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Meat allergy and allergens

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Introduction

Despite the fact that some animal products are well established food allergens, such as milk and eggs, allergy to meat itself has historically been considered to be quite rare. Case reports of allergy to mammalian and avian meat became more commonplace starting about 20 years ago, which in part also coincides with an increasing appreciation of food allergy in general[1]. IgE-mediated reactions to many different types of meat have now been reported. The list includes beef, pork, lamb, and poultry, but also a host of others including kangaroo[2], whale[3], seal[3] and crocodile[4]. A number of relevant allergens have been identified and characterized, and we have an increasing an appreciation of the natural history of meat allergy and relevant cross-sensitizations. About 10 years ago a new form of delayed anaphylaxis to red meat was recognized which relates to the oligosaccharide Gal- α 1,3Gal- β 1,4GlcNAcR (α -Gal)[5]. This allergy, which is often known as the α -Gal syndrome, has challenged many traditional paradigms of how we think about food allergy[6]. The present review considers various forms of meat allergy with a special emphasis on mammalian meat, aiming to highlight several advances over the last decade.

Immunology and epidemiology

Good estimates of the prevalence of meat allergy do not exist. Reactions to mammalian meat are more common than for avian meat, at least anecdotally, but neither is common. Mammalian meat allergy was once largely thought to be restricted to children, most commonly those with atopic dermatitis or cow's milk allergy[7], but now is equally appreciated in adults. Part of the explanation relates to the fact that several different forms of meat allergy have now been recognized. There is significant regional variation in meat allergy, which is likely a function of differences in local dietary habits, but other environmental factors are also important. This is dramatically highlighted by the realization that IgE sensitization to α -Gal is mediated by bites from certain hard ticks. Thus, for example, there is a markedly higher rate of allergic reactions to mammalian meat in the southeastern United States, an area endemic with *Amblyomma americanum* (lone star ticks), as compared to other parts of the country[8].

The mechanisms and routes of exposure that lead to anaphylactic sensitization have been an active area of inquiry for over a century dating back to the pioneering work of Richet and

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Portier[9]. For some food allergens, such as peanut, there has been convincing evidence that allergy results from epicutaneous sensitization[10, 11], but for many food allergens the route of sensitization is incompletely understood. For 'primary' mammalian and avian meat allergy the suggestion is that the inciting exposure is via the GI tract. However, many allergens can also be present in airborne particles or skin products[12] leading to the possibility of respiratory or cutaneous sensitization. Indeed, examples of syndromes where sensitization is established to have occurred outside the GI tract include: pork-cat[13], bird-egg[14] and α -Gal syndromes[8] (see table I). Generally these forms of allergy disproportionately impact adults and older children compared to primary meat allergy, however young children can also be affected. Fish-chicken syndrome is a more recently described entity that likely involves cross-sensitization from GI exposure [15].

Biology and biochemistry

Serum albumin constitutes one of the most important contributors to both mammalian and avian meat allergy. In contrast, the oligosaccharide α -Gal is selectively present only on mammalian tissue and IgE antibody to an equivalent oligosaccharide has not been described in avian allergy. Other less commonly identified allergens include immunoglobulin, myosin light chain kinase, parvalbumin, enolase and aldolase (see Table II) although this list is certainly not complete[16].

Albumins:

Serum albumins are ~ 70 kD α -helical proteins that are highly conserved in sequence and conformation across many animals, including mammals and birds[17] (see Table III). Serum albumins have multiple biologic functions but importantly they can cross capillary endothelia and are present in epithelia. Thus, in addition to being present in mammalian foods such as meat, milk and eggs, animal pelts and bird feathers also contain serum albumins, with the implication that inhalant and cutaneous exposure can occur [18]. There are several consequences that can result from the multitude of different sources of animal albumin. One is that it is common for subjects with beef allergy to have a co-existing milk allergy. Indeed, this was reflected among 28 young Italian children with beef allergy where 26 were sensitized specifically to Bos d 6 and all of these had immediate reactions upon milk challenge[19]. Bird-egg syndrome represents a situation where primary sensitization to an avian serum albumin occurs via a respiratory route but subsequently subjects develop allergic symptoms upon ingestion of poultry[20, 21].

