophils, eosinophils, and, to a lesser extent, tissue-resident mast cells infiltrate the bite site soon after tick bites occur (14, 46, 47). Allergic responses to tick bites promote the development of acquired resistance to future tick infestation, interfering with the transmission of numerous tick-borne pathogens in several mammalian hosts (43). It has also been hypothesized that the allergic response helps rid epithelial surfaces of these multicellular ectoparasites (48).

Initially, investigators hypothesized that adult ticks transmitted mammal-derived alpha-gal Ags to human hosts during a blood meal (11). Subsequent reports described individuals developing alpha-gal–sIgE after bites from larval stage ("seed") ticks that had never had a blood meal from a mammalian host, suggesting that alpha-gal is an endogenous tick component (41, 49). Indeed, alpha-gal has been found in the saliva, salivary glands, hemolymph, and gastrointestinal tracts of ticks, independent of whether they have consumed a blood meal (41, 50). Galactosyltransferase genes encoding for the enzyme necessary for alpha-gal production are present in ticks (11), and alpha-gal expression is upregulated in tick saliva the longer ticks feed on blood from humans (41) and other mammals (51). Some have proposed that upregulation of alpha-gal in tick saliva serves as a form of molecular mimicry deployed to avoid triggering a massive clearing immune response by the mammalian host (51).

Tick mouth parts, including barbed telescoping chelicerae, penetrate the epidermis, securing the tick to the skin (52). This structure and the tubular, blood-drawing hypostome induce trauma to the skin. The potential introduction of pathogenic bacteria present in tick gut and saliva (53–55) could disrupt skin microbiota. These cumulative hits to the epithelial barrier may trigger the production of alarmins IL-33, IL-25, and thymic stromal lymphopoietin (TSLP) by skin epithelial cells, generating a microenvironment that drives Th2/type 2 immune responses (27, 56) (Fig. 1). Yet, individuals with atopic dermatitis do not spontaneously develop AGS, despite skin barrier disruption, exposure to alpha-gal in skin microbiota, and epigenetic DNA modifications in skin epithelium and lymphocytes that promote alarmin and type 2 cytokine release (57, 58). This suggests that the physical breach and interference with skin microbiota induced by tick bites, coupled with alpha-gal in tick saliva secreted into the host, may trigger distinct epigenetic programs in epithelial cells or resident cutaneous immune cells that favor alpha-gal sIgE production.

Inflammatory factors or adjuvants in clinically relevant ticks may also promote production of alpha-gal–sIgE. Chandrasekhar et al. (59) demonstrated that a synthetic alpha-gal glycoprotein (alpha-gal BSA) injected s.c. with tick whole-body extract (TWBE) from the lone star tick induced polyclonal, tick-specific, and alpha-gal–sIgE responses in alpha-galactosyltransferase–deficient (AGKO) mice that cannot produce alpha-gal. Lone star TWBE had adjuvant effects similar to the classic adjuvant alum, when sensitizing mice to tick-independent Ags. The adjuvant effects of TWBE depended on signaling through MyD88, an adaptor molecule downstream of the TLRs, including TLR2, TLR4, TLR5, and TLR9, through which TWBE had a robust stimulatory effect (59).

We developed a mouse model showing that s.c. injection of tick salivary gland extract (TSGE) from sheep blood–fed lone star ticks, independent of an exogenous alpha-gal glycoprotein or glycolipid, induced alpha-gal–sIgE in AGKO mice. Mice sensitized with TSGE developed 190-fold higher levels of total IgE compared with controls, and alpha-gal–sIgE went from undetectable to 158.4 pg/ml. In addition, AGKO mice sensitized with lone star TSGE demonstrated a drop in core body temperature, among other allergic signs, after oral challenge with pork kidney, a well-established source of alpha-gal glycoproteins, as well as pork fat (60). Surprisingly, circulating alpha-gal sIgE has been found in dogs naturally infested with ticks, even though as nonprimate mammals they possess

