

The glycan did it: how the α -gal story rescued carbohydrates for allergists – a US perspective

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Key words

alpha-gal – IgE – red meat allergy – delayed anaphylaxis – cetuximab – glycan

Abstract

IgE antibodies to carbohydrate epitopes on allergens are thought to be less common than IgE antibodies to protein epitopes and also of much less clinical significance. Our recent work, however, has identified a novel IgE antibody response to a mammalian oligosaccharide epitope, galactose-alpha-1,3-galactose (alpha-gal). IgE to alpha-gal has been associated with two distinct forms of anaphylaxis including delayed allergic reactions after eating beef, pork or lamb. IgE antibody responses to

alpha-gal have now been found globally. Therefore, establishing the mechanism of the specific IgE antibody response to alpha-gal will be an important aspect to address as this area of research continues.

Cite this as Commins SP, Jerath MR, Platts-Mills T. The glycan did it: how the α -gal story rescued carbohydrates for allergists – a US perspective. *Allergo J Int* 2016;25:44–8

DOI: 10.1007/s40629-016-0097-1

Introduction

IgE antibodies to carbohydrate epitopes on allergens are thought to be less common than IgE antibodies (Ab) to protein epitopes and also of much less clinical significance (see www.allergen.org) [1, 2, 3]. Thus, when anaphylactic reactions to the monoclonal antibody cetuximab were recognized as a major regional complication of this cancer treatment the initial investigation focused on finding IgE antibodies to a protein epitope [4, 5]. It was a surprise when it became clear that these reactions were causally related to pre-existing IgE antibodies specific for glycosylation present on cetuximab (**Fig. 1**) [4]. This discovery, published in 2008, has led our research in several directions, most significant for this review is that the IgE Ab to galactose-alpha-1,3-galactose (α -gal) also bind a wide range of non-primate mammalian products including beef, pork, and lamb. An understanding of the tissue distribution of α -gal led us to recognize and describe a delayed food allergy to mammalian meat and those studies have opened up investigations of how the IgE response to α -gal is different from typical IgE responses directed towards protein allergens [6, 7].

Cetuximab-induced hypersensitivity reactions

In clinical trials for the monoclonal antibody (mAb) cetuximab, which is specific for the epidermal growth factor receptor (EGFR), it became clear that the antibody was causing hypersensitivity reactions in a group of southern US states that were not evident elsewhere [5]. These reactions to cetuximab developed rapidly during the first infusion of the antibody and occasionally proved fatal [4, 5, 8]. In part because our

Abbreviations

Ab	Antibodies
CCDs	Cross-reactive carbohydrate determinants
EGFR	Epidermal growth factor receptor
α -gal	Galactose-alpha-1,3-galactose
Ig	Immunoglobulin
LDL	Low density lipoprotein
mAb	Monoclonal antibody
NGNA	N-glycolyl neuraminic acid

Submitted

June 17, 2015

Accepted

September 21, 2015

group had developed the IgE fluorometric enzyme immunoassay or CAP assay to cetuximab using the streptavidin technique, we were subsequently asked to investigate the reactions to cetuximab [9]. Through a series of collaborations, we demonstrated that the patients who had reactions to cetuximab also had IgE antibodies specific for this molecule before they started treatment [4]. Characterization of cetuximab glycosylation revealed 21 distinct oligosaccharide structures, of which approximately 30% have one or more alpha-1,3 linked galactosyl residues (**Fig. 1**) [10]. Analysis of the IgE antibodies to cetuximab demonstrated that these antibodies were, in fact, specific for the α -gal residues on the heavy chain portion of the mAb [10].

The red meat connection and α -gal

Also in the 2006–2008 time period, we evaluated a number of patients who had presented with episodes of generalized urticaria, angioedema, or recurrent anaphylaxis. Despite there being no clear immediate cause for their symptoms, several of the patients reported that they felt the reactions might be due to eating meat three to five hours earlier. Prick tests were performed with commercial extracts of beef, pork, or lamb and produced small wheals that often would be reported as negative (**Fig. 2**). However, given the compelling history described by the patients, we extended our analysis to intradermal skin testing with commercial meat extracts or prick skin tests with fresh meat extracts both of which demonstrated strong positive results (**Fig. 2**) [7]. In some patients with IgE to α -gal, positive responses have been seen to gelatin-based colloids on skin testing. The skin findings were confirmed with blood tests for specific IgE Ab to α -gal and mammalian antigens [7].

The characteristics of reactions due to α -gal IgE are different from typical food allergic reactions. While common complaints include both gastrointestinal symptoms and hives, patients do not develop any symptoms until several hours after eating red meat. In fact, many reactions are delayed for four to six hours or even longer. Although the most common reported and observed symptom that heralded a reaction was pruritus, symptoms can progress to be severe or even life threatening. Many patients do not report any symptoms prior to the onset of a reaction and, equally, symptoms do not occur with every exposure to red meat. In each known instance, the onset of allergic reactions due to α -gal represented a break in oral tolerance where the patient had tolerated mammalian meat in the past, often for over 25 years.

