Anaphylaxis syndromes related to a new mammalian cross-reactive carbohydrate determinant

Scott P. Commins, MD, PhD, and Thomas A. E. Platts-Mills, MD, PhD *Charlottesville, Va*

Anaphylaxis is a severe allergic reaction that can rapidly progress and occasionally be fatal. In instances in which the triggering allergen is not obvious, establishing the cause of anaphylaxis is pivotal to long-term management. Assigning cause is limited, however, by the number of known exposures associated with anaphylaxis. Therefore identification of novel causative agents can provide an important step forward in facilitating new, allergen-specific approaches to management. In contrast to the view that carbohydrate-directed IgE has minimal, if any, clinical significance, recent data suggest that IgE antibodies to carbohydrate epitopes can be an important factor in anaphylaxis that might otherwise appear to be idiopathic. Here we review the evidence relating to carbohydrates in food allergy and anaphylaxis and discuss the implications of a new mammalian cross-reactive carbohydrate determinant. (J Allergy Clin Immunol 2009;124:652-7.)

Key words: *Anaphylaxis, cross-reactive carbohydrate determinant, α-gal, glycosylation*

Abbreviations used
CCD: Cross-reactive carbohydrate determinant
α-gal: Galactose-α-1,3-galactose
Neu5Gc: *N*-glycol neuraminic acid
sIgE: Specific IgE

Investigation of severe hypersensitivity reactions occurring during the first infusion of the monoclonal antibody cetuximab demonstrated that these reactions were caused by pre-existing IgE antibodies to the carbohydrate galactose-α-1,3-galactose (α-gal) present on the Fab portion of the monoclonal antibody.[1] The obvious implication is that the glycosylation of therapeutic recombinant molecules can create a risk for severe hypersensitivity reactions. We have now found that these IgE antibodies to α-gal occur in the sera of patients in an area of the southeastern U.S. and bind to a wide range of mammalian allergens.[1,2] In addition, it has become clear that IgE antibodies specific for α-gal are associated with an unusual form of delayed anaphylaxis which occurs 3-6 hours after eating mammalian meat.[2] Due to the significant delay in symptoms, many of these reactions were regarded as 'spontaneous' or 'idiopathic' anaphylaxis.

In those cases of anaphylaxis in which the cause is known, the symptoms appear to be mediated by a specific IgE (sIgE) response to the causative allergen that leads to systemic release of histamine and other mediators from mast cells and basophils. This process might lead to shock with generalized urticaria, laryngeal edema, lower-airway obstruction, and hypotension. Establishing the cause of recurrent anaphylaxis is a critical aspect of treatment because the identification of causal allergens allows the use of either avoidance or immunotherapy in the management. The most frequent allergens involved in anaphylactic reactions are proteins found in peanuts, tree nuts, fish, shellfish, and bee and wasp venoms, as well as drug haptens and latex. Carbohydrates in the form of complex oligosaccharides are also present on many foods and can be the target of anti-glycan IgE responses. Because these carbohydrate moieties can be present on multiple different types of proteins, they are prone to extensive cross-reactivity. These epitopes are called cross-reactive carbohydrate determinants (CCDs); however, until recently the clinical significance of IgE antibodies directed against them has been unclear.

OVERVIEW OF CCDS

Role in producing clinical symptoms

The study of glycosylation on proteins as a target for IgE in relation to food antigens began in the 1970s when a Japanese group reported the structure of a protease from pineapple stem.[3] It was subsequently shown that this protease, bromelain, carried an oligosaccharide with 2 structural features that had not been found in mammalian glycoproteins: core α1,3-fucose and xylose (Fig 1). In fact, xylose and core-3–linked fucose might be the most common carbohydrate epitopes recognized by human IgE antibodies. Subsequently, the binding of IgE to Api m 1 (honeybee venom phospholipase A_2) in sera of patients with bee venom allergy was shown to be inhibited by glycopeptides from pineapple stem bromelain.[4]

Current estimates are that 15% to 30% of allergic patients generate specific anti-glycan IgE.[5] This frequent occurrence of serum IgE to CCDs contrasts with their modest effect on skin tests and their apparent inability to produce clinical symptoms. Historically, a study by van der Veen et al[6] is often cited as evidence against a significant clinical effect of carbohydrate-directed IgE. In that study basophils from patients who had IgE antibodies to a carbohydrate moiety on peanut but without clinical symptoms were found to release histamine only after stimulation with very

From the Asthma and Allergic Diseases Center, University of Virginia Health System, Charlottesville, Va.

