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Cetuximab-Induced Anaphylaxis and IgE Specific for Galactose-α-1,3-Galactose

Christine H. Chung, M.D., **Beloo Mirakhur, M.D., Ph.D.**, **Emily Chan, M.D., Ph.D.**, **Quynh-Thu Le, M.D.**, **Jordan Berlin, M.D.**, **Michael Morse, M.D.**, **Barbara A. Murphy, M.D.**, **Shama M. Satinover, M.S.**, **Jacob Hosen, B.S.**, **David Mauro, M.D., Ph.D.**, **Robbert J. Slebos, Ph.D.**, **Qinwei Zhou, Ph.D.**, **Diane Gold, M.D.**, **Tina Hatley, M.D.**, **Daniel J. Hicklin, Ph.D.**, and **Thomas A.E. Platts-Mills, M.D., Ph.D.**

From the Division of Hematology/Oncology, Department of Medicine (C.H.C., E.C., J.B., B.A.M.), the Department of Cancer Biology (C.H.C., R.J.S.), and the Department of Otolaryngology (R.J.S.), Vanderbilt University School of Medicine, Nashville; Bristol-Myers Squibb, Plains-boro, NJ (B.M., D.M.); Stanford University School of Medicine, Menlo Park, CA (Q.-T.L.); the Department of Medicine, Duke University Medical Center, Durham, NC (M.M.); Asthma and Allergic Diseases Center, University of Virginia, Charlottesville (S.M.S., J.H., T.A.E.P.-M.); Im-Clone Systems, Branchburg, NJ (Q.Z., D.J.H.); Channing Institute, Harvard University, Boston (D.G.); and Allergy and Asthma Clinic of Northwest Arkansas, Bentonville (T.H.).

Abstract

BACKGROUND—Cetuximab, a chimeric mouse–human IgG1 monoclonal antibody against the epidermal growth factor receptor, is approved for use in colorectal cancer and squamous-cell carcinoma of the head and neck. A high prevalence of hypersensitivity reactions to cetuximab has been reported in some areas of the United States.

METHODS—We analyzed serum samples from four groups of subjects for IgE antibodies against cetuximab: pretreatment samples from 76 case subjects who had been treated with cetuximab at multiple centers, predominantly in Tennessee, Arkansas, and North Carolina; samples from 72 control subjects in Tennessee; samples from 49 control subjects with cancer in northern California; and samples from 341 female control subjects in Boston.

RESULTS—Among 76 cetuximab-treated subjects, 25 had a hypersensitivity reaction to the drug. IgE antibodies against cetuximab were found in pretreatment samples from 17 of these subjects; only 1 of 51 subjects who did not have a hypersensitivity reaction had such antibodies ($P<0.001$). IgE antibodies against cetuximab were found in 15 of 72 samples (20.8%) from control subjects in Tennessee, in 3 of 49 samples (6.1%) from northern California, and in 2 of 341 samples (0.6%) from Boston. The IgE antibodies were shown to be specific for an oligosaccharide, galactose-α-1,3-galactose, which is present on the Fab portion of the cetuximab heavy chain.

CONCLUSIONS—In most subjects who had a hypersensitivity reaction to cetuximab, IgE antibodies against cetuximab were present in serum before therapy. The antibodies were specific for galactose-α-1,3-galactose.

RECOMBINANT MONOCLONAL ANTIBODies have an increasing role in the treatment of cancers, inflammatory bowel disease, rheumatoid arthritis, and asthma.[1-3] These agents can cause rapidly developing, severe hypersensitivity reactions.[4-7] Cetuximab (Erbitux, Bristol-Myers Squibb and ImClone Systems), a chimeric mouse–human IgG_1 monoclonal antibody

Address reprint requests to Dr. Platts-Mills at the Asthma and Allergic Disease Center, University of Virginia Medical School, Box 801355, Charlottesville, VA 22908, or at tap2z@virginia.edu..

Drs. Chung and Mirakhur contributed equally to this article.