Three observations led us to investigate whether IgE antibodies to α -gal were present in the sera of adult patients reporting reactions to beef:

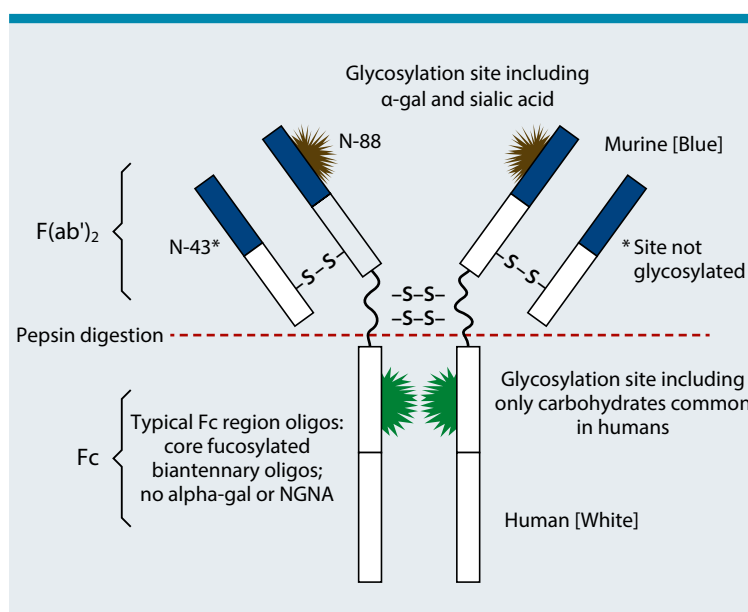


Fig. 1: As reported by Qian et al. [10], there were 21 distinct oligosaccharide structures present on cetuximab. These oligosaccharides differ mainly in degree of sialylation with N-glycolyl neuraminic acid (NGNA) and extent of galactosylation. Of the 21 oligos identified, approximately 30% had one or more alpha-1,3 linked galactosyl residues as measured by peak area on time-of-flight mass spectroscopy analysis. Approximately 12% had an NGNA residue and a very small amount of high mannose. Both α -gal and NGNA were found only in the Fab portion.

1. α -gal is known to be present on both tissues and meat from non-primate mammals [11],
2. the antibodies causing reactions to cetuximab were directed against α -gal [4],
3. the geographical distribution of the reactions to cetuximab overlapped the same geographical area where the red meat reactions were occurring [4, 5, 7].

Not surprisingly, the patients' sera tested positive for IgE to beef, pork, lamb, cat, and dog, but not to non-mammalian meat such as turkey, fish, or chicken [7, 12]. The presence of α -gal was confirmed using beef thyroglobulin and α -gal-linked human serum albumin that were each bound to sepharose beads [7]. Pre-incubation of either glycosylated antigen with appropriate sera reduced the specific IgE binding to beef, pork, lamb, cat, and dog by > 75% [7].

Sensitization and symptoms of delayed anaphylaxis

Currently, it is our hypothesis that bites from ectoparasitic ticks are the sensitizing event that leads to the development of sIgE to the oligosaccharide α -gal, which results in a loss of tolerance to non-primate mammalian meat and related food products in some individuals [13]. We do not



Fig. 2: Representative skin prick and intradermal test results in a patient with IgE antibodies to α -gal. Prick tests (left side) were performed with a lancette on the volar surface of the arm with commercially available skin testing reagents at 1 : 20 wt/vol after histamine reactivity (+) was verified. Intradermal testing (right) was performed with 0.03 mL of a 1 : 100 dilution of commercially available reagents (ie, 1:2,000 wt/vol) with a 25-gauge needle. Negative controls were 50 % glycerin/saline for skin prick tests and buffered saline for intradermal tests.

believe that every person bitten by a tick ends up with an IgE response and one of our goals is to determine whether there are inherent factors that create a risk for the IgE response. While the α -gal IgE response represents sensitization to a glycan, one of the notable differences when compared to IgE antibodies to plant-derived cross-reactive carbohydrate determinants (CCDs) is that α -gal antigen exposure more reliably leads to symptoms [1, 2, 3, 14, 15, 16].

Patients with IgE to α -gal typically report symptoms beginning three to six hours after eating meat [7]. In formal challenge studies using pork or beef, hives and other symptoms were delayed at least two hours after meat ingestion [17]. More recent work has shown that the time frame to reaction can be decreased with co-factors such as exercise or alcohol [18]. Delayed reactions after eating meat are distinct from the reactions to cetuximab, which develop rapidly and symptoms often peak within 20 minutes of initial administration of the drug [4, 5, 8]. In keeping with the more rapid time frame, in vitro responses of basophils isolated from subjects with delayed meat reactions show activation with glycoproteins within 20–30 minutes [17].