Disclosure of potential conflict of interest: T. A. E. Platts-Mills has received research support from ImClone and Phadia. S. P. Commins has declared that he has no conflict of interest.

Received for publication July 14, 2009; revised August 13, 2009; accepted for publication August 18, 2009.

Reprint requests: Scott P. Commins, MD, PhD, Allergy Division, PO Box 801355, University of Virginia Health System, Charlottesville, VA. E-mail: spc7w@virginia.edu.

0091-6749/$36.00

doi:10.1016/j.jaci.2009.08.026

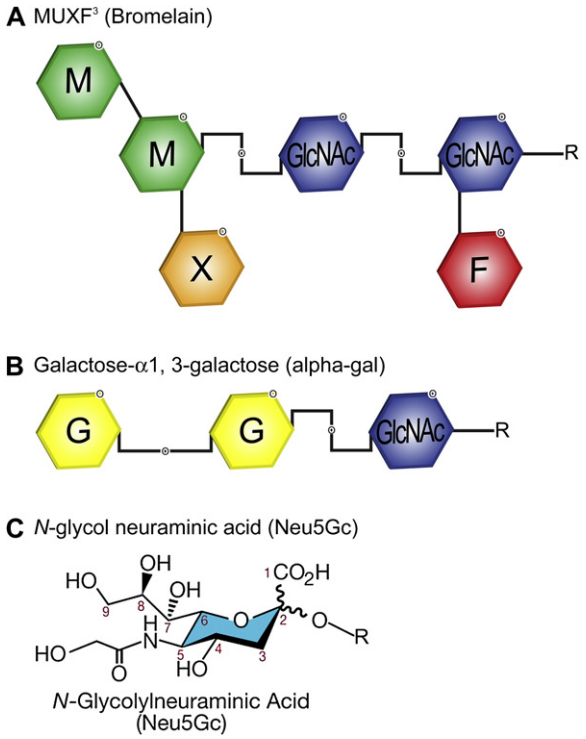

FIG 1. Plant and mammalian glycans. **A**, Structure of MUXF[3] (bromelain), which contains the structural features of xylose and core α1,3-fucose. Xylose and core α1,3-fucose are the main motifs for CCD-directed IgE and are the essential part of 2 independent epitopes. **B**, Structure of the α-gal epitope found in lower mammals and depiction of the important α(1→3) linkage. **C**, Structure of Neu5Gc that, like α-gal, is found in lower mammals but not human subjects.

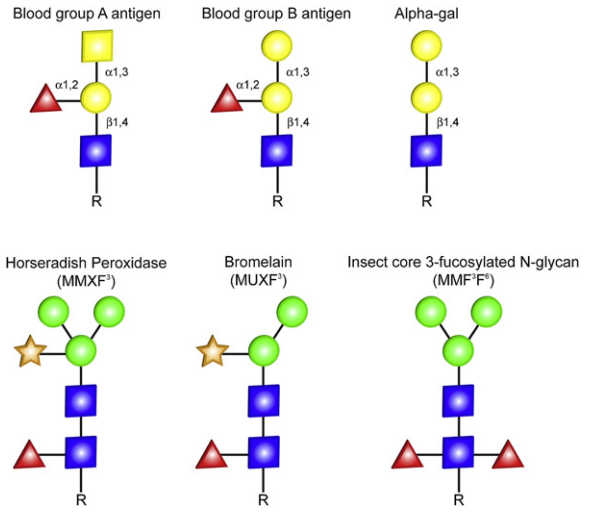

FIG 2. Comparison of representative glycans referenced in the text. The oligosaccharide structures are shown in the symbolic depiction suggested by the Consortium of Functional Glycomics, such that the *blue squares* represent N-acetylglucosamine, *green circles* represent mannose, *yellow* symbolizes galactose, while *orange squares* and *red triangles* are xylose and fucose, respectively. Note that the lack of a core fucose residue separates the structure of blood group B antigen from α-gal.