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against the epidermal growth factor receptor (EGFR), is approved for use in metastatic colorectal cancer and squamous-cell carcinoma of the head and neck.[2,6,8-10] According to the drug's product label, severe hypersensitivity reactions to cetuximab occur in 3% of patients. However, higher rates and clusters of cases have been reported in North Carolina, Arkansas, Missouri, Virginia, and Tennessee.[6,9,11] A recent study showed that 22% of patients who were treated with cetuximab in Tennessee and North Carolina had severe hypersensitivity reactions.[11] In contrast, rates of hypersensitivity reactions were lower (<1%) in most centers in the Northeast.[11] A review of case reports on hypersensitivity reactions to cetuximab revealed that many such reactions occurred within minutes after the patient's first exposure to the drug and were compatible with IgE-mediated anaphylaxis.[11-13]

We investigated the hypothesis that severe hypersensitivity reactions occurring during the initial infusion of cetuximab are mediated by preexisting IgE antibodies against cetuximab. Using a recently developed assay,[14] we found such IgE antibodies in serum samples from case subjects and control subjects. Our results indicate that these antibodies, which are present before treatment, are a cause of severe hypersensitivity reactions to cetuximab. The antibodies are specif for an oligosaccharide, galactose-α-1,3-galactose, which is present on the Fab portion of the cetuximab heavy chain. Such IgE antibodies also bind to a range of mammalian proteins, a finding that is consistent with the expression of galactose-α-1,3-galactose on proteins from most nonprimate mammals. We also found that there is a high prevalence of the IgE antibody in areas of the United States where anaphylactic reactions to cetuximab have occurred.

METHODS

STUDY SUBJECTS

In addition to the samples from subjects who had received cetuximab therapy, we analyzed samples from three distinct locations in the United States to investigate the geographic differences in rates of hypersensitivity reaction (Table 1). In group 1, serum samples were available from 76 subjects with cancer who had received cetuximab and whose clinical response had been documented. The case reports were retrospectively evaluated in a blinded manner at Vanderbilt University Medical Center (VUMC), in Nashville. We used a prespecified case definition to determine the presence or absence of a hypersensitivity reaction within 2 hours after the administration of cetuximab and, if present, to score the severity of the reaction. The serum samples that we evaluated included 35 pretreatment samples from VUMC. These samples were obtained from all subjects who had been treated at VUMC for colorectal cancer or cancer of the head and neck between June 2005 and December 2006; of these subjects, 10 had a hypersensitivity reaction that met our case definition.

Group 1 also included 41 samples from subjects at the other centers, including subjects with a history of an adverse event after cetuximab treatment and a nonrandom selection of subjects with no such report. Fourteen of the subjects with an adverse event did not meet our case definition of a hypersensitivity reaction and were categorized as having had no hypersensitivity reaction. The serum samples included those from five subjects at Duke University Medical Center, in Durham, North Carolina (three of whom had a hypersensitivity reaction), and from nine subjects at the Allergy and Asthma Clinic of Northwest Arkansas, in Bentonville, Arkansas (four of whom had a hypersensitivity reaction). Medical reports and serum samples from 27 subjects (8 of whom had a hypersensitivity reaction) were collected from Bristol-Myers Squibb clinical trials at multiple sites.

Groups 2, 3, and 4 were the source of the control serum samples. Group 2 consisted of 72 healthy volunteers at a yearly cancer-screening event held at VUMC, who were matched with subjects with cancer at VUMC for age, sex, race or ethnic group, and smoking status. Group 3 consisted of 49 subjects with cancer of the head and neck (3 of whom had received cetuximab)

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who had presented at the Stanford University Medical Center, in Stanford, California. Group 4 consisted of 341 female control subjects who were mothers of children in a large cohort study in Boston.[15] Cohorts 3 and 4 were included as representative samples from areas in which there had been a low prevalence (<1%) of hypersensitivity reactions during cetuximab treatment. The screening of 21 subjects with recurrent anaphylaxis who had presented at the University of Virginia Allergy Clinic identified 11 subjects with positive results on testing for IgE antibodies against cetuximab; serum from 6 of these subjects was used to develop the assays and evaluate specificity.

Representatives of Bristol-Myers Squibb and ImClone Systems reviewed the manuscript, which was written by Drs. Chung, Mirakhur, and Platts-Mills. The study was approved by the institutional review board at each center. Each subject provided written informed consent.

