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Review article

Alpha-gal syndrome: Implications for cardiovascular disease

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ABSTRACT

Alpha-gal syndrome (AGS) refers to a potentially life-threatening allergy to the molecule galactose- α1,3-galactose (gal), which is expressed on most mammalian tissues but, importantly, is not expressed by humans. This syndrome can manifest as an allergic reaction to mammalian meat products, but other sources of mammalian tissue can also provoke an immune response, including injectable and implantable medical products. This syndrome has been linked to coronary atherosclerosis, and medical products that express gal are routinely used in cardiology and cardiac surgery. This article seeks to discuss potential implications of alpha syndrome as it relates to cardiovascular health and to heighten awareness in the cardiovascular community about this emerging public health issue.

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PREVALENCE OF GALACTOSE- α1,3-GALACTOSE

Most mammalian species (including New World monkeys, cows, pigs, goats, horses, sheep, rabbits and mice) express the galactose- α1,3-galactose (gal) disaccharide sugar on cells and tissue surfaces[1–4]. Gal expression results from the catalytic activity of the α1,3-galactosyltransferase enzyme encoded by the glycoprotein α1,3-galactosyltransferase gene (GGTA1)[1–3,5]. Certain mammalian species, such as catarrhines (humans, apes, and Old World monkeys), do not have a functional GGTA1 gene[6–8] and correspondingly do not express gal[1,3,4]. Additionally, gal has been documented to be absent in fish, amphibians, reptiles, and birds[3,9,10]. The function of gal is unknown[3], but it is clearly not essential for survival[1,3].

PREVALENCE OF IgM, IgG, AND IgA ANTI-GAL ANTIBODIES

Mammalian species that do not produce gal such as humans and Old World primates have been well documented to possess natural anti-gal antibodies[1–3,11,12]. It has been reported that these natural antibodies occur as different isotypes, including IgM, IgG, and IgA[1,2,13]. In humans, anti-gal antibodies are among the most abundant immunoglobulins, with some studies reporting that 1–3% of circulating immunoglobulins are directed against gal[3,11–15]. Anti-gal immunoglobulin titers may be attenuated or amplified by various factors; a vegetarian diet reduces titers while implantation of bioprosthetic heart valves increases titers[16,17].

PREVALENCE OF IgE ANTI-GAL ANTIBODIES

More recently, van Nunen, Commins and others[18–24] have described a unique population with high titers of anti-gal IgE. Anti-gal IgE develops in a subset of people after an index exposure to gal. On re-exposure to gal, this subset of people can develop a severe IgE-mediated hypersensitivity reaction that can manifest as anaphylaxis (including urticaria, tachycardia, angioedema, syncope, and hypotension) with many patients requiring emergency care.

The condition, termed "alpha-gal syndrome" (AGS), is incited by exposure to gal through tick bites, even in patients who previously tolerated exposure to gal through red meat consumption. Although the Lone Star tick is the culprit in the United States, bites from certain other tick species around the world cause a similar hypersensitivity to gal[23–31].

The National Institute of Health (NIH) recently highlighted AGS and noted that it is often unrecognized or misdiagnosed[32]. For AGS patients, a tick bite can lead to a hypersensitivity reaction that characteristically manifests as anaphylaxis three to six hours after consumption of mammalian meat products, even in patients who previously tolerated red meat for their entire lives[20,22,33].

Others who have elevated anti-gal IgE levels (allergen specific positivity to gal) due to a tick bite remain asymptomatic after red meat consumption but may manifest anaphylaxis after exposure to injected or implanted mammalian derived medical products[19,34–37]. For this reason, allergists have described patients as allergen negative (alpha-gal-specific IgE levels below a cutoff value; typically 0.1 or 0.35 kUA/L), allergen positive (alpha-gal-specific IgE levels above the cutoff value), and patients with alpha-gal syndrome (alpha-gal-specific IgE levels above the cutoff value and a history of clinical anaphylaxis after red meat consumption).