Cross-reactivity between albumins from different species is common, but most often involves phylogenetically similar sources. Thus, cases of albumin-related allergy to both mammal and bird products are very rare [22, 23]. Serum albumin cross-reactivity is a key feature in pork-cat syndrome, where primary sensitization to cat serum albumin, also known as Fel d 2, leads to allergic reactions upon ingestion of pork products containing pork serum albumin, i.e. – Sus s 1. Interestingly, some of these subjects also react to beef, which likely reflects further epitope spreading of the IgE response to include Bos d 6[13]. Although historically the syndrome has been called 'pork-cat', some have advocated that because cat sensitization precedes the allergic reaction to pork, that 'cat-pork' would be a more apt name[24, 25]. Other examples of clinically relevant albumin cross-reactivity have been

described in case reports. One such recent example involved a woman with respiratory allergy to dog, who reported anaphylaxis to horse meat. Her ensuing work-up revealed elevated IgE responses to dog extract as well as the serum albumins to dog (Can f 3) and horse (Equ c 3). Supporting a diagnosis that would be consistent with 'dog-horse' is the fact that inhibition studies supported primary sensitization to Can f 3 [26].

Serum albumins are generally considered heat labile, and as such the frequency and severity of reactions are likely reduced by consuming well-cooked animal products [7, 27]. Other approaches, such as freeze-drying, may be even more helpful for reducing allergenicity [27, 28].

α -Gal:

When considering α -Gal it is important to realize that it was first appreciated as a 'B like' blood group antigen by Landsteiner[29]. Indeed, it shares structural features with the blood group B antigen (Fig 1), and is the target of abundant 'natural' IgM, IgG and IgA antibodies in immunocompetent humans[30]. The oligosaccharide is present in many mammalian foods, including meat, internal organs (such as kidney or tripe), milk and other dairy, and gelatin[31], but also other products such as the monoclonal antibody cetuximab, anti-venom and the zoster vaccine [32–34]. Among the features that distinguish α -Gal syndrome from other IgE-mediated meat allergies (see Table IV) is the fact that reactions are delayed, typically occurring 3–6 hours after a relevant exposure[35]. From a clinical perspective this is an important characteristic and helps distinguish reactions related to α -Gal from those caused by IgE to other allergens. Anecdotally, we have seen several patients in our clinic for evaluation of possible α -Gal syndrome where the correct diagnosis involved IgE to bovine serum albumin, pork serum albumin or gelatin. The case of gelatin is notable because some preparations contain α -Gal and therefore it is possible to have IgE-mediated reactions occurring to either the gelatin itself or the α -Gal component [31, 34, 36].

In the ten years since α -Gal was first identified as an important meat allergen there remain several important unanswered questions (see Table V.) The mechanisms that contribute to the delay in clinical symptoms with α -Gal remain poorly understood. Importantly, this delay has been demonstrated in prospective meat challenge studies where *ex vivo* basophil activation occurred with similar kinetics [35]. The explanation that seems most plausible involves the time required for processing, digestion and transit of α -Gal epitopes to target tissues. While a number of recent studies have focused on α -Gal containing glycoproteins, including in meat (see Table VI)[37–39], α -Gal linked glycolipids are also well established in other mammalian cells and tissues[40]. The kinetics of lipid metabolism, which involves packaging into chylomicrons and transit through lymphatics and the thoracic duct before entering the bloodstream, suggests the possibility that α -Gal-containing lipids are particularly important in the delayed allergic response. Indeed, this hypothesis also fits with the observation that lean meat, particularly venison, is less likely to trigger reactions in α -Gal allergic subjects than fatty cuts.