high concentrations of a peanut extract. Those results contrasted with the much lower concentrations needed to produce histamine release from basophils of patients with sIgE to peanut protein and documented clinical peanut allergy. Based on these differences, it was concluded that anti-glycan IgE detected by using *in vitro* methods was clinically irrelevant or required high concentrations to produce degranulation, possibly because of its monovalent nature. Mari[5] reached a similar conclusion when testing of more than 4,500 patients with possible inhalant allergy for reactivity to bromelain revealed that 23% had positive *in vitro* results, whereas only 0.1% had positive reactions on skin testing.

A clinically benign role for CCDs is now being questioned, though, as several studies have shown the ability of anti-CCD IgE to trigger mediator release from basophils.[7] Careful analysis of the van der Veen et al[6] study reveals that those patients were primarily sensitized to grass pollens, and their anti-glycan IgE antibodies were more likely induced as part of this sensitization and only cross-reactive with peanut glycans (mannose). Thus, a high concentration of peanut extract might have been required to achieve basophil stimulation because the IgE antibodies bound poorly to, or were partially cross-reactive against, carbohydrates present on peanut. In the aforementioned study which examined the prevalence of bromelain sensitization, skin testing to bromelain revealed few positive results. However, this might be expected given that degranulation requires cross-linking on the surface of the mast cell and bromelain contains only 1 oligosaccharide chain per molecule. It has also been suggested that antibodies to relatively uncharged carbohydrate epitopes would have low affinity,[8] thus rendering skin testing less reliable. However, the evidence that IgE antibodies to CCDs are of low affinity is poor, and recent work has indicated that these antibodies have affinities comparable with IgG antibodies.[9] Our studies have shown that IgE antibodies specific for the carbohydrate galactose-α-1,3-galactose (α-gal) are capable of eliciting serious, even fatal reactions to the mAb cetuximab.[1] We subsequently extended that observation by demonstrating that IgE antibodies to α-gal are associated with an unusual form of delayed anaphylaxis, which follows 3 to 6 hours after eating meat that carries α-gal (eg, beef, pork, or lamb).[2] In contrast to previously described CCD motifs of xylose and core-3–linked fucose, which are common in plants and insects (see Fig 2), the α-gal epitope is abundantly expressed on cells and tissues of nonprimate mammals. This expression pattern makes α-gal potentially clinically relevant either as a food allergen (eg, beef, pork, or lamb) or as an inhaled allergen (eg, cat or dog).

Presence of CCDs can complicate immunoassays

In most cases CCDs are likely to have a marginal effect on *in vitro* test results. However, in certain subgroups of patients CCD reactivity might have clinical relevance, and an awareness of a possible CCD response might therefore be of great value when making diagnoses. For instance, if the patient was not originally sensitized to the allergen tested but the carbohydrate epitopes recognized by the patient's IgE are cross-reactive, the positive test result might not have the same clinical relevance. In particular, the glycan epitopes present in a latex extract can bind carbohydrate-directed IgE present in the serum of a patient with pollen allergy not originally sensitized to latex, resulting in a positive *in vitro* assay for IgE to latex. At times, if a patient is sensitized to the allergen tested by means of immunoassay, the presence of carbohydrate-directed IgE in addition to anti-peptide IgE can result in a higher quantitative result, suggesting a more severe sensitization than is actually the case. Moreover, a

recent study underscores the high occurrence of clinically irrelevant results for peanut sIgE in patients sensitized to grass pollen who have no symptoms related to peanuts.[10] Some have suggested that tests for CCD sIgE should be carried out systematically to improve the *in vitro* diagnosis of certain allergies; at least all *in vitro* tests should be evaluated together with the clinical history.