The reported prevalence of individuals in the United States with elevated allergen-specific titers of anti-gal IgE (i.e., allergen positive) has been reported to be in the range

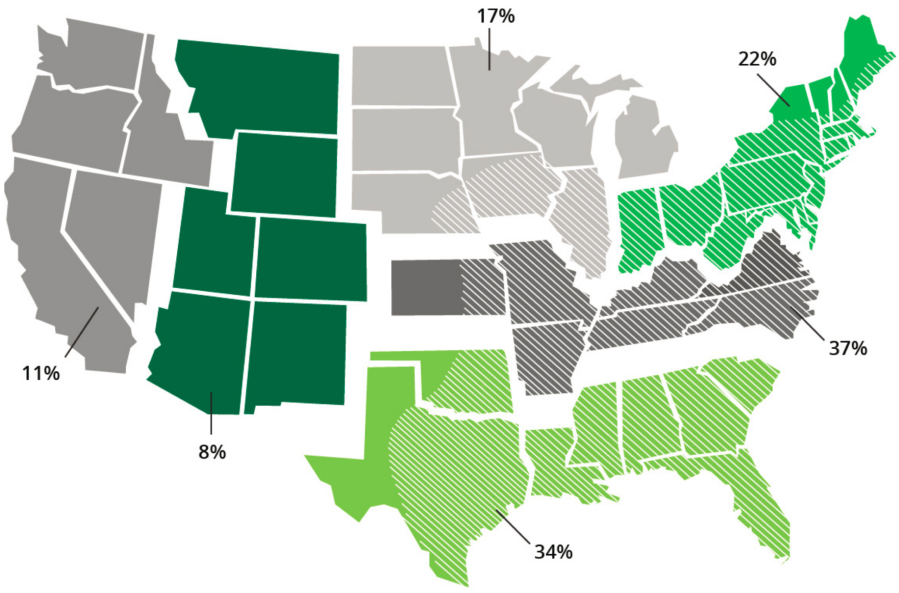

Figure 1. Surveillance for IgE to alpha-gal. Percent positive rates are presented for IgE to alpha-gal within each of six regions in the United States, 2012–2013 (7300 samples). Percentages refer to the percentage of samples submitted for testing that tested positive. Diagonal white lines on the map represent the known geographic distribution of the Lone Star tick (from Olafson, P. Ticks and the mammalian meat allergy. USDA Beef Research, (2015)).

of 8% to 46%, with highest prevalence within the geographic distribution of the Lone Star tick (Figure 1)[21,38–41]. Similar prevalence rates have been reported in other regions around the world (Table 1)[27,42,43].

Children within the geographic distributions of certain ticks are projected to have allergen positive prevalence comparable to the adult population[33]. As one might expect, hunters and forest service workers have been reported to have a prevalence that is more than twice that of the general population[21,43]. It appears that the prevalence of AGS equates to 10% of the allergen-positive population. Thus, in the southeastern United States, approximately 3% of the general population exhibits anaphylaxis after consumption of mammalian meat.

UNIQUE CHARACTERISTICS OF THE ALLERGEN-POSITIVE POPULATION

Allergen-positive patients have been identified to have higher titers of anti-gal IgG, with more IgG subtype 1 and less IgG subtype 2 than allergen negative populations[44]. Furthermore, allergen-positive patients manifest a significant difference in coronary artery disease when compared to an allergen-negative cohort[38]. This suggests that IgE sensitization to alpha gal may be a novel modifiable risk factor for coronary atherosclerosis, especially in patients 65-years and younger. By eating mammalian food products, patients sensitized to gal may be contributing to coronary artery disease.

PUTATIVE MECHANISMS OF IgE SENSITIZATION

A central question is why some individuals who tolerate gal exposure through red meat consumption for years go on to develop an allergy to gal after a tick bite. Wilson et al[45] have proposed that tick-induced α-gal sensitization is due to an activation of the innate immune system through one of at least three possible mechanisms. First, the damage and local trauma from the bite may release local damage-associated molecular

Table 1 Prevalence of α-gal allergen positivity (e.g., Alpha-gal-specific IgE titers fall above the threshold for positivity).