The complete α -Gal epitope is considered to be the trisaccharide form (i.e - Gal- α 1,3Gal- β 1,4GlcNAcR), however multiple studies have shown that the two terminal galactoses are the major binding determinant[41–43]. Indeed, this is why we often refer to the α -Gal

epitope as galactose- α -1,3-galactose. It should be pointed out, however, that the antibody repertoire to α -Gal is broad and, at least in studies that investigated anti-Gal IgG, some antibodies can also bind the B antigen [40, 41, 44]. An implication of the heterogeneous specificity in anti-Gal antibodies is that differences in the quantity and/or quality of the IgE antibody repertoire may impact whether a sensitized subject experiences allergic symptoms upon a relevant ingestion. The point here is really two-fold: i) in population studies many individuals produce IgE to α -Gal but do not have allergic symptoms, and ii) the extent of IgE affinity maturation and epitope spreading is likely a factor in distinguishing allergic subjects from those that are sensitized but tolerant. The former point is perhaps best exemplified by a recent report of high-risk forest workers from southwest Germany where 58 of 300 subjects were sensitized to α -Gal (cut-off of 0.35 IU/mL), but only 5 of these had symptoms consistent with α -Gal syndrome, i.e – over 90% of the sensitized subjects in the cohort did not report relevant symptoms[45]. The latter point is suggested by recent work from Jappe et al. where subjects with α -Gal syndrome had broad reactivity to a number of different α -Gal-containing epitopes[46]. Another possibility that could explain why many subjects who are sensitized to α -Gal do not report symptoms, or do not report symptoms with every meat ingestion, is that there can be significant heterogeneity in the complexity of α -Gal linked oligosaccharide structures. For example, α -Gal can be present on mono-, bi- or tri- antennary oligosaccharides, as shown in Figure 3 for Cetuximab. It is possible that IgE binding is favored when multiple α -Gal epitopes are in close proximity, which was supported by *in vitro* experiments that compared IgE binding to cetuximab F(ab')₂ and Fab fragments with purified Gal- α 1,3Gal- β 1,4GlcNAc polysaccharide. However, the details of the complexity of α -Gal-linked oligosaccharides have not been established for meat itself.

Any discussion about the relevance of a food allergen needs to consider the stability of the epitope during food preparation and transit of the digestive tract. Results of prick-to-prick tests comparing raw or cooked meat (beef and pork) in α -Gal subjects suggest that heating may have some effect on allergenicity[47]; on the other hand α -Gal epitopes on glycoproteins in pork kidney retained reactivity to a specific monoclonal antibody despite heating for 10 minutes at 95^o C[38]. Using beef thyroglobulin as a model, Apostolovic et al. have shown that α -Gal peptides remain intact after *in vitro* pepsin digestion. Not only was IgE binding maintained, but the glycopeptides also stimulated basophils obtained from α -Gal allergic (but not non-allergic) subjects [39].

Immunoglobulin:

A few studies have described immunoglobulin as a target of IgE in meat allergic subjects, however the clinical relevance is less clear-cut than for albumin and α -Gal[7, 48]. For example, among ten Japanese children with atopic dermatitis and reported beef allergy, seven had a strong signal to BSA using IgE immunoblots but three did not[48]. The sera from these three subjects recognized ~60 and 200 kD glycoproteins. The fact that binding was inhibited by bovine gamma globulin suggested that immunoglobulin was the target of this IgE. One possibility is that a specific glycosylation(s) could explain this binding, although this has not been directly addressed [49]. Alpha-Gal could represent one such glycosylation, although the evidence for its presence on mammal (non-human) IgA or IgM is stronger than for IgG[50, 51].

Allergen sources

The majority of studies investigating meat allergy have relied on natural sources of allergens. One exception, which relates to pork-cat syndrome, is that a recombinant feline serum albumin, i.e. - Fel d 2, has been developed by Phadia/Thermo-Fisher. As such, commercial assays that use ImmunoCAP currently incorporate a recombinant Fel d 2 that has been expressed in a yeast system. This recombinant is absent of any N-linked glycosylation and is reported to have a native folding pattern (Jonas Lidholm, PhD, personal communication).

Approaches to meat allergy testing with focus on *in vitro* diagnostics

Investigation of a suspected case of meat allergy often requires a multi-faceted approach, with component diagnostics playing an important role. Skin testing can be helpful, although sensitivity can be limiting with standard prick testing. Alternatives include attempting prick-to-prick with fresh food sources or cautious use of intradermal testing. A shortcoming of the prick-to-prick approach is that there can be substantial variability in food preparations and results are not well validated. In our experience intradermal testing with commercially available beef, pork and lamb extracts can be done safely and correlates well with clinically relevant α -Gal allergy. However, *in vitro* tests can eliminate the risks associated with intradermal testing and variability with prick-to-prick testing, and can be required to confirm a diagnosis when identifying a specific allergen is important.

