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The alpha-gal story: Lessons learned from connecting the dots

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Activity Objectives

1. To describe the history and presenting symptoms of galactose-alpha-1,3-galactose (alpha-gal).
2. To describe the pathophysiology of the IgE-mediated response to alpha-gal in human subjects.
3. To list the diagnostic approach to alpha-gal.

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Anaphylaxis is a severe allergic reaction that can be rapidly progressing and fatal, and therefore establishing its cause is pivotal to long-term risk management. Our recent work 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 2 distinct forms of anaphylaxis: (1) immediate-onset anaphylaxis during first exposure to intravenous cetuximab and (2) delayed-onset anaphylaxis 3 to 6 hours after ingestion of mammalian food products (eg, beef and pork). Results of our studies and those of others strongly suggest that tick bites are a cause, if not the only significant cause, of IgE antibody responses to alpha-gal in the southern, eastern, and central United States; Europe; Australia; and parts of Asia. Typical immune responses to carbohydrates are considered to be T-cell independent, whereas IgE antibody production is thought to involve sequential class-switching that requires input from T cells. 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. (J Allergy Clin Immunol 2015;135:589-96.)

Key words: *Anaphylaxis, delayed reaction to red meat, galactose-alpha-1,3-galactose*

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Hypersensitivity in the allergic setting refers to immune reactions stimulated by soluble antigens that can be rapidly progressing and, in the case of anaphylaxis, are occasionally fatal. Because the number of known exposures associated with anaphylaxis is limited, identification of novel causative agents is important in facilitating both education and other allergen-specific approaches that are crucial to long-term risk management. Within the last 10 years, several seemingly separate observations were recognized as related, all of which resulted from the development of antibodies to a carbohydrate moiety on proteins in which exposure differed from airborne allergens but

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Abbreviations used
Alpha-gal: Galactose-alpha-1,3-galactose
LDL: Low-density lipoprotein
RMSF: Rocky Mountain spotted fever
VLDL: Very low-density lipoprotein

that were nevertheless capable of producing anaphylactic and hypersensitivity reactions. Our recent work has identified these responses as being due to a novel IgE antibody directed against a mammalian oligosaccharide epitope, galactose-alpha-1, 3-galactose (alpha-gal).[1] This review will present the history and biology of alpha-gal and discuss the evidence that the IgE response to alpha-gal is different from typical IgE responses directed toward protein allergens.

CETUXIMAB-INDUCED HYPERSENSITIVITY REACTIONS

In 2004, ImClone and Bristol-Myers Squibb were investigating an mAb (cetuximab) specific for the epidermal growth factor receptor in clinical trials for the treatment of metastatic colorectal cancer. Early in those studies, it became clear that the antibody was causing hypersensitivity reactions; however, these reactions were occurring primarily in a group of southern US states (Table I). These reactions to cetuximab developed rapidly, and symptoms often peaked within 20 minutes after or during the first infusion of the antibody and occasionally proved fatal.[1-3] Because of delays in marketing, it was not until 2006 that the true severity of the reactions became obvious.[2]

At this time, our group began preliminary experiments examining the IgE response to this molecule. Dr Hatley, who was working in Bentonville, Arkansas, convinced our group to develop a new version of the IgE fluorometric enzyme immunoassay or CAP assay to cetuximab using the streptavidin technique. In this assay streptavidin is coupled to the solid phase of the CAP to provide a matrix for the binding of biotinylated novel or purified allergens.[4]

We were subsequently asked to investigate the reactions to cetuximab, in part because we had already developed the IgE assay to cetuximab. In collaboration with Dr Chung from Nashville, Dr Mirakhur from Bristol-Myers Squibb, and Dr Hicklin from ImClone, we demonstrated that the patients who had reactions to cetuximab also had IgE antibodies specific for this molecule before they started treatment.[1] The question remained as to what epitope the IgE antibody was recognizing on the cetuximab molecule.

Early work by Karl Landsteiner discovered that all human subjects had antibodies to a blood group "B-like" oligosaccharide found on nonprimate red blood cells.[5] That antigen was subsequently identified as alpha-gal, which represents a major transplantation barrier between primates and other mammals.[6-8] Antibodies against alpha-gal are present in all nonimmunocompromised human subjects, and some early studies suggested that the IgG antibodies against alpha-gal constituted about 1% of circulating immunoglobulins in human subjects, apes, and Old World monkeys.[9] Recent work in our laboratory with specific assays for IgG antibodies suggests that the percentages are not this high. As discussed below, the fact that all nonprimate mammals, including mice, can make oligosaccharides that are foreign to human subjects is an important component of our story.

CARBOHYDRATE ANALYSIS OF CETUXIMAB

Glycosylation of proteins is a posttranscriptional modification that can play key roles in many processes, including protein folding, protein stability, intracellular trafficking, and cellular adhesion, as reviewed by Hurtado-Guerrero and Davies.[10] Characterization of cetuximab glycosylation, as measured based on peak area on time-of-flight mass spectrometric spectra, revealed 21 distinct oligosaccharide structures, of which approximately 30% have 1 or more alpha-1,3–linked galactosyl residues.[11] Analysis of the IgE antibodies to cetuximab demonstrated that these antibodies were specific for the oligosaccharide residues on the heavy chain of the Fab portion of the mAb. From the known glycosylation of the molecule at amino acids 88 and 299 (Fig 1),[11] alpha-gal was identified as the relevant epitope. Of the total alpha-gal in cetuximab, most of it is located in the Fab domain (Fab domain: 990 nmol alpha-gal/μmol IgG vs Fc domain: 140 nmol alpha-gal/μmol IgG).[11] Recent mass spectrometric analysis indicates that glycosylation of cetuximab might be more complex than previously thought, containing both dianternary and trianternary structures.[12] Synthesis of alpha-gal requires the gene encoding alpha-1, 3-galactosyltransferase. In human subjects and higher primates this gene is not functional, and therefore these species cannot produce alpha-gal, which in turn makes it possible for these animals to initially make IgG and IgM antibodies directed toward this oligosaccharide.[7,13] How IgE to alpha-gal gets made and the nature of the IgE response will be considered later.

Of considerable importance to the development of biologics, in particular mAbs, is the observation that murine cell lines, such as NS0 and Sp2/0, can synthesize galactose in an alpha-1,3 linkage such that alpha-gal is present on the molecules. Sp2/0 was the cell line used to produce cetuximab. In those subjects with IgE to alpha-gal (≥0.35 IU/mL), reactions are likely to occur directed against this mAb.[3]

THE RED MEAT CONNECTION

During this same time period (2006-2008), we evaluated a number of patients, most of whom spent a significant amount of time outdoors, who had presented with episodes of generalized urticaria, angioedema, or recurrent anaphylaxis. The importance of the time spent outdoors was not clear at that time. There was no obvious immediate cause for the symptoms, but in several cases the patients reported that they believed the reactions might be due to consumption of meat 3 to 5 hours earlier. Skin prick tests were performed with commercial extracts of beef, pork, or lamb and produced small wheals only 2 to 4 mm in diameter that often would be interpreted as negative results. However, given the compelling history described by the patients, we extended our analysis to intradermal skin testing with commercial meat extracts or skin prick tests with fresh meat extracts, both of which demonstrated strong positive results.[14] These results were confirmed with blood tests for specific IgE antibody to red meats.[14]

Although not published, similar sensitivity to red meat had been previously noted in Georgia. Starting in 1989, Mrs Sandra Latimer, together with Dr Antony Deutsch from Athens, Georgia,