Carbohydrate epitope on cat IgA is α-gal

In 2006, Adedoyin et al[11] reported that IgE antibody specific for cat IgA, which is present in the serum of cat-sensitized patients, bound to a glycan moiety localized on the α-chain. In addition, they reported that these carbohydrates are also present on IgM from cat, as well as on IgM from many different mammalian species, but not human immunoglobulins. Unexpectedly, IgE antibodies to cat IgM and cat IgA showed complete cross-reactivity, whereas cat IgG did not, suggesting an identical oligosaccharide on the 2 former immunoglobulin classes.[12] As the first mammalian carbohydrate IgE epitope described, it was of major interest to identify the structure responsible for the broad cross-reactivity. Recent collaboration between our group and the Swedish group has established that the IgE-binding oligosaccharide on cat IgA is α-gal.[12]

Necessity for understanding glycosylation in the production of recombinant molecules

The cell type used for expression of a recombinant therapeutic glycoprotein has significant implications for the presence, number, and diversity of protein-linked oligosaccharides attached during the synthesis and secretion of the molecule. From a pharmacologic perspective, the potential for changes in glycosylation to influence the activity, serum half-life, or immunogenicity of the recombinant protein is an obvious cause for concern. Studies have shown, for example, that variations exist in the glycosylation pattern of tissue plasminogen activator isolated from different cell lines.[13] The most commonly used production cell lines for mAbs are CHO, NS0, and Sp2/0, and each of these can add sugar residues that are not present in normal serum-derived IgG (reviewed by Jefferis[14]). As recent studies have shown,[1] a particular concern is the addition of galactose in an α(1→3) linkage by NS0 and Sp2/0 cells, such that α-gal is formed. In human subjects and higher primates, the gene encoding alpha-1,3-galactosyltransferase is not functional, and therefore these species cannot produce α-gal; by contrast, the α-gal–negative animals make IgG antibodies specific for this oligosaccharide.[8] The implications of antibodies directed against the mAb are that the response to treatment might be influenced by accelerated clearance of the molecule or of sensitization potentially causing reactions on re-exposure. In the case of cetuximab, which carries α-gal, the patients who reacted had sIgE before the exposure, and anaphylaxis occurred during the first infusion.[1]

In addition to α-gal, the production cell lines can add an α(2→3)–linked *N*-glycol neuraminic acid (Neu5Gc; Fig 1, *C*) that is not present in human subjects and might have immunogenic properties. CHO cells in particular can add N-acetylneuraminic acid in α(2→3) linkage rather than the α(2→6) linkage found in human subjects.[14] Moreover, there is new evidence that fucose residues (or the absence of such sugars) on IgG Fc might influence activation of FcγRIIa and FcγRIIIa. The FcγRs might have differential glycosylation patterns themselves. Thus knowledge and awareness of the oligosaccharides present on all recombinant molecules (not only mAbs) is critical to understanding the cause of infusion reactions. Although glycosylation of the Fc portion of the molecule (Asn 297, see Fig 3) is known to play a significant role in Fc binding and the activation of antibody-dependent cellular cytotoxicity, it is not clear that the same is true for glycosylation on the Fab side. Although current knowledge limits the ability to change glycosylation patterns, it is possible to change the amino acid sequence by genetic engineering so that the glycosylation sites on the Fab are not present.

IgE TO A MAMMALIAN CCD

Prior evidence

As discussed, although IgE antibody to CCDs is known to be common, until recently, the clinical data were almost uniformly negative. Thus although patients are exposed to plant products (either inhaled or oral) that carry a CCD to which they have IgE antibodies, they do not report symptoms. In addition, investigation of sera from patients in Europe with chronic urticaria did not identify a significant number of cases with IgE antibodies to plant-derived CCDs (Rob Aalberse, personal communication). Other carbohydrate antigens that are recognized as immunogens in human subjects include A and B blood group substances. Until the past few years, however, no evidence existed for a significant mammalian CCD. Recent work by Wong et al[15] using deglycosylation techniques hinted at the existence of another CCD in mammalian tissue. Our description of IgE to α-gal in a cohort of patients who reported delayed symptoms after eating mammalian meat fits the results of Wong et al, and α-gal might well be the first clinically relevant CCD that is specifically found in mammalian tissue.