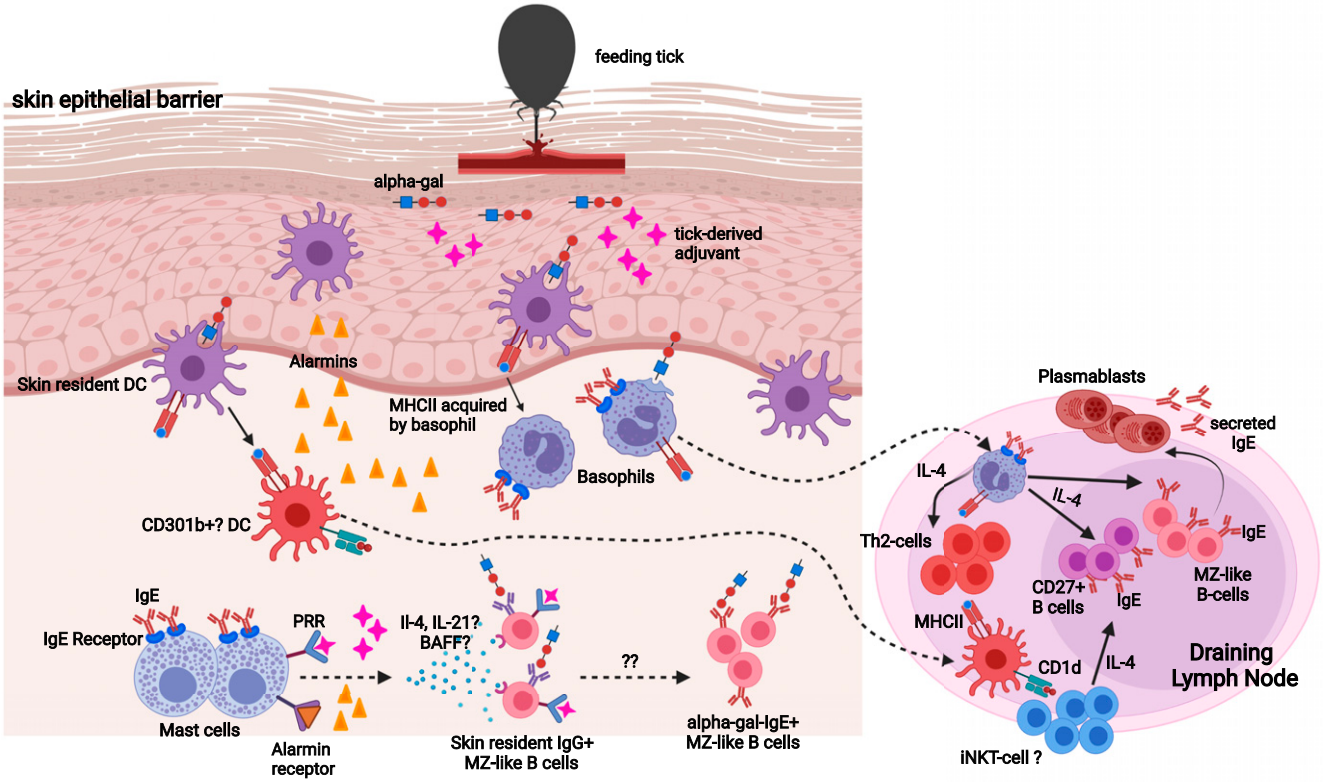

FIGURE 1. Sensitization phase in AGS. During feeding, tick mouth parts induce physical trauma to the skin epithelial barrier while introducing alpha-gal, potentially pathogenic bacteria, and adjuvants present in tick saliva. This may trigger the production of alarmins, such as IL-33, IL-25, and TSLP, by skin epithelial cells, generating a microenvironment that favors the generation of Th2/type 2 immune responses. Skin-resident and migrating DCs take up alpha-gal glycoproteins and glycolipids. Migrating DCs, possibly expressing CD301b$^+$, traffic to draining lymph nodes to present peptide Ag to naive CD4$^+$ T cells. DCs may also present alphac-gal glycolipid complexed with CD1d to IL-4–producing iNKT cells. IL-4–secreting basophils may acquire peptide–MHC-II complexes from DCs or express endogenous peptide–MHC-II complexes, which they present to CD4$^+$ T cells, inducing Th2 responses. Tick saliva adjuvants binding to pattern recognition receptors (PRRs) may induce basophil and mast cell–derived IL-4, IL-21, and B cell activation factor (BAFF), which can promote class switching of clonally restricted, skin-resident, IgG$^+$ MZ-like B cells (in pink) that recognize alpha-gal and accumulate in skin. MZ-like and other heterogeneous CD27$^+$ alpha-gal–specific B cell populations (purple) accumulate in draining lymph nodes, and basophil-derived IL-4 may promote class switching to alpha-gal–sIgE$^+$ B cells that eventually differentiate into IgE-secreting plasmablasts.

functional galactosyltransferase enzymes critical for synthesizing endogenous alpha-gal. Hodžić et al. (61) found that 50% of the banked serum samples from clinically healthy, naturally tick-infested dogs had detectable alpha-gal sIgE whose levels correlated with tick salivary gland protein sIgM and sIgE levels. These animal models of alpha-gal sensitization and allergy, coupled with observational studies of humans, support the idea that tick bites from clinically relevant species can induce alpha-gal sIgE, predisposing to the development of AGS (15).