CASE DEFINITION AND GRADING SYSTEM

Our case definition and grading of hypersensitivity reactions were based on documented symptoms listed in the National Cancer Institute Common Toxicity Criteria, version 3.[11,16] The characteristics of a grade 1 reaction were transient flushing or rash with a fever of less than 38°C (100.4°F); those of a grade 2 reaction were rash or flushing, urticaria, and dyspnea with or without a fever of more than 38°C; and those of a grade 3 reaction were rash, dyspnea, and hypotension. A grade 4 reaction was anaphylaxis. Among 25 subjects who were judged to have had a hypersensitivity reaction, investigators identified 13 mild reactions (grade 1 or 2) and 12 severe reactions (grade 3 or 4) (Table 1). All treatment decisions were made by the local physicians before the serum samples were assayed for IgE antibodies.

EVALUATION OF ANTIGENS

Cetuximab, which is produced by expressing clone C225 in the mouse myeloma cell line SP2/0, was provided by ImClone Systems.[8,17] A variant of cetuximab, CHO-C225, which is produced in Chinese hamster ovary (CHO) cell lines, was also obtained from ImClone. CHO cells do not produce α-1,3-galactosyltransferase and, for this reason, have a pattern of glycosylation that differs from that of cetuximab.[17,18] This monoclonal antibody, which was purified by means of the techniques used for cetuximab, had the same affinity for EGFR as did cetuximab. The F(ab′)2 and Fc fragments of cetuximab were prepared by digestion with pepsin and papain, respectively, followed by purification over a protein A column. The molecular weights of these molecules were confirmed by sodium dodecyl sulfate–polyacrylamide-gel electrophoresis. Antigens were biotinylated with the use of sulfosuccinimidyl 6-(biotinamido) hexanoate (EZ-Link, Pierce Biotechnology).[14]

Rituximab (Genentech), an anti-CD20 monoclonal antibody, and infliximab (Centocor), a monoclonal antibody against tumor necrosis factor α, were obtained commercially. The reagent galactose-α-1,3-galactose-β;-1,4-*N*-acetylglucosamine-β;-spacer-biotin was purchased from Glyco-Tech. Mouse IgG was obtained from Immunology Consultants. Fel d 1, a cat allergen, was purified by affinity chromatography with the use of the monoclonal antibody clone 6F9.[19]

IMMUNOCAP IgE ASSAYS

ImmunoCAP is a variation of the radioallergosor-bent test in which IgE antibodies that have bound to antigen on the solid phase are detected with a secondary enzyme-labeled anti-IgE antibody.[14,20] Total and specific IgE antibodies were measured with the use of either ImmunoCAP (Phadia U.S.) or the modified assay with streptavidin-coated ImmunoCAP.[14] All assays on serum samples from subjects who had received cetuximab were performed at the University of Virginia and analyzed in a fashion that was blinded to the scoring of subjects' hypersensitivity reactions. Cetuximab was biotinylated, and approximately 5 μg was added to

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each streptavidin-coated ImmunoCAP before serum was added. The assays were performed with the ImmunoCAP250 instrument, and the results were expressed as international units (IU) per milliliter (with 1 IU equivalent to approximately 2.4 ng). The threshold value for a positive reaction was 0.35 IU per milliliter. The streptavidin Immuno-CAP technique was also used to measure IgE antibodies against CHO-C225, the F(ab′)2 and Fc fragments, galactose-*α*-1,3-galactose, mouse IgG, rituximab, infliximab, and Fel d 1. ImmunoCAP assays were used to test selected serum samples for IgE antibodies against allergens from dust mites, cats, dogs, German cockroaches, grass pollen, ragweed pollen, beef, pork, and cow's milk.

STATISTICAL ANALYSIS

The limiting factor in our study was the number of serum samples available from subjects who had a hypersensitivity reaction. Using consistent grading criteria, we identified 25 such subjects, who were matched with sequential controls (for subjects from Tennessee) or with nonrandom controls (for subjects from centers in other states). We compared the results for IgE antibodies in these 25 subjects with results in 51 subjects who did not have a hypersensitivity reaction, using chi-square analysis, and expressed the results as the natural logarithm of the odds ratio. We compared quantitative measures of IgE antibodies against cetuximab and IgE antibodies against galactose-*α*-1,3-galactose and cat, beef, grass, pollen, and dust-mite allergens with the use of Spearman's rank-order correlation. Statistical analyses were performed with SPSS software, version 13.0 (SPSS). A two-sided P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