IgE antibody to α-gal

Over the last 3 years, it has become increasingly clear that there are many cases of "delayed anaphylaxis to red meat" occurring in an area of the United States that includes primarily Virginia, North Carolina, Tennessee, Arkansas and Missouri but also extends into the surrounding states.[2] The basis for these reactions appears to be an IgE antibody response to the oligosaccharide α-gal, awareness of which was triggered by severe hypersensitivity reactions to the mAb cetuximab.[1] These reactions to beef, pork, or lamb present several features that are strikingly different from established teaching on food allergy. First, this form of allergy to meat develops in adult life. Second, the reactions do not start until several hours after eating meat. Third, the patients generally have negative or very weak wheal responses to skin prick tests with meat extracts.[2,16] Because of these features, in many cases the patient's histories had previously been dismissed by other physicians, including allergists. Here we discuss the various syndromes and evidence in the literature that support the unique relevance of IgE to α-gal as a significant factor in this anaphylactic syndrome.

The mammalian pattern: IgE to beef, pork, cat, dog, and milk

In 2005, Mamikoglu[17] reported the existence of 18 patients in a southeastern state (Arkansas) whose sera were positive by means of ImmunoCAP testing for IgE to beef, pork, cat, dog, and milk. This pattern of positive titers to mammalian allergens could well

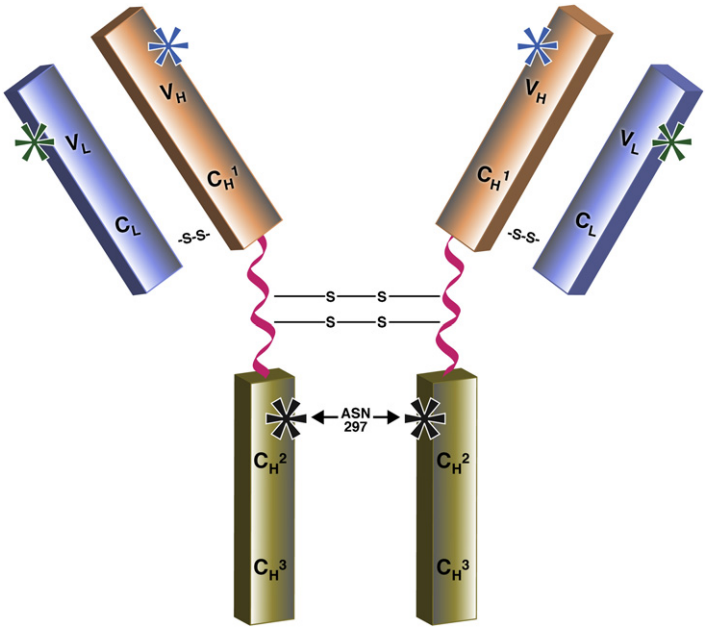

FIG 3. Structure of an IgG antibody molecule showing potential sites of glycosylation. The IgG-Fc region contains C_H2 domains that are glycosylated through covalent attachment of oligosaccharide at asparagine 297 (*Asn 297*, indicated by *black asterisks*). The oligosaccharide at Asn 297 is integral to the IgG-Fc structure, forms multiple noncovalent interactions with the protein surface of the C_H2 domain, and is important to the function of the molecule; therefore the site cannot be engineered out. In addition to the conserved glycosylation site at Asn 297, 15% to 20% of polyclonal human IgG molecules bear N-linked glycosylation within the IgG-Fab region. Although there is no consensus sequence for N-linked oligosaccharide within the constant domain of the light chain or the C_H1 domain of the heavy chain, glycosylation can be present in the variable regions of the κ and λ (*V_L, green asterisks*) or heavy chains (*V_H, blue asterisks*) and sometimes both. For example, the amino acid sequence of cetuximab has potential glycosylation sites at Asn 43 of the light chain and at Asn 88 and Asn 299 of the heavy chain, but the glycosylation site at Asn 43 is not glycosylated. The biopharmaceutical industry and others have shown that differences in glycosylation products and manipulation of oligosaccharide placement (or deletion) can have significant effects on therapeutic efficacy for recombinant mAbs. *S—S* denotes a disulfide bond.