Cellular players involved in sensitization to alpha-gal. **Dendritic cells.** Dendritic cells (DCs) are important players in the initiation of immune responses to food. The presence of alpha-gal on an Ag impacts DC function. Immature monocyte-derived DCs take up proteins glycosylated with alpha-gal more readily than proteins lacking alpha-gal and degrade alpha-gal glycoproteins at a slower rate (62). This may have implications for the efficiency and rate of DC-mediated processing and presentation of alpha-gal Ags to T cells. Sensitization to alpha-gal starts with a cutaneous insult, and notably, tick saliva from the clinically relevant *Ixodes ricinus* species has been shown to hamper maturation and migration of skin DCs to draining lymph nodes, disrupting DC ability to promote Th1 or Th17 responses and favoring Th2 responses (63). The role of cutaneous CD301b$^+$ DCs in the AGS sensitization phase will be critical to address because cutaneous CD301b$^+$ DCs have been shown to migrate to draining lymph nodes, in a chemokine receptor CCR7- and CCR8-dependent manner, to initiate CD4$^+$ Th2 cell differentiation after cutaneous allergen exposure (64).

Basophils and mast cells. DCs do not secrete the IL-4 needed to direct naive CD4$^+$ T cells toward a type 2/Th2 response (65). This opens the door for alternative immune cells, such as basophils, to serve as APCs and sources of IL-4 that can promote Th2 differentiation. Basophils produce IL-4 after TLR stimulation (66) and express the costimulatory molecules CD40, CD56, and CD86 (65, 67). They can acquire peptide–MHC class II (MHC-II) complexes used for Ag presentation from DCs (65). They also express endogenous MHC-II and have been shown to endocytose, process, and present peptide Ags to naive CD4$^+$ T cells in vitro (67). In addition, basophils were shown to be essential for Th2 differentiation in vitro and in vivo in a mouse model of s.c. immunization with the papaya cysteine protease papain (67). Basophils can also produce TSLP (65), which, together with other alarmins classically produced by disrupted epithelial barriers, creates a tissue microenvironment that favors Th2/type 2 immune responses (56). Human mast cell lines and primary mast cells isolated from eosinophilic allergic polyps are also capable of producing cytokines important for Ab isotype

switching, independent of IgE-FcεRI signaling. Mast cells secreted B cell activating factor, IL-4, and IL-21, a cytokine classically associated with T follicular helper cells that regulate B cell class switching (68). Triggered by tick-associated adjuvants and host alarmins, these mast cell–derived cytokines could stimulate B cell class switching into alpha-gal–sIgE$^+$ B cells that eventually differentiate into IgE-secreting plasmablasts.

CD4$^+$ Th2 cells. Repetitive tick infestations have been shown to generate a Th2 cytokine profile in mice (69) and increase the frequency of CD4$^+$ Th2 cells compared with Th1 cells in humans (14). Tick saliva contains factors, including PGs, sphingomyelinase, and cysteine protease inhibitors, that appear to promote type 2/Th2 responses (70, 71). CD4$^+$ T cells stimulated with tick saliva from *Ixodes scapularis* produce significantly more IL-4 and IL-13 than IFN-γ, the canonical type 1 cytokine (72, 73). Chandrasekhar et al. (59) showed that CD4$^+$ T cells were essential for the generation of a Th2-skewed Ab profile after s.c. injection with *A. Americanum* TWBE. Elevations in total IgE and tick-sIgE and IgG1 were abrogated in mice whose CD4$^+$ T cells were depleted before tick exposure (59). In addition, we recently showed that the transcriptional immune profiles in circulating PBMCs from alpha-gal–sensitized human subjects were distinct from controls without detectable alpha-gal–sIgE. There was increased expression of genes associated with Ag presentation, MHC-II surface expression, and cytokines and chemokines associated with itch and allergic dermatitis, including *IL-13RA*, which encodes the receptor for the Th2 cytokine IL-13 (19). These findings suggest that the generation of alpha-gal–sIgE may depend in part on MHC-II–mediated Ag presentation to CD4$^+$ Th2 cells, although to date, this has not been formally demonstrated.