Region	Location	Reference	Threshold for positivity (Anti-gal IgE allergen titer)	Prevalence of positivity
United states	Southeastern US	Commins[21]	≥0.35 IU/ml	20%
	North Carolina	Commins[21]	≥0.35 IU/ml	20%
	North Carolina	Burk[39]	≥0.35 kUA/L	22%
	Tennessee ,	Commins[21]	≥0.35 IU/ml	22%
	Virginia	Wilson[38]	≥0.1 kU/L	26.3%
	Virginia	Commins[21]	≥0.35 IU/ml	18%
	Boston	Commins[21]	≥0.35 IU/ml	<1%
	Northern California	Commins[21]	≥0.35 IU/ml	2%
Africa	Kabati, Kenya (rural)	Commins[21]	≥0.35 IU/ml	76%
	Thika, Kenya (industrial town)	Commins[21]	≥0.35 IU/ml	29%
South America	Esmeraldas Province, Ecuador	Commins[21]	≥0.35 IU/ml	37%
Europe	Germany General population	Fischer[43]	≥0.1 kUA/L	15%
	Germany hunters and forest service workers	Fischer[43]	≥0.1 kUA/L	35%
	Spain	Gonzalez-Quintela[42]	≥0.1 kUA/L	5.5%
	Denmark	Gonzalez-Quintela[42]	≥0.1 kUA/L	8.1
	Norrbotten, Sweden (age 18 y)	Commins[21]	≥0.35 IU/ml	<1%
	Sweden (10%),	Apostolovic[27]	Not reported	10%

patterns (DAMPs) that activate innate immune cells, leading in turn to activation of the adaptive immune system, including the formation of plasma cells that produce gal-specific IgE through T-cell-dependent germinal center reactions (or possibly through a T-cell independent process, though this is unlikely). Second, the tick bite may introduce gal while at the same time introducing tick-associated microbes that could act as pathogen-associated molecular patterns (PAMPs) and likewise cause the innate immune system to direct an adaptive immune response. According to these theories, gal is an innocent bystander that is swept up at the scene of an immune response initiated by local damage or by microbes. Third, it has been proposed that gal itself could be perceived as a PAMP and initiate a response in its own right.

CLINICAL IMPLICATIONS OF GAL IN WHOLE ORGAN XENOTRANSPLANTATION

The fact that gal has been confirmed on mammalian cells and tissues has significant clinical implications in whole organ xenotransplantation (i.e., pig to human, pig to Old World primate)[1,2,4]. This is due to the fact that gal is the major antigen expressed on pig cells and tissues to which natural anti-gal antibodies bind[3,9,13,46]. The binding of anti-gal antibodies to gal activates the complement system within minutes to hours of discordant tissue, cell, or organ transplantation and the host effectively rejects the transplanted material[2,4].

CLINICAL IMPLICATIONS OF GAL IN HUMAN THERAPEUTIC PRODUCTS

The health concerns for alpha-gal IgE positive patients (especially patients remaining asymptomatic after meat consumption) who may be under consideration for mammalian derived medicinal products has been well stated by Fischer et al[43]:

> "In our opinion, clinical tolerance to mammalian meat and innards cannot be considered the same as clinical tolerance to intravenous application of alpha-gal-containing drugs. Due to this potential risk, a special warning regarding the intravenous administration of alpha gal-containing drugs may be needed in all individuals who display alpha-gal-IgE positivity."

