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Invited Review Article

Alpha-Gal-containing biologics and anaphylaxis

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ABSTRACT

Cetuximab, the IgG1 subclass chimeric mouse-human monoclonal antibody biologic that targets the epidermal growth factor receptor (EGFR), is used worldwide for the treatment of EGFR-positive unresectable progressive/recurrent colorectal cancer and head and neck cancer. Research has shown that the principal cause of cetuximab-induced anaphylaxis is anti-oligosaccharide IgE antibodies specific for galactose-α-1,3-galactose (α-Gal) oligosaccharide present on the mouse-derived Fab portion of the cetuximab heavy chain. Furthermore, it has been revealed that patients who are allergic to cetuximab also develop an allergic reaction to mammalian meat containing the same α-Gal oligosaccharide owing to cross-reactivity, and the presumed cause of sensitization is tick bites: *Amblyomma* in the United States, *Ixodes* in Australia and Europe, and *Haemaphysalis* in Japan. The α-Gal-specific IgE test (bovine thyroglobulin-conjugated ImmunoCAP) or CD63-expression-based basophil activation test may be useful to identify patients with IgE to α-Gal in order to predict risk for cetuximab-induced anaphylactic shock. Investigations of cetuximab-related anaphylaxis have revealed three novel findings that improve our understanding of immediate-type allergy: 1) oligosaccharide can serve as the main IgE epitope of anaphylaxis; 2) because of the oligosaccharide epitope, a wide range of cross-reactivity with mammalian meats containing α-Gal similar to cetuximab occurs; and 3) tick bites are a crucial factor of sensitization to the oligosaccharide. Nonetheless, taking a medical history of tick bites and beef allergy may be insufficient to prevent cetuximab-induced anaphylaxis, and therefore blood testing with an α-Gal-specific IgE test, with high sensitivity and specificity, is necessary to detect sensitization to α-Gal.

Introduction

Recombinant monoclonal antibody biologics are categorized into chimeric mouse-human antibodies, humanized antibodies, and human antibodies depending on the molecular production process. Cetuximab is a chimeric mouse-human mAb and IgG_1 subclass biologic that is composed of mouse-derived variable regions and human-derived constant regions to target the epidermal growth factor receptor (EGFR).[1] Cetuximab is used globally for the treatment of EGFR-positive unresectable progressive/recurrent colorectal cancer and head and neck squamous cell carcinoma and has been demonstrated to prolong progression-free survival in patients with these cancers.[2–5] More than 80% of patients receiving cetuximab develop dermatological symptoms such as acneiform eruption, xeroderma, and perionychia owing to its inhibitory effect on EGFR functions.[1] In addition, severe hypersensitivity reactions are observed in 3% of patients.[1] Some lethal cases due to severe infusion reaction after cetuximab administrating have also been reported.[6,7]

Cetuximab-induced anaphylaxis

After the approval of cetuximab for use in metastatic colorectal cancer and squamous-cell carcinoma of the head and neck, higher rates of hypersensitivity reactions were reported in the Southeast of the U.S., especially Tennessee, Arkansas, and North Carolina.[2,4,8,9] In contrast, the rates of hypersensitivity reactions were much lower in Massachusetts or northern California in the U.S.[9] Chung *et al.* studied IgE antibodies against cetuximab in 76 patients who received cetuximab therapy in Tennessee, Arkansas, and North Carolina and found the 25 patients developed hypersensitivity reactions to cetuximab; 17 of the 25 patients with hypersensitivity had cetuximab-specific IgE before treatment, whereas only one of the remaining 51 patients who did not develop hypersensitivity had cetuximab-specific IgE.[9] Cetuximab-specific IgE was also found in the sera of 15 of 72 control subjects (20.8%) in Tennessee, despite the fact that none of these subjects had ever received cetuximab.