Unconventional and semivariant T cells. Classically, CD4$^+$ Th2 cells recognize peptide, and not glycan, MHC-II complexes. Thus, unconventional T cells with the ability to recognize exogenous glycolipid Ag (γ-δ T cells, invariant NKT [iNKT] cells, and mucosal-associated invariant T cells) (74) may factor into the generation of alpha-gal–sIgE. The most evidence that exists supports a potential role for iNKT cells, which recognize glycolipid Ags in the context of the MHC-I–like molecule CD1d. In peach allergy, for example, the Pru p 3 lipid-ligand acts as an adjuvant, promoting sensitization to the allergen Pru p 3, through a CD1d-mediated interaction (75). iNKT cell activation and type 2 cytokine skewing have also been demonstrated in cow's milk allergy (76) and eosinophilic esophagitis (77). Both mouse and human iNKT cells can recognize the alpha-gal glycolipid isoglobotrihexosylceramide presented in the context of CD1d (78). Alpha-gal–sIgM and –sIgG produced by human B cells depend in part on B cells interfacing with iNKT cells via CD1d (79). In addition, we showed that the median frequency of circulating iNKT cells expressing the early activation marker CD69 was 2.5-fold higher in alpha-gal–sensitized individuals (with no reported recent tick bite; no mammalian meat ingestion) than in control participants seronegative for alpha-gal–sIgE. We identified a weak positive linear correlation between the frequency of circulating CD69$^+$, activated iNKT cells, and alpha-gal sIgE levels (19). Activated iNKT cells also accumulate in the spleen and liver of AGKO mice sensitized with TSGE and challenged orally with alpha-gal–rich pork kidney (O.I. Iweala, S.K. Choudhary, and S.P. Commins, unpublished observations). Studies exploring the potential role for iNKT cells in alpha-gal allergy are in their infancy, but these findings suggest that CD1d-restricted, activated, unconventional iNKT cells may promote AGS.

B cells. As the cells ultimately responsible for producing IgE, there is considerable interest in delineating the role for B cells in the development of mammalian meat allergy. B cell–intrinsic MyD88 expression was critical for tick-sIgE production after sensitization with TWBE in a mouse model of lone star tick-induced IgE responses (59). This highlighted a role for TLR signaling through MyD88 in B cells in the generation of immune hypersensitivity responses to an AGS-associated tick. There is also evidence that circulating B cells in alpha-gal–sensitized, mammalian meat–allergic individuals are distinct from B cells in nonallergic control subjects. When we examined bulk PBMCs using multiplex gene transcription array, we found differential expression of genes associated with B cell function in patients sensitized and allergic to alpha-gal compared with control subjects. These included upregulation of BCL-6, a transcription factor expressed both in germinal center B cells and in T follicular helper cells, and CD70, a ligand of the B cell marker CD27 that can regulate IgG synthesis and enhance B cell–mediated IgE production (19). Cox et al. (80) also identified distinct B cell immunophenotypes linked to alpha-gal–sIgE production in patients with AGS. CD27$^+$ memory B cells from participants with AGS expressed lower IgM but higher amounts of IgD and the chemokine receptors CXCR4 and CCR6 compared with nonallergic control subjects. The memory B cells were also enriched for IgE-secreting B cells (80).

The observation that alpha-gal–sIgE can wane fairly rapidly over time if patients avoid ingesting red meat or getting tick bites (42) has led some to postulate that alpha-gal–sIgE is produced by circulating plasmablasts rather than longer-lived, bone marrow–resident plasma cells (5, 73). Preliminary studies suggest that although rare, circulating IgE$^+$, alpha-gal$^+$ CD27 high, CD38 high, CD138$^-$ B cell plasmablasts are detectable in individuals allergic to mammalian meat (81, 82). The transcriptional signatures and functional phenotypes of these cells require additional investigation, but the early evidence suggests that a specialized B cell subset with an unconventional memory phenotype drives alpha-gal–sIgE production. This alpha-gal–sIgE$^+$ unconventional B cell population may include marginal zone (MZ) B cells, found in spleen (mice and human), lymph nodes (human), and intestinal Peyer's patches (human) (83). They can mount polyclonal Ab responses to T cell–dependent protein and T-independent carbohydrate Ags. MZ-like, class-switched IgG$^+$ B cells with clonally restricted BCRs are also detectable in human skin. These heterogeneous CD22$^+$CD27$^-$ mature and CD22$^+$CD27$^+$ memory B cells accumulate in the skin 3–7 d after intradermal injection with varicella zoster virus or *Candida* Ag (84). Thus, tick bites may effectively act like intradermal injections of Alpha-gal and tick factors. Alpha-gal and tick factors could activate and expand MZ-like, skin-resident, alpha-gal–specific B cells by simultaneously engaging BCRs and B cell pattern recognition receptors. Activated B cells surrounded by basophil and mast cell–derived IL-4 and other cytokines could subsequently class-switch to IgE (Fig. 1).