Others[25] concur and have identified case studies that highlight different classes of medicinal products that "may prove risky in people who are gal sensitized [allergen positive]"[25]. These include:

- Drugs including cetuximab[37], heparin[47],
- Gelatin including capsules[48], tablets[36], suppositories, colloids[49], and vaccines[50,51]
- Collagen including corneal shields[52], hemostatic agents[47] or other scaffolds
- Magnesium stearate[36]
- Mammalian derived heart valves[34,35,54]

Although some health care providers observe that AGS patients may tolerate administration of gal containing therapeutics, these patients require unique and special care that places additional economic burden while exposing them to potential harm[55,56].

IN VITRO OR *IN VIVO* TESTING IN LABORATORY ANIMALS OF MEDICINAL PRODUCTS

The understanding of the health implications for the allergen-positive population is new, and emerging. For medicinal products currently in use, Muglia indicates[53]:

> "Pharmaceutical manufacturers do not currently test products for alpha-gal content. Additionally, they are not required by the Food and Drug Administration to report changes in inactive ingredients on the package insert."

> "Manufactures do not report alpha-gal content in their package inserts or test for alpha-gal content in products. Inactive ingredient information can change at any time, and the FDA does not require manufacturers to disseminate this information."

As one might expect, and prior to human use, many of the medical products including bioprosthetic heart valves are routinely evaluated in sheep[57], rabbits[58], etc. However, these models do not challenge the gal-mediated immune response because these animals all naturally express the gal molecule and therefore do not produce anti-gal antibodies[59]. The only appropriate model would involve animal subjects that produce anti-gal antibodies such as Old World non-human primates (NHP). Although NHPs have been well documented to have anti-gal IgG and IgM antibodies, corresponding anti-gal IgE values particularly after a challenge with the lone star tick (or comparable tick, or tick extract) were not noted in the scientific literature.

Given the growing awareness of this issue, surgeons have demanded medical manufactures eliminate gal from medicinal products[60]. Similarly, NIH-NIAID Director Anthony S. Fauci specifically identified the alpha gal allergy as "a serious and growing public health problem that urgently requires more research."[32]

CARDIOVASCULAR MEDICINAL PRODUCTS

The persistence of gal on acellular xenografts derived from bovine or porcine sources has been implicated in both acute and chronic responses. The current standard of care with bioprosthetic valves is crosslinking the collagen matrix with glutaraldehyde that reduces antigenicity by "hiding" or "masking" antigens including gal[16,61]. Unfortunately,

the glutaraldehyde treatment obliterates the natural regenerative properties of the graft and residual gal remains[17,59,62,63]. The persistent exposure of the recipient's immune system to gal is implicated in failure of current heart valves by calcification via a chronic IgM/IgG response[17,59,62–64].

More concerning for AGS patients receiving heart valves is that the acute IgE response has been linked to immediate post-operative anaphylaxis,[35] blood culture negative endocarditis,[54,65] and rapid destruction of the valve[34,54]. In addition, anaphylaxis during other cardiac procedures have been attributed to administration of heparin (derived from porcine intestines) or the use of the hemostatic agent Gelfoam (derived from porcine skin)[36,47,56]. Given the widespread use of heparin, it is of special concern and it must be noted that there have been very few documented cases of anaphylaxis due to heparin use. Although screening patients for anti-gal IgE titers prior to cardiovascular surgery may be an effective tactic to identify the optimum surgical intervention in order to prevent operative or immediate post-operative anaphylaxis, Hawkins documented several years after implantation of a bioprosthetic valve an allergen negative patient may seroconvert to allergen positive (after a tick bite) and subsequently acutely reject the implanted valve[34].

DECELLULARIZATION STRATEGIES TO REMOVE GAL

Some entities have tried to remove gal via decellularization[17,57] to remove immunogenic components including gal; however, these results have not been successful[61,66,67]. Allergists, particularly those examining the gal epitope on food products, have identified substantial flaws in any strategy intended to remove gal from tissue matrices via fluid washes. Raw, boiled, fried, beef or pork products have been examined to understand persistence of the gal epitope to typical cooking methods and regardless of treatment, alpha gal persisted[31,68].