There are nuances of *in vitro* IgE testing which are important to consider. For example, there are unique strengths and weaknesses that come with the use of extract versus component assays, or singleplex versus multiplex assays (see Table VII). For extract tests an important caveat relates to the fact that many meat allergens represent a minor fraction of the extract. As a consequence, the result of the extract assay may be an underestimation of the magnitude of the IgE response to the specific allergen[52]. Component tests do not suffer this limitation and, additionally, can be particularly helpful for identifying the relevant epitope of a mammalian meat allergy. This is also true for identifying allergens that may be involved in cross-sensitization. In addition to extract tests, component tests that are particularly helpful in the evaluation of a putative mammalian meat allergy include: α -Gal, Bos d 6, Sus s 1, Fel d 2 and gelatin.

The assay for α -Gal warrants additional consideration. The commercially available assay involves beef thyroglobulin conjugated on the solid-phase and is available through Phadia/Thermo-Fisher, and in the United States through Viracor-IBT (Lees Summit, MO). Beef thyroglobulin is heavily glycosylated with reports suggesting the possibility of 8–11 α -Gal linkages[39, 53]. Research studies have also often used the monoclonal antibody cetuximab conjugated by the streptavidin technique to the solid-phase. Importantly, the performance of the two assays correlate closely as demonstrated in Fig 2, and reported by European colleagues[46]. Despite the close correlation, the conclusion of Jappe et al. was that the cetuximab assay may be the more sensitive of the two assays[46]. Another assay that has been used experimentally uses α -Gal-conjugated to human serum albumin on the solid-phase, and this too had similar performance with the cetuximab assay[54].

Vaccine candidates

The idea of desensitization to food allergens has recently gained traction in the allergy community, though meat has been little studied in this regard. While we are aware of a recent case report describing successful desensitization in two α -Gal cases[55], we have not undertaken it and would not recommend desensitization outside of research settings. An intriguing question is whether the continued consumption of foods that contain small amounts of α -Gal, such as some dairy products, could be protective for those that are allergic to beef, but this has not been adequately addressed.

Conclusion and Future directions

Despite traditionally being considered rare, meat allergy is being increasingly recognized in subjects of all ages. In part this may reflect an increasing incidence, but also an appreciation that regional differences in exposure can have a major impact on prevalence of the disease. The increase has occurred at the same time as increases in other allergic diseases. The development of *in vitro* diagnostics has helped define important syndromes in meat allergy, i.e.- α -Gal and pork-cat, and has been an important tool in clinical practice for confirming diagnosis. Identification of relevant allergens has had important consequences for disease management. This includes tailored dietary information to the patient, but also insight into the exposures and underlying mechanisms that lead to and/or promote ongoing sensitization.

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Figure 1.
Comparison of structure of α -Gal and blood Group B antigen

Figure 2. Correlation of sIgE to cetuximab (IU/mL) and beef thyroglobulin (IU/mL) in 34 subjects with α -Gal syndrome and 11 control subjects. Modeled with linear regression ($p < 0.001$).

Figure 3.

Cetuximab is a chimeric IgG1 monoclonal antibody produced in a mouse cell line (SP2/0). Demonstrated here are representative bi- and tri-antennary glycans with terminal α -Gal epitopes that are often present on the variable region of the heavy chain (V_H). Other glycans with α -Gal are also possible. Glycans are very common on the constant heavy chain domain 2 (C_H2) but only rarely do these have terminal α -Gal epitopes. When present on the Fc α -Gal epitopes are most frequently mono-antennary. Anti-Gal IgE can bind to α -Gal epitopes on Fab, but not Fc because the tertiary structure of the antibody precludes exposure of these epitopes[54, 56].

Table 1.