Effector phase in AGS

Intestinal absorption of alpha-gal and delayed allergic reactions to mammalian meat in AGS. One of the notable characteristics of AGS is the delay between eating red meat and

symptoms of an allergic reaction (4, 5). Our mouse model of AGS has also recapitulated this delay in allergic responses to mammal fat compared with conventional food-protein allergens (60). A potential explanation is that alpha-gal molecules are somehow delayed in reaching the bloodstream (4, 85, 86). This could be because of how patients with AGS process alpha-gal in its glycolipid and glycoprotein forms. When Steinke et al. (87) conducted metabolic profiling of patients with AGS and non–alpha-gal–allergic control subjects, they showed significant differences in metabolic pathways for lipids, proteins, and carbohydrates between the groups. Even before oral pork challenge, investigators found that participants with detectable serum alpha-gal sIgE differed significantly in lipid and fatty acid metabolism pathways compared with non-AGS control subjects, although the reason for this remains unclear. After oral pork challenge, the differences in lipid and fatty acid metabolism between AGS and control participants increased; lipid blood levels did not increase above baseline in AGS participants for several hours, compared with 2 h for control subjects (87). In addition, an in vitro simulation of beef digestion and intestinal transport demonstrated that only alpha-gal in glycolipid, not glycoprotein, form could effectively cross a simulated intestinal epithelial barrier composed of Caco-2 cells (85). Alpha-gal glycolipids were incorporated into chylomicrons to traffic through enterocytes (85). Lipids are metabolized more slowly than proteins (85), which, coupled with the alterations to the metabolism of patients with AGS (87), likely contributes to the delay in allergic reaction (Fig. 2).

However, another report assessing digestion and transport of alpha-gal–containing compounds across a Caco-2 cell monolayer had contrasting results: mammalian proteins glycosylated with alpha-gal could pass through the mock intestinal epithelium. However, significantly smaller amounts of alpha-gal glycoproteins were detected than proteins without alpha-gal (88). Differences in the form and concentration of alpha-gal used could explain these conflicting results. Nevertheless, both studies demonstrated that alpha-gal glycoproteins are hindered in moving across the intestinal epithelium. In addition, glycosylating a mammalian protein with alpha-gal increased both its resistance to digestion in vitro and the amount detected in the endosomal fraction of the Caco-2 lysates, even after 24 h of incubation (88). These results suggest that delays in the digestion and transport of alpha-gal glycoproteins across the intestinal epithelium may also contribute to delayed allergic responses to alpha-gal (Fig. 2).

Alpha-gal glycoproteins and glycolipids activate allergy effector cells. In the effector phase of IgE-mediated food allergies, Ag cross-links IgE bound to FcεRI on the surface of mast cells and basophils, triggering degranulation and the release of inflammatory mediators. The roles of mast cells, basophils, and the signaling molecules downstream of alpha-gal–sIgE–FcεRI complexes in these cells during the effector phase of AGS are not entirely clear.

Basophils. Basophils circulate in the bloodstream, making them more accessible to retrieve from human study subjects than tissue-dwelling mast cells. Given their central role in the immune responses to ticks associated with the development of AGS, basophil responses to alpha-gal stimulation have been widely studied. In alpha-gal–allergic participants undergoing open mammalian meat challenge, basophils upregulated the activation marker CD63, with maximal expression 4 h after