Many proteins were identified as having the alpha gal epitope attached, including heat stabile proteins that were confirmed to be reactive to allergen positive serum[31,68]. Similarly, Bovine thyroglobulin (BTG) that is well known to be decorated with gal was subjected to a simulated digestion process. Although the BTG was broken down into many different smaller proteins, gal persisted and was reactive to allergen positive patient serum[69]. Others have identified gal to be inherent or bound to the collagen matrix including collagen and laminin – the major structural components of the extracellular matrix (ECM)[70].

Of note, Mullins has described the extreme conditions employed to produce gelatin intended as a human therapeutic that "uses a combination of acid and alkaline hydrolysis, followed by heat extraction at temperatures up to 90 °C, then sterilized at temperatures >100 °C"[49]. Regardless, gal persisted in these gelatin-derived colloids and resulted in anaphylaxis in AGS patients after intravenous exposure[49]. Perhaps, decellularization may be effective at removing unbound or soluble proteins that have gal. However, any decellularization strategy intense enough to remove gal chemically bound to ECMs to prevent adverse reactions in the allergen positive population would need to break chemical bonds subsequently degrading the matrix to the point of obliterating its biomechanical properties and rendering any resulting ECM useless.

ESTABLISHMENT OF AN ENGINEERED PIG THAT DOES NOT HAVE GAL

Revivicor, Inc. (Blacksburg, VA) has utilized its expertise in somatic cell nuclear transfer (SCNT), in combination with gene targeting techniques, to establish a unique proprietary