be explained by IgE to α-gal and is mirrored in the 24 patients that we recently reported.[2] It seems unlikely that cross-reactivity to a protein epitope on serum albumin would explain the reported mammalian pattern because no current reports exist to link dog dander allergy to meat allergy. Alternatively, α-gal is present on proteins from the epithelia and milk of all lower mammals, which is consistent with the reported pattern.

Similar anaphylaxis syndromes not caused by a CCD

There are additional types of cross-reaction in patients with meat allergy:

1. Cross-reactivity between meats from different animal species and animal dander: The probability of cross-reaction is increased the more closely related the animals are evolutionarily, such that patients allergic to beef might react to mutton or pork but not poultry or fish. In some cases this cross-reactivity has been shown to be due to IgE specific for common determinants on serum albumin, and this has been referred to as the pork-cat syndrome.[18] A recent publication specifically addressed whether IgE to α-gal was present in patients with pork-cat syndrome and found no evidence for sIgE to α-gal, supporting the central role of serum albumin in the pork-cat syndrome.[16]
2. Cross-reactivity between beef and cow's milk: DBPCFC studies of children allergic to beef have shown immediate reactivity to cow's milk.[19] Again, sensitivity to BSA was the main predictive marker of this cross-reactivity and that of a subset of patients with cow's milk allergy also reacting to beef.

Desensitization possibilities

Modulation of the anaphylactic responses should be possible if IgE to α-gal has similar properties to more classical, peptide-directed IgE. Indeed, preliminary experiments in our laboratory have found that basophils and mast cells activate and release histamine, respectively, in response to the carbohydrate antigen in appropriately sensitized patients. Additionally, desensitization to cetuximab has been performed in a patient who had a grade 3 hypersensitivity reaction during the initial infusion. The patient's serum was tested and found to have IgE antibody to α-gal, but there was a clinical need for cetuximab therapy, and desensitization was successfully performed.[20]

EVIDENCE RELATED TO TICKS

A recent report on 25 patients from Sydney, New South Wales, documents an association between tick bite reactions and red meat allergy.[21] These 25 patients reported clinical reactions

ranging from urticaria to anaphylaxis, and 10 (40%) of the 25 patients reported a delayed onset of 4 hours or longer after ingestion of mammalian meat.[21] In this report from Australia by Van Nunen et al,[21] nearly all of the patients described large local reactions to tick bites. Likewise, greater than 90% of patients with IgE to α-gal in Virginia report tick or chigger bites. Interestingly, screening of sera from a separate cohort of Australian patients also with tick bites and reactions to red meat showed the presence of IgE antibody to α-gal in 9 of 13 patients with meat allergy (Mullins, Commins, James, and Platts-Mills, unpublished data).

The normal pattern of allergic disease is a primary exposure that gives rise to an IgE antibody response, and subsequent exposure to the same antigen then gives rise to symptoms. However, there are some well-documented situations in which exposure to one foreign antigen gives rise to an IgE response that cross-reacts with an apparently different antigen that might be encountered through a different route. The obvious example is birch pollen exposure giving rise to IgE antibodies to Bet v 1, which cross-reacts with the closely related proteins of apple, hazelnut, or the cherry-derived allergen Pru a 1. These reactions are due to close structural similarity between the proteins. There are also examples in which insect stings can sensitize to a CCD present on pollens. Thus honey bee venom induces IgE responses to the oligosaccharide MUXF[3], which is present on the pineapple protein bromelain and also on proteins derived from grass pollen.[4] Therefore there is an existing model of an arthropod bite or sting in the skin inducing IgE antibodies to a carbohydrate antigen.