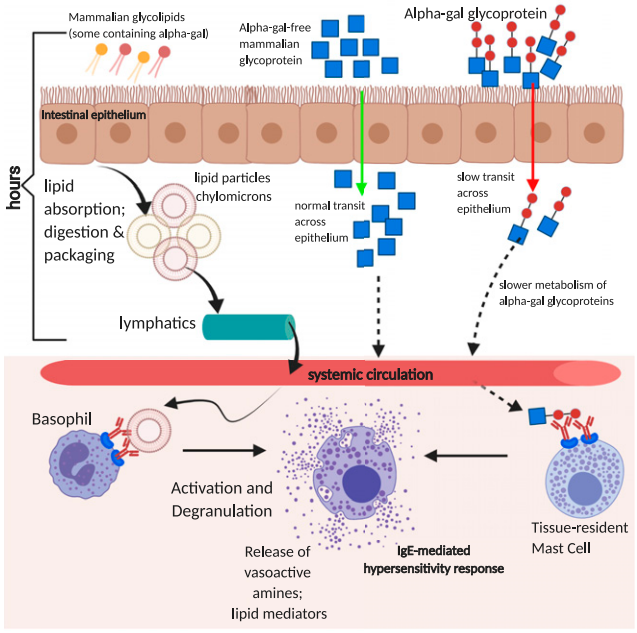

FIGURE 2. Slow transit and metabolic processing of alpha-gal glycolipids and glycoproteins by the intestinal epithelium may contribute to delayed allergic responses to alpha-gal. Alpha-gal glycoproteins are hindered in moving across the intestinal epithelium, and glycosylating a mammalian protein with alpha-gal may increase its resistance to digestion and trafficking time through the cellular endosomal compartments. After prolonged digestion and metabolism of alpha-gal glycoproteins and additional packaging of alpha-gal glycolipids into chylomicrons or other lipid particles, multimeric alpha-gal glycans (alpha-gal glycolipid or glycoprotein) cross-link alpha-gal sIgE–FcεRI complexes on circulating basophils and tissue-resident mast cells. The subsequent activation and degranulation generate vasoactive amines, lipid mediators, and cytokines that drive the IgE-mediated hypersensitivity response.

meat ingestion. This also correlated with the appearance of clinical symptoms (89). Basolateral media of Caco-2 cells containing chylomicrons with alpha-gal glycolipid also activated basophils from an alpha-gal–allergic subject (85). Using an indirect basophil activation test, we were able to show that alpha-gal glycoproteins and glycolipids activated basophils sensitized with plasma from individuals allergic to alpha-gal. Omalizumab, a mAb against IgE, impaired alpha-gal–mediated basophil activation (86), indicating that activation is IgE dependent. These reports highlight a unique role for a glycan, both in glycolipid and glycoprotein forms, activating allergy effector cells through surface-bound IgE in the context of food allergy.

Basophil activation tests have also been studied as a confirmatory test for the diagnosis of AGS. For example, basophils from alpha-gal–sIgE seropositive red meat–allergic patients stimulated with alpha-gal–rich cetuximab upregulated expression of cell surface activation markers. This mirrored positive percutaneous and intradermal test results in these patients using cetuximab (90). The basophil activation test has also been explored as a surrogate for clinically observed oral red meat challenges in patients with AGS, which are currently the gold standard to distinguish between alpha-gal–sensitized and alpha-gal/mammalian meat–allergic individuals (91). Taken together, these reports illustrate that basophils sensitized with alpha-gal sIgE are activated after exposure to alpha-gal, likely playing a significant role in driving allergic reactions in AGS.

Mast cells. Although the function of basophils in AGS has become clearer, there is less known about the role of mast cells in AGS reactions after mammalian meat ingestion. In conventional food protein allergy, percutaneous skin prick testing using the extracts of the allergenic food serves as one in vivo test of mast cell function in humans. When cutaneous mast cells sensitized with food-sIgE contact food proteins scratched onto the skin, the food protein triggers mast cell degranulation, histamine release, and a corresponding wheal and flare response on the skin at the scratch site. Skin prick testing using standardized mammalian meat extracts frequently results in negative or very small wheals in patients with AGS (8). However, intradermal testing, a more sensitive in vivo test of allergen-induced mast cell activation that takes advantage of the higher density of mast cells in the dermis compared with the epidermis, is positive in patients with AGS (8). Notably, intradermal skin testing for food allergy is considered high risk for inducing anaphylaxis and is performed only under research protocols. In the case of AGS, intradermal testing provided initial evidence demonstrating that cutaneous mast cells in patients with AGS could be activated by alpha-gal glycoproteins (8).

Serum tryptase serves as a marker for mast cell degranulation in humans but is not consistently elevated during severe allergic reactions to food protein (92). Similarly, only 30% of participants with AGS with allergic symptoms after oral red meat challenge experienced elevations in serum tryptase, peaking 4 h after meat ingestion (89). Although it was not statistically significantly different, in lone star TSGE-sensitized mice challenged with pork kidney homogenate, there was a 4-fold increase in levels of mouse mast cell protease-1, a serum marker for mast cell degranulation in mice, compared with controls (60). The need for both human and animal studies linking mast cell activation to clinical AGS symptoms remains.