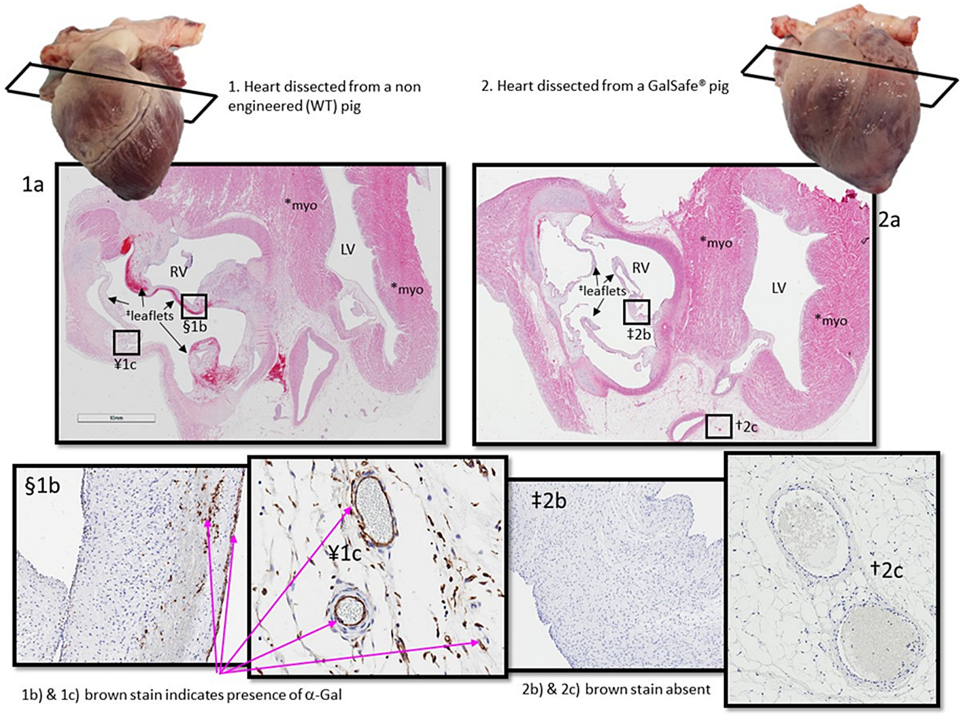

Figure 2. Evaluation of the H&E slides (transverse section at level of the tricuspid/ atrioventriculure valve) confirmed that the gross morphological characteristics of the GalSafe® heart (2a) is indistinguishable from a heart derived from a WT pig (1a) and confirmed normal morphology for heart tissue layers and structures (e.g., *myocardium, blood vessels and valve leaflets) with no observed differences between GalSafe® and WT genotypes. The cross section of heart valves from WT and GalSafe® pigs were stained with GS-isolectin B4 (brown) to detect gal. Gal is present (+) on the WT heart valve and is indicated by the brown stain present (pink arrows) in the representative enlarged images of the (1b) valve leaflet and (1c) blood vessels, respectively. Gal was not detected (–) in comparable areas, (2b) leaflet and (2c) blood vessels from the GalSafe® pigs.

genetically engineered (GE) pig, GalSafe®, that has both alleles of GGTA1 inactive, meaning that gal is not detectable in these pigs[71,72]. The GalSafe® pig is phenotypically normal[73,74] to a comparable non-engineered pig except for its genetically engineered trait (Figure 2). In addition, GalSafe® pigs produce anti-gal IgM and IgG[75]. Of note, Revivicor has demonstrated safety and efficacy of the GalSafe® pig by essentially completing all necessary steps for regulatory approval of the pig[76] with the FDA-CVM (data on file) that provides a foundation for pursuing various raw materials for fabrication into acellular scaffolds (tissue grafts without viable cells) for distribution as implantable human use medical product. Any tissue derived from the GalSafe® pig including heart valves, pericardium, vascular conduits and others may serve as materials for human use medical products.

IMPLICATIONS FOR CARDIOLOGY AND CARDIAC SURGERY

Gal hypersensitivity should be recognized as a relevant issue to cardiac surgeons and cardiologists. As noted above, patients may be at increased risk for severe coronary artery disease if they are sensitized to gal. As a result, consumption of red meat may be a modifiable risk factor that could decrease morbidity and mortality in patients who are sensitized to gal.

The fact that patients can be exposed to gal through implantation of bioprosthetic heart valves and other devices presents a different set of challenges, especially for

surgeons. Future studies should better characterize the relationship between elevated anti-gal antibody titers and bioprosthetic valve function. For example, patients with bioprosthetic valves known to have elevated anti-gal antibody titers should undergo echocardiography to evaluate valve function. At the same time, a non-human primate model of gal sensitization should be developed so that gal sensitization as it relates to bioprosthetic valves and other devices can be studied in a controlled manner. Non-human primate work should investigate new methods of decellularization and other processing techniques designed to mitigate the immunogenicity of gal. Importantly, non-human primate work should also directly compare the implantation of bioprosthetic heart valves from bioengineered animals such as GalSafe® pigs to the implantation of valves from wild type animals in non-human primates sensitized to gal. In the future, the standard of care may be implantation of bioprosthetic valves only if they are free of gal expression.

In the meantime, identifying patients with a gal allergy is important so that they can be properly managed in the perioperative period. Before implanting a gal-containing medical device (especially in areas endemic to pathogenic species sensitizing to gal), patients should be asked about food allergies, and especially about an allergy or intolerance to red meat or pork. They should also be asked about an allergy to the drug cetuximab, as this has been implicated in alpha gal allergy. If time permits, a referral to an allergist for specific testing may be appropriate. For patients suspected or confirmed to be sensitized to gal, the medical record should be updated to reflect this allergy. Manning and colleagues have recently written a thorough review of anesthesia considerations for patients with alpha gal syndrome, including a list of common perioperative drugs and other products that may contain alpha gal and should be avoided in patients with an alpha gal allergy[77]. While alpha gal sensitization is a relatively newly described condition, its potentially serious implications demand increased education efforts and vigilance in the perioperative period, especially in regions like the southeast United States where a high prevalence of sensitization exists.

CONFLICT OF INTEREST STATEMENT

John Bianchi and Anneke Walters are employees of Revivicor Inc, which develops genetically modified pigs, including gal-safe pigs. No other author has a conflict of interest.

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