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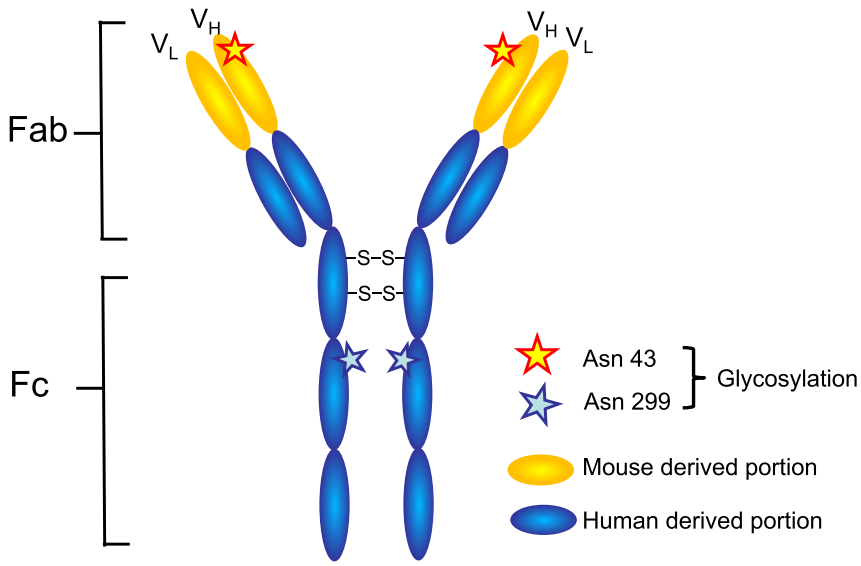

Fig. 1. Structure of cetuximab showing glycosylation sites. Cetuximab is a chimeric mouse-human monoclonal antibody biologic with galactose-α-1,3-galactose (α-Gal) oligosaccharide bound possibly to the Asn 43 glycosylation site of V_H of the mouse-derived Fab portion.

However, these antibodies were found in only three of 49 (6.1%) control subjects with cancer of the head and neck in California and in two of 341 (0.6%) control subjects in Boston, Massachusetts. These investigations demonstrated that even control subjects with no history of cetuximab therapy may harbor cetuximab-specific IgE, and the proportion of people with cetuximab-specific IgE highly differs among regions.

Chung and colleagues also identified that cetuximab-specific IgE reacts with the oligosaccharide, galactose-α-1,3-galactose (α-Gal), which is present on the Fab portion of the cetuximab heavy chain (Fig. 1). Intriguingly, cetuximab is produced by the mouse cell line SP2/0, which expresses the gene encoding α-1,3-galactosyltransferase.[9] Another type of cetuximab, which is produced by the Chinese hamster ovary (CHO) cell line, has no reactivity with sera of patients exhibiting anaphylaxis related to cetuximab[9] owing to its lack of α-1,3-galactosyltransferase. These findings highlight carbohydrates as an important epitope playing a role in immediate-type hypersensitivity, which is groundbreaking as carbohydrates have been considered to be minimally involved in allergic symptoms.[10–12] Of note, such frequent adverse events have not been observed for infliximab, another chimeric mouse-human monoclonal antibody biologic targeting TNFα that is produced using the CHO cell line.

α-Gal allergy and cross-reactivity

In 2009, Commins *et al.* reported that the cause of delayed-onset red meat allergy is IgE specifically reacting to α-Gal by investigating 24 patients who reported anaphylaxis or urticaria after eating beef, pork, or lamb.[13] The IgE in these patients' sera reacted with beef, pork, lamb, cow's milk, cat, and dog but did not react with chicken, turkey, and fish. Because the α-Gal epitope is abundantly expressed on the cells and in tissues of non-primate mammals but not in tissues of humans and monkeys, the α-Gal-specific IgE explains the allergic reaction to a wide range of mammals.[14]

Subsequently, Commins *et al.* suggested a relationship between tick bites and production of IgE antibodies against α-Gal based on the epidemiologic finding that the area in which cetuximab-induced anaphylaxis is more common overlaps with the endemic area of Rocky Mountain spotted fever, which is mediated by *Amblyomma americanum* (*A. americanum*).[15] In addition, they monitored serum α-Gal-specific IgE levels in three subjects with tick bites and confirmed the relationship. Furthermore, they reported a positive correlation between history of tick bites and titer of α-Gal-specific IgE, and a significant correlation between anti-α-Gal IgE titer and IgE against proteins extracted from *A. americanum*, indicating that the production of IgE antibodies against α-Gal is caused by *A. americanum* bites.