Neither serum elevations of mast cell mediators, the percutaneous and intradermal skin prick testing, nor the mouse model of AGS formally prove that alpha-gal–induced mast cell activation is IgE dependent. Nor do they prove that signaling pathways downstream of alpha-gal sIgE–FcεRI complexes are the same as pathways activated by conventional protein allergens. Additional studies formally investigating this are needed. We have found that primary human mast cells derived from skin mast cell progenitors or adipose tissue stem cells (93) and sensitized with alpha-gal–sIgE seropositive plasma can be activated by alpha-gal glycoproteins (O.I. Iweala and C. Kepley, unpublished observations). Interestingly, skin-derived, but not lung-derived, mast cells reacted to alpha-gal in vitro (O.I. Iweala and C. Kepley, unpublished observations). This may underlie the finding that >90% of patients with AGS describe cutaneous symptoms after red meat ingestion, nearly three times higher than those who report respiratory symptoms (16, 19, 94).

Alpha-gal glycolipids can activate appropriately sensitized basophils (85, 86), but it remains unclear whether alpha-gal glycolipid can activate mast cells. Mast cells have receptors for low-density lipoprotein (95). In mouse models, low-density lipoprotein, high-density lipoprotein, and very low-density lipoprotein induce mast cell activation (96). Because dietary alpha-gal is found in lipoprotein particles (85, 97), it is possible that alpha-gal glycolipid in these particles could activate human mast cells in AGS. Human mast cell lines (98), primary human mast cell culture (93), and emerging technology to make human monoclonal sIgE (99) stand as promising tools in the quest to determine whether alpha-gal glycans can activate human mast cells.

Emerging therapies for AGS

Currently, there is no cure for AGS. Individuals with AGS are simply counseled to avoid red meat, mammalian products, and, in some cases, dairy (4, 5). There is no one-size-fits-all approach for these avoidance diets, because tolerance to mammalian products varies from patient to patient (4). Allergies to conventional food-protein allergens, such as peanut, tree nuts, and shellfish, can develop in both childhood and adulthood and almost never resolve spontaneously (28). By contrast, alpha-gal–sIgE has been shown to wane with time in children and some adults who avoid repeat tick bites from ticks associated with AGS development (42). This means that, in some individuals, AGS can spontaneously resolve, allowing the reincorporation of mammalian meat and food products into the diet (4). More studies are required to establish how long alpha-gal–sIgE persists and whether this correlates with the absence of bound alpha-gal–sIgE on basophils and mast cells. In addition, there are currently no available biomarkers to predict persistence or waning of alpha-gal–sIgE.

Avoidance can be difficult for patients with AGS, because many foods, cosmetics, and medications contain alpha-gal in the form of mammal-derived ingredients. Severe allergic symptoms and anaphylaxis after accidental alpha-gal ingestion are treated with i.m. epinephrine (4). For patients who continue to report mild allergic symptoms even after strict alpha-gal avoidance, oral antihistamines and mast cell stabilizers such as cromolyn are used to treat and to prevent symptoms associated with unknown, accidental alpha-gal ingestion (5). Therapies for food allergy, AGS included, are in their infancy. There is only one Food and Drug Administration–approved therapy for the treatment of food allergy, oral immunotherapy (OIT) specific for peanut allergy (29). OIT involves feeding an allergic individual increasing doses of food allergen until a treatment dose is reached and administered daily; it has been shown to alter cellular and Ab responses to food allergen (39). Although a case report detailed successful desensitization to alpha-gal in a child using dose escalation of beef, followed by daily beef ingestion (100), there are currently no Food and Drug Administration–approved, standardized OIT protocols to treat AGS. Anecdotal, clinical observations suggest that AGS is more likely to resolve in patients who tolerate moderate amounts of dairy in their diet (4, 5). Informed by this observation, a small, nonrandomized, non–placebo-controlled, unblinded pilot study involving seven participants with AGS evaluated the safety of using cow's milk containing 6 mg alpha-gal over 36 mo as daily OIT to treat AGS (https://clinicaltrials.gov/ct2/show/results/NCT02350660). No serious adverse events were reported, but it is unclear how much mammalian meat participants tolerated on challenge, because the study was terminated early as a result of funding. These findings suggest that OIT to treat AGS will likely be tolerated, but additional studies are clearly required to assess the utility of alpha-gal OIT in managing AGS.