Meat allergy syndromes

Source	Allergy syndrome	Major Allergen(s)	Route/Mode
Mammalian Meat	Pork-cat	Serum albumins	Respiratory exposure to cat serum albumin in dander
	α -Gal	Gala.1–3Gal β 1–4GlcNAcR	Skin via tick bites
Avian meat	Bird-egg	Serum albumin	Respiratory exposure to bird serum albumin in feathers
	Fish-chicken	Parvalbumin, Aldolase, Enolase	Oral

Table II.

Important allergens from representative mammalian and avian meat sources

Source	Allergen name	Biochemical name
Bovine	Bos d 6	Serum albumin
	Bos d 7	Immunoglobulin
	α -Gal	Gal- α 1,3Gal- β 1,4GlcNAcR *
Chicken	Gal d 5	Serum albumin
	Gal d 7	Myosin light chain kinase
	Gal d 8	α -parvalbumin
	Gal d 9	β -enolase
	Gal d 10	Aldolase

* α -Gal linkages have been described on a number of mammalian glycoproteins and glycolipids[17–19]

Table III.Common serum albumin allergens in animals (from allergen.org, WHO/IUIS)

Common name	Species	Allergen	Molecular Weight (kD)
Domestic cattle	<i>Bos domesticus</i> *	Bos d 6	67
Dog	<i>Canis familiaris</i>	Can f 3	69
Guinea pig	<i>Cavia porcellus</i>	Cav p 4	66
Domestic horse	<i>Equus caballus</i>	Equ c 3	67
Cat	<i>Felis domesticus</i>	Fel d 2	69
Chicken	<i>Gallus domesticus</i>	Gal d 5	69
Domestic pig	<i>Sus scrofa</i>	Sus s 1	60

* traditionally referred to as *Bos Taurus*, but studies have not been done on native cow species

Table IV.

Ways that α -Gal syndrome differs from traditional IgE-mediated food allergies

I.	Primary sensitization is mediated through the skin via tick bites (not oral exposure)
II.	Allergy onset is usually in adults
III.	The major B cell epitope is an oligosaccharide
IV.	Anaphylactic reactions are delayed, usually > 2 hours
V.	Skin prick tests are not sufficiently sensitive

Table V.Unanswered questions regarding the mechanism of reactions in α -Gal syndrome

Does the delay reflect time required for processing and digestion?
Is there a difference between the response to α -Gal-containing glycolipid and glycoproteins?
Is the complexity of the oligosaccharide, i.e. - mono vs bi-antennary, relevant?
Are multiple adjacent α -Gal moieties necessary for Fc ϵ RI cross-linking?

Table VI.

α -Gal linked glycoproteins recognized by IgE in subjects with red meat allergy[37]

Protein	Molecular Weight (kD)
Alpha-enolase *	47.3
Beta-enolase	47.1
Aspartate aminotransferase	46.4
Creatine kinase M-type	43
Lactate dehydrogenase A	35.6
Carbonic anhydrase 3	29.4
Triosephosphate isomerase	26.7

* Glycoproteins in bold retained IgE binding after heat treatment.

Table VII.Considerations for *in vitro* IgE testing in meat allergy

	Extract --vs.-- Component		Singleplex -- vs. -- Multiplex	
Strength	<ul style="list-style-type: none"> > Readily available in commercial assays > Useful for initial screen 	<ul style="list-style-type: none"> > Greater sensitivity and specificity for identifying IgE epitope > Helpful for establishing syndromes with cross-sensitization 	<ul style="list-style-type: none"> > More allergen on the solid-phase & thus greater sensitivity > Both extracts and components can be conjugated to solid-phase > Quantitative 	<ul style="list-style-type: none"> > Can test > 100 allergens with single test > Standard panel has multiple serum albumins
Weakness	<ul style="list-style-type: none"> > IgE result can be underestimate for minor allergens > Cannot identify specific epitopes 	<ul style="list-style-type: none"> > Not all components are commercially available > Minor impurities can be amplified (in singleplex format) 	<ul style="list-style-type: none"> > Some components are not available in this format > Multiple tests likely required 	<ul style="list-style-type: none"> > Less sensitive, particularly in the presence of high levels of specific IgG₄ or IgG₁ > Cannot use extracts on solid-phase > Standard panel may not include α-Gal > Semi-quantitative