Following this report, studies from Australia, France, and Spain suggested that tick bites, likely by *Ixodes holocyclus* (*I. holocyclus*) or *Ixodes ricinus* (*I. ricinus*), are involved in the development of red meat allergy through sensitization to α-Gal.[16–18] Hamsten *et al.* directly demonstrated the presence of α-Gal in the gastrointestinal tract of tick *I. ricinus*, by immunostaining with a polyclonal mouse anti-α-Gal antibody and patient serum IgE positive for α-Gal.[19] Further, they analyzed 39 patients in Sweden with a history of allergic reactions after consumption of mammalian meat and IgE against α-Gal and found that most of the patients had a history of repeated tick bites and IgE antibodies against *I. ricinus*.[20] In addition, they screened 143 healthy donors and found that 10% harbored IgE antibodies against α-Gal, indicating a high rate of sensitivity to α-Gal not only in the Southeast of the U.S. but also in the Stockholm area of Sweden. Notably, 37 of the 39 patients with red meat allergy had B-negative blood type.[20] ABO blood antigens are determined by oligosaccharides, and B antigen is composed of an α-Gal structure combined with a fucose residue.[11] Individuals with B antigen already harbor an oligosaccharide structure that resembles α-Gal; therefore, B blood type individuals are considered to be more tolerant of sensitization against α-Gal.

α-Gal allergy in Japan

From 2005 to 2013, we examined 30 patients with red meat allergy at Shimane University Hospital in Japan.[21,22] Most of the patients with red meat allergy developed allergic reactions at least 3 h after red meat ingestion, similar to the reports from the U.S., Australia, or Europe.[13–18] Sekiya *et al.* also reported a case of delayed anaphylactic reaction to mammalian meat in Kanagawa.[23] Although the specific mechanism for this delayed reaction remains to be determined, it is considered to be attributed to the principal antigen epitope in red meat allergy is an oligosaccharide. We previously analyzed extracts from beef and identified laminin γ-1 and the collagen α-1 (VI) chain as α-Gal-bound causative beef

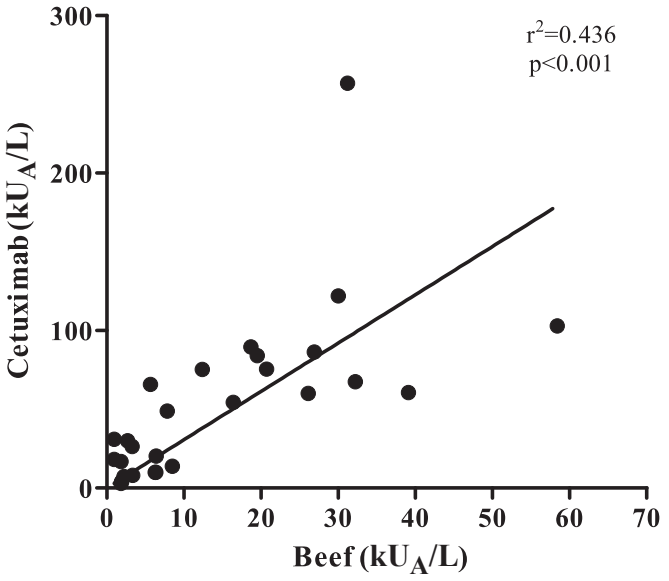

Fig. 2. Correlation of specific IgE value to beef and cetuximab in patients with red meat allergy. Value of specific IgE to beef and cetuximab measured in sera of patients (n = 19) with the CAP-fluorescent enzyme immunoassay (CAP-FEIA) system was plotted. A significant correlation was observed between these two parameters (p<0.001, Spearman's rank correlation coefficient). Modified from the study by Chinuki *et al.*[21]

allergens.[22] Most of the patients were shown to harbor IgE against cetuximab when biotinylated cetuximab was immobilized onto streptavidin ImmunoCAP and used to detect cetuximab-specific IgE, although none of them had ever received cetuximab treatment. The binding of patient serum IgE to soluble beef protein was decreased in inhibition tests with cetuximab. The amount of IgE bound to cetuximab was significantly correlated with that to beef in the patients (Fig. 2), suggesting that the major IgE-binding epitope of beef allergen in these patients is α-Gal. In addition, almost all patients with red meat allergy were B-negative blood type. Furthermore, IgE specific for beef and pork was detected, but IgE specific for chicken was not detected in almost all our subjects.