Ag-independent therapies to treat food allergies are attractive to patients with multiple food allergies or who cannot tolerate side effects associated with OIT, including allergic reactions (29). Biologics, including mAbs omalizumab and dupilumab, are under active investigation as adjuncts to OIT or independent

food allergy treatments in their own right (27). Omalizumab in particular binds the Fc portion of IgE, preventing it from binding to FCεRI on mast cells and basophils and hampering alpha-gal–induced activation of basophils sensitized with alpha-gal plasma in vitro (86). A small prospective study of 14 patients with AGS, started on omalizumab to treat chronic urticaria that persisted even after appropriate alpha-gal avoidance diets, showed a decline in frequency and severity of participants' urticaria and symptom improvement in those reporting accidental or intentional ingestion of mammalian products (S. Commins, unpublished observations). This suggests that omalizumab may serve as a therapeutic option for patients with AGS with persistent allergic symptoms despite alpha-gal avoidance diets.

Conclusions

Red meat allergy in AGS disrupts our current understanding of the mechanisms of pathogenesis driving food allergy. In food protein allergy, protein allergens cross-link sIgE–FcεRI complexes on sensitized mast cells and basophils typically after minimal digestion or processing. By contrast, current evidence suggests that after prolonged digestion and metabolism of alpha-gal glycoproteins and additional packaging of alpha-gal glycolipids into chylomicrons and other lipid particles, these multimeric forms of alpha-gal cross-link alpha-gal–sIgE–FcεRI complexes. This may explain the delay in allergic symptom onset.

Bites from select hard-bodied ticks, including *Amblyomma americanum* in the United States, induce elevations in alpha-gal–specific and total IgE levels. Sensitization to alpha-gal likely involves several of the same innate and adaptive immune cells classically associated with allergic sensitization in food protein allergy, including DCs, $CD4^+$ Th2 cells, and $CD27^+$ memory B cells. However, there may also be roles for cells not commonly associated with allergic sensitization to protein allergen, including basophils, iNKT cells, unconventional MZ B cells, and IgE^+ plasmablasts. Questions remain regarding the mechanisms of pathogenesis behind AGS. Unraveling these immunologic mechanisms will enhance and expand our understanding of food allergy overall.

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Institutional History

- Assistant Professor, University of North Carolina, Chapel Hill, 2018–current.
- Clinical Instructor, University of North Carolina, Chapel Hill, 2017–2018.
- Postdoctoral Fellow, University of North Carolina, Chapel Hill, 2017–2018.
- Clinical Fellow, University of North Carolina, Chapel Hill, 2015–2017.
- Medical Instructor, Duke University, Durham, NC, 2014–2015.
- Attending Physician, Durham VA Medical Center, 2014–2015.
- Hospitalist, Duke Regional Hospital, 2013.
- Internal Medicine Residency, Massachusetts General Hospital, Boston, MA, 2010–2013.
- M.D., Harvard Medical School, Boston, MA, 2010.
- Ph.D., Harvard University, Cambridge, MA, 2009.
- A.B., Harvard University, 2002.

Research Interests

- Mechanisms of pathogenesis behind alpha-gal mammalian meat (red meat) allergy/alpha-gal syndrome
- Epigenetic regulation of IgE Ab responses
- Mast cell inhibition in the treatment of allergic disease

I have been interested in medicine and biomedical research since high school. My first exposure to bench research was under the mentorship of Drs. Dan Camerini-Otero and Peter Romanienko in the Biochemistry Branch of National Institute of Diabetes and Digestive and Kidney Diseases as a Howard Hughes Medical Institute-NIH Summer Fellow. I was also exposed to bench research in industry as a United Negro College Fund–Merck Undergraduate Scholar. While in college, I witnessed the challenges of health care delivery in resource-poor settings first-hand when I interned in a sexually transmitted disease prevention clinic in Cambodia and assisted my physician-father in his impromptu rural clinic in our village in Abia State, Nigeria. These experiences pushed me toward research with

possible applications in underresourced nations, influencing my decision to get both an M.D. and a Ph.D. in the fields of mucosal immunity and vaccination under the mentorship of Dr. Cathryn R. Nagler. After completing my internal medicine residency at Massachusetts General Hospital and a combined clinical and research allergy/immunology postdoctoral fellowship with Dr. Scott Commins and the University of North Carolina Food Allergy Initiative, I joined the faculty at UNC-Chapel Hill School of Medicine

As an allergist, a clinical immunologist, and a physician-scientist fully committed to an academic research career, I have benefited and continue to benefit from top-notch mentorship. My mentors nurture and challenge me. They reinforce my sense of belonging in science. As a result, part of my career mission is to transfer my enthusiasm for basic and translational immunology to a diverse set of learners and mentees, inspiring and energizing them to make significant contributions to the field of immunology. I want to ensure that my mentees recognize that our field benefits from their ideas and perspectives, and that they, too, belong in science.

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