Interestingly, immediate anaphylactic and large hypersensitivity reactions to tick bites have been reported with several different species of ticks.[22] These IgE-mediated responses are due to tick salivary proteins[22] and are distinct reactions from the delayed, food-induced anaphylactic and urticarial reactions that we have reported. Clinically, these hypersensitivity reactions are reported to occur within minutes of a tick bite and have been reported both with *Ixodes holocyclus* and with the pigeon tick *(Argas reflexus)*.[22,23] Thus, based on current evidence, it appears that tick bites can give rise to IgE responses to both carbohydrates and to tick-derived proteins. This should not be a surprise because ticks are well recognized for their potent adjuvant and immunogenic effects.[24]

COMMENTARY AND FUTURE WORK

Although the oligosaccharide α-gal has only recently been recognized as a target for IgE antibodies, it has long been recognized as a significant factor in transplant immunology. In the 1930s, Karl Landsteiner recognized the existence of a blood group B–like substance on mammalian cells, which was the target of agglutinating antibodies in human sera. This substance was probably α-gal because blood group B antigen and the α-gal epitope differ only in that blood group B antigen has a fucose-linked α(1→2) to the penultimate galactose (Fig 2). Other distinct but equally interesting work is unfolding from Varki and his colleagues, showing that the carbohydrate Neu5Gc is found in high amounts in lamb, pork, and beef; intermediate amounts in cow's milk; and low/undetectable levels in poultry and fish. That group found anti-Neu5Gc antibodies of IgA, IgM, and IgG isotypes in patient sera and that these antibodies represented up to 0.25% of total circulating IgG levels in some subjects.[25] This falls into the ranges of IgG antibodies to α-gal, but there was no direct correlation between the 2 antibody levels in a given individual.[25] We have screened a large number of sera for IgE antibody to Neu5Gc, but the results were negative (Platts-Mills, James, and Commins, unpublished data). Certainly, the finding of IgE to α-gal raises the issue that there might be other naturally occurring nonprotein epitopes, such as other sialic acid–based sugars (eg, Lewis X), carbohydrates on horseradish peroxidase, and hydroxyl proline–linked arabinogalactan that could be the target of IgE responses.

The discovery of IgE antibodies to the oligosaccharide α-gal has made it possible to investigate several novel aspects of allergic disease. One obvious issue is that the glycosylation of therapeutic recombinant molecules, particularly mAbs, can create a risk for severe hypersensitivity reactions. The syndrome of "delayed anaphylaxis to beef" has changed our approach to what would normally be regarded as "spontaneous" or "idiopathic" anaphylaxis in a large area of the southeastern United States. In the future, an understanding of the factors that control the delay might provide real insight into the factors that control anaphylaxis. At this time, there remain several major unexplained issues:

1. Is IgE to α-gal induced by ticks, and if so, is it related to a certain species?
2. Why are the reactions to red meat delayed?
3. Why is this IgE antibody that binds α-gal on dog and cat proteins and is present in high titers in the serum not related to asthma or immediate nasal symptoms?

REFERENCES

1. Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. N Engl J Med 2008;358:1109-17.
2. Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD, et al. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose-alpha-1,3-galactose. J Allergy Clin Immunol 2009;123:426-33.
3. Ishihara H, Takahashi N, Oguri S, Tejima S. Complete structure of the carbohydrate moiety of stem bromelain. An application of the almond glycopeptidase for structural studies of glycopeptides. J Biol Chem 1979;254:10715-9.
4. Aalberse RC, Koshte V, Clemens JG. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. J Allergy Clin Immunol 1981;68:356-64.
5. Mari A. IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. Int Arch Allergy Immunol 2002;129:286-95.
6. van der Veen MJ, van Ree R, Aalberse RC, Akkerdaas J, Koppelman SJ, Jansen HM, et al. Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. J Allergy Clin Immunol 1997;100:327-34.
7. Foetisch K, Westphal S, Lauer I, Retzek M, Altmann F, Kolarich D, et al. Biological activity of IgE specific for cross-reactive carbohydrate determinants. J Allergy Clin Immunol 2003;111:889-96.
8. Galili U. The alpha-gal epitope and the anti-Gal antibody in xenotransplantation and in cancer immunotherapy. Immunol Cell Biol 2005;83:674-86.
9. Jin C, Hantusch B, Hemmer W, Stadlmann J, Altmann F. Affinity of IgE and IgG against cross-reactive carbohydrate determinants on plant and insect glycoproteins. J Allergy Clin Immunol 2008;121:185-90.e2.
10. Guilloux L, Morisset M, Codreanu F, Parisot L, Moneret-Vautrin DA. Peanut allergy diagnosis in the context of grass pollen sensitization for 125 patients: roles of peanut and cross-reactive carbohydrate determinants specific IgE. Int Arch Allergy Immunol 2009;149:91-7.
11. Adedoyin J, Gronlund H, Oman H, Johansson SG, van Hage M. Cat IgA, representative of new carbohydrate cross-reactive allergens. J Allergy Clin Immunol 2007;119:640-5.
12. Gronlund H, Adedoyin J, Commins SP, Platts-Mills TA, van Hage M. The carbohydrate galactose-alpha-1,3-galactose is a major IgE-binding epitope on cat IgA. J Allergy Clin Immunol 2009;123:1189-91.