Japanese spotted fever is a rickettsial infection mediated by ticks and prevalent in Shimane Prefecture located in the western part of Japan, and its dominant vector is *Haemaphysalis longicornis* (*H. longicornis*).[24] We demonstrated that the salivary glands of *H. longicornis* contain α-Gal-bearing proteins, and the serum IgE of patients with red meat allergy binds to several soluble proteins extracted from *H. longicornis* salivary glands.[25] These findings suggest that sensitization to α-Gal in this area is caused by tick *H. longicornis*. Importantly, Hashizume *et al.* demonstrated that 50% of patients who had more than two tick bites were sensitized to α-Gal.[26] These cases were associated with *Amblyomma testudinarium* tick bites. As *I. holocyclus* is known to be responsible for the majority of tick bites in humans worldwide including Japan,[23,27] it is conceivable that sensitization to α-Gal could occur by tick bites anywhere in Japan (Fig. 3).

In 2013, we experienced a high incidence of anaphylaxis in 13 patients with head and neck cancer who had been administered cetuximab for the first time in the Department of Otolaryngology-Head and Neck Surgery of Matsue Red Cross Hospital located in the eastern part of Shimane Prefecture. Four of the 13 patients developed anaphylactic shock and were found to have IgE against α-Gal (determined by bovine thyroglobulin-conjugated ImmunoCAP, Thermo Fisher Scientific) and cetuximab. The other nine patients who did not develop anaphylactic shock showed negative IgE against these allergens. Ten Japanese cases of cetuximab-induced anaphylactic shock, including the four cases at Matsue Red Cross Hospital, are detailed in Table 1. Almost all patients resided in the western part of Japan. Of the 10 cases, only two had tick bites, and one had a beef allergy according to their past histories. The reason why many patients did not have a history of tick bite is that the ticks inject various substances into the host to facilitate feeding, including proteins that anchor the mouth to the skin of the host as well as enzymes, vasodilators, and anti-inflammatory, anti-hemostatic, and immunosuppressive substances, so it is likely that the patients have not noticed the tick bites.[25] Beef-specific IgE was detected in eight cases, and α-Gal-specific IgE was detected in all 10 cases. The results of α-Gal-specific IgE corresponded to the IgE Western blot results in these 10 cases (data not shown). These findings indicate that the α-Gal-specific IgE test is useful to identify

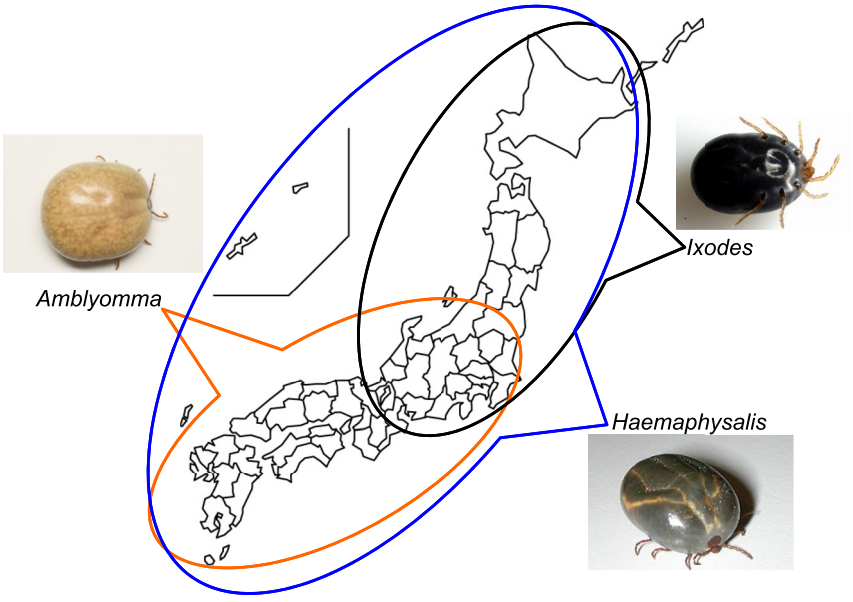

Fig. 3. Distribution of three ticks, *Amblyomma*, *Ixodes*, and *Haemaphysalis* in Japan. *Amblyomma* is found mainly in the western part, and *Ixodes* is found in the northern part; *Haemaphysalis* is present across all of Japan.

Table 1
Japanese cases of cetuximab-induced anaphylactic shock.