Skin test responses, whether prick or intradermal, to cetuximab are also rapid [19]. Thus, the delay in response after eating meat does not reflect an inability of basophils or mast cells to be activated by these glycoproteins. On the contrary, we believe that the results to date suggest the allergenic form of the oligosaccharide enters the circulation several hours after eating. Lipids would make a likely form of the allergen given the absorption and processing that must occur before these particles enter the bloodstream (Fig. 3) [20]. In fact, α -gal is present on both glycoproteins and glycolipids (including chylomicrons) [11]. Our current concept is that the most likely explanation for the delay in symptoms is that the allergenic form of α -gal exists as a glycolipid.

Our studies have shown that during a challenge, circulating basophils assessed ex vivo upregulate

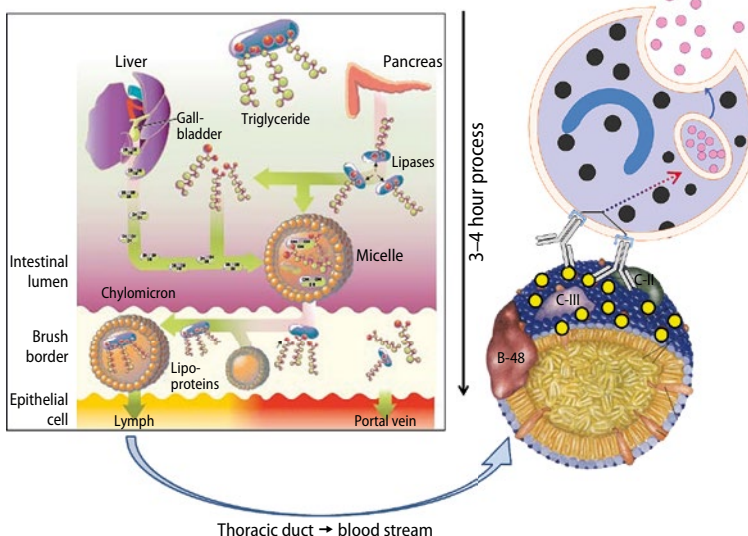


Fig. 3: Absorption, trafficking, packaging, and digestion of dietary lipids. When the ingested lipids are from a non-primate mammalian, α -gal glycolipid moieties may be present (represented as yellow dots on the surface of a lipid particle). In the presence of IgE to α -gal, crosslinking of IgE receptors can occur and lead to basophil/mast cell degranulation (pink dots).

the expression of CD63 in a similar time frame as the patients develop symptoms [17]. Surprisingly, a proportion of non-allergic controls also demonstrated upregulation of CD63, although they do not experience any symptoms. Evidence that basophils and mast cells have receptors for low density lipoprotein (LDL) was reported many years ago [21, 22]. One potential explanation for this enigmatic finding is that the existing IgG or IgM response specific for α -gal (known as “anti-Gal” [23]) present in all immunocompetent individuals is binding α -gal glycolipids and activating basophils to upregulate CD63. The implication is that lipid particles with α -gal on the surface can cause pro-inflammatory mediator release not only in individuals with IgE Ab to α -gal but also in those who are non-allergic. Recent studies have shown that anti-Gal may not be present or at least present in a highly variant degree [24] than previously shown by some studies [23] (but certainly not all [25, 26]), thus the activation of basophils in control subjects could be reflective of the anti-Gal variability. In addition, data have clearly shown that host blood group significantly affects the anti-Gal IgG response, which may also be reflective of the initial observation that patients with B or AB blood group appear to be under-represented in those diagnosed with the red meat allergy [6].

Conclusion

The finding that IgE to α -gal explains two novel forms of anaphylaxis has not only changed several established rules about allergic disease, but has opened up at least two new areas of research. The results provide evidence that: IgE responses to an oligosaccharide can induce significant or severe allergic symptoms, ticks can induce high titer food specific IgE responses in adult life breaking long-held periods of oral tolerance, and also that eating mammalian products carrying this epitope does not give rise to any symptoms during the few hours or more.

Like so many new findings, this area of research provides both challenges and opportunities. The delay in onset of symptoms following eating red meat is best explained by delayed arrival of the relevant form of antigen in the circulation, but the question remains as to what form of glycoprotein or more likely glycolipid takes three hours or more to appear in the circulation. Finally, the often-rapid production of IgE antibodies to α -gal after tick bites provides a striking model of an ecto-parasite-induced IgE response. This parasite only enters through the skin and the tick saliva contains a wide variety of agents that could act as antigens and/or as adjuvants. However, it remains a striking challenge to identify why the response is so ro-

bust and why it is directed so consistently against the α -gal disaccharide.

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Conflict of interest

Dr. Platts-Mills holds a patent on the use of streptavidin solid phase to evaluate IgE antibodies to recombinant molecules. The rest of the authors declare no conflict of interest.

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DOI: 10.1007/s40629-016-0097-1

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