13. Buelens K, Hillmayer K, Compernolle G, Declerck PJ, Gils A. Biochemical importance of glycosylation in thrombin activatable fibrinolysis inhibitor. Circ Res 2008; 102:295-301.
14. Jefferis R. Glycosylation as a strategy to improve antibody-based therapeutics. Nat Rev Drug Discov 2009;8:226-34.
15. Wong KN, Ong TC, Wang X, Lee WSe, Lim PA, Wang DeY, Chew FT. Deglycosylation of meat extracts reduced the binding of cross-reactive antibodies to meat. XVth Annual Congress of the European Academy of Allergology and Clinical Immunology, Vienna, Austria, June 10-14, 2006.
16. Jacquenet S, Moneret-Vautrin DA, Bihain BE. Mammal meat anaphylaxis: clinical relevance of anti-galactose-alpha-1,3-galactose IgE confirmed by skin tests to cetuximab. J Allergy Clin Immunol In press. 2009;124:603-5.
17. Mamikoglu B. Beef, pork, and milk allergy (cross reactivity with each other and pet allergies). Otolaryngol Head Neck Surg 2005;133:534-7.
18. Fuentes Aparicio V, Sanchez Marcen I, Perez Montero A, Baeza ML, de Barrio Fernandez M. Allergy to mammal's meat in adult life: immunologic and follow-up study. J Investig Allergol Clin Immunol 2005;15:228-31.
19. Werfel SJ, Cooke SK, Sampson HA. Clinical reactivity to beef in children allergic to cow's milk. J Allergy Clin Immunol 1997;99:293-300.
20. Jerath MR, Kwan M, Kannarkat M, Mirakhur B, Carey L, Valgus J, et al. A desensitization protocol for the mAb cetuximab. J Allergy Clin Immunol 2009;123:260-2.
21. Van Nunen SA, O'Connor KS, Clarke LR, Boyle RX, Fernando SL. An association between tick bite reactions and red meat allergy in humans. Med J Aust 2009;190:510-1.
22. Gauci M, Loh RK, Stone BF, Thong YH. Allergic reactions to the Australian paralysis tick, *Ixodes holocyclus*: diagnostic evaluation by skin test and radioimmunoassay. Clin Exp Allergy 1989;19:279-83.
23. Hilger C, Bessot JC, Hutt N, Grigioni F, De Blay F, Pauli G, et al. IgE-mediated anaphylaxis caused by bites of the pigeon tick *Argas reflexus*: cloning and expression of the major allergen Arg r 1. J Allergy Clin Immunol 2005;115:617-22.
24. Francischetti IM, Sa-Nunes A, Mans BJ, Santos IM, Ribeiro JM. The role of saliva in tick feeding. Front Biosci 2009;14:2051-88.
25. Tangvoranuntakul P, Gagneux P, Diaz S, Bardor M, Varki N, Varki A, et al. Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. Proc Natl Acad Sci U S A 2003;100:12045-50.