Case	Age (years) Sex	Primary disease	Food allergy history	History of tick bites	Beef-specific IgE (kU_A/L)	α-Gal-specific IgE (kU_A/L)
1	67 Female	Laryngeal cancer	None	None	<0.34	1.33
2	81 Male	Oropharyngeal cancer	Flatfish eggs	None	2.14	6.19
3	60 Male	Laryngeal cancer	None	None	0.48	6.62
4	67 Male	Epipharyngeal cancer	None	None	1.34	3.30
5	62 Male	Rectal cancer	Beef, pork, flatfish eggs	None	8.11	16.4
6	74 Male	Cancer of the floor of the mouth	None	None	1.04	4.64
7	81 Male	Laryngeal cancer	None	Yes	3.74	6.50
8	74 Male	Hypopharyngeal cancer	None	Yes	2.99	11.5
9	50 Male	Rectal cancer	Kiwi fruit, pineapple, oyster	Unknown	1.28	24.9
10	66 Male	Buccal mucosal cancer	None	None	<0.34	0.493

the patients with IgE to α-Gal in order to predict risk for cetuximab-induced anaphylactic shock.

Basophil activation test for α-Gal allergy

The diagnosis of red meat allergy by clinical history is challenging because hypersensitivity reactions to red meat are delayed by several hours after ingestion of red meat. In addition, the sensitivity of the skin prick test and serum-specific IgE test to meat extracts is limited.[27] Thus, skin prick tests and the CD63-expression-based basophil activation test with cetuximab were recommended for the patients with an assumed red meat allergy.[28] In contrast, another study revealed that the CD203c-expression-based basophil activation test is not always suitable for diagnosing hypersensitivity to cetuximab, particularly with lower-grade symptoms.[29] Commins *et al.* monitored CD63-expression-based basophil activation during the open red meat challenge tests for the patients with IgE to α-Gal and found that *in vivo* activation of basophils was strongly correlated with the appearance of clinical symptoms.[30] Recently, Mehlich *et al.* reported that the %ratio of the CD63-expression/anti-FcεRI-based basophil activation test can be used to differentiate patients with α-Gal syndrome and asymptomatic α-Gal sensitization.[31] In addition, a passive sensitization-basophil activation test was introduced to possibly identify patients with IgE to α-Gal.[32] In this examination, basophils from an unrelated donor are isolated and stripped of surface-bound IgE by using an acid treatment, and serum from patients is incubated and allowed to bind to the basophils. Although the basophil activation test is not readily available to most practicing allergists, the CD63-expression-based basophil activation test may be useful to identify patients with IgE to α-Gal in order to predict risk for cetuximab-induced anaphylactic shock.

Conclusion

According to the reports revealing a high incidence of cetuximab-related anaphylaxis in the Southeast of the U. S., three novel findings have improved our understanding of immediate-type allergy: 1) oligosaccharide can serve as the main IgE epitope of anaphylaxis; 2) because of the oligosaccharide epitope, a wide range of cross-reactivity with mammalian meats containing α-Gal similar to cetuximab occurs; and 3) tick bites are a crucial factor of sensitization to the oligosaccharide.

Based on these reports, the following statements were added to the Japanese drug product label for cetuximab in July 2015: “One of the reported mechanisms of anaphylaxis induced by this drug is due to IgE antibodies to α-Gal. IgE antibodies to α-Gal were reportedly detected in patients with red meat allergy (beef, etc.) and in patients who have received tick bites. Among these groups, anaphylaxis has reportedly been induced by cetuximab in patients with beef allergy.” This suggests that a history of beef allergy is a predictive factor for cetuximab-related severe infusion reaction; however, based on our analysis, cetuximab-induced anaphylaxis cannot be avoided in most cases by only taking a medical history of tick bites and beef allergy (Table 1). IgE antibodies to α-Gal were detected in most cases before cetuximab treatment as described above, and therefore blood testing before treatment, such as α-Gal-specific IgE assay (ImmunoCAP), IgE western blotting with cetuximab, or possibly the CD63-expression-based basophil activation test, is necessary to detect sensitization to α-Gal. The α-Gal-specific IgE test, established by using bovine thyroglobulin (ImmunoCAP), is highly sensitive and specific for identifying subjects with IgE to α-Gal,[33,34] and thus we hope that this test will be adopted for clinical use early in the near future.

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Conflict of interest
The authors have no conflict of interest to declare.

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