SERUM ASSAYS FOR IgE ANTIBODIES

Serum samples that were positive for IgE antibodies against cetuximab had antibody titers ranging from 0.38 to 140.00 IU per milliliter. Table 2 shows results for 6 subjects who had anaphylaxis after receiving cetuximab, 11 subjects who had no reaction to cetuximab, and 6 who had recurrent anaphylaxis or angioedema unrelated to cetuximab treatment. Evidence that the assay detected IgE antibodies against cetuximab included the detection of these antibodies by the monoclonal anti-IgE antibody used with the ImmunoCAP assay, demonstration that more than 95% of the IgE antibodies bound to the F(ab′)2 portion of cetuximab, and the finding that absorption of the serum with the use of a monoclonal anti-IgE antibody depleted binding to cetuximab and total levels of IgE in parallel (Table 2).

PREEXISTING IgE ANTIBODIES

Of a total of 538 serum samples from the four groups, 38 contained IgE antibodies against cetuximab (Fig. 1). Among the 76 selected subjects who had received cetuximab, 25 had a hypersensitivity reaction; of these subjects, 17 had a positive test for IgE antibodies against cetuximab in pretreatment serum, whereas only 1 of 51 subjects who did not have a hypersensitivity reaction had such antibodies before treatment with cetuximab ($\log_e$ of the odds ratio, 4.7; $P<0.001$). The sensitivity and specificity of a positive assay for IgE antibodies for any hypersensitivity reaction were 68% and 98%, respectively. For severe hypersensitivity reaction, these values were 92% and 90%, respectively. Subjects with IgE antibodies against cetuximab had a higher rate of severe hypersensitivity reaction than did subjects without such antibodies ($P=0.03$ by Fisher's exact test). Among the eight subjects who were reported to have had a hypersensitivity reaction but had negative results on the IgE assay, seven had grade 1 or 2 reactions, and only one subject had a grade 3 reaction. Five of the eight subjects were rechallenged; of these subjects, one had a second hypersensitivity reaction, and four completed treatment without further reactions. Of the subjects who were subsequently found to have IgE antibodies against cetuximab, 17 had discontinued therapy.

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Among control subjects in Tennessee, 15 of 72 serum samples (20.8%) had positive results on testing for IgE antibodies against cetuximab. In these samples, both the prevalence and titers of IgE antibodies against cetuximab were similar to those in samples from the treated subjects (Fig. 1). Among subjects with cancer of the head and neck in California and female control subjects in Boston, 3 of 49 serum samples (6.1%) and 2 of 341 (0.6%), respectively, had IgE antibodies against cetuximab (Fig. 1). These low rates in cohorts 3 and 4 parallel the low rates of hypersensitivity reactions that were reported with cetuximab treatment in those regions.[11]

CHARACTERIZATION OF THE EPITOPE ON CETUXIMAB

Given that the IgE antibodies were specific for the Fab portion of the heavy chain of cetuximab, the relevant epitope could be a mouse amino acid sequence or an oligosaccharide on this segment of the molecule (Fig. 2). The absence of binding to other chimeric monoclonal antibodies (e.g., rituximab and infliximab) and the absence of IgE antibodies against cetuximab in 25 samples from allergic subjects who had IgE antibodies against mouse proteins[21] argue against the role of a mouse amino acid sequence (Table 3). The Fab portion of the cetuximab heavy chain is glycosylated at N88 with a range of sugars, including galactose-α-1,3-galactose and a sialic acid, *N*-glycolylneuraminic acid (NGNA).[17] To test whether the IgE antibodies were specific for the oligosaccharides, samples containing IgE antibodies against cetuximab were assayed for IgE antibodies that could bind to CHO-C225. These assays were negative for 11 cetuximab-treated subjects and for 5 of the 6 subjects who had an anaphylactic reaction after receiving cetuximab (Table 3). In addition, in 150 samples from groups 1 and 2, as well as those listed in Table 3, assays for IgE antibodies against galactose-α-1,3-galactose correlated with results for antibodies that bound to cetuximab ($r = 0.92$, $P<0.001$). Most of the positive samples also contained IgE antibodies against cat, dog, and beef proteins but not against mite allergens or pollens (Table 3, and Table 1 of the Supplementary Appendix, available with the full text of this article at www.nejm.org).

The correlation with IgE antibodies against mammalian proteins is consistent with the presence of galactose-α-1,3-galactose on proteins of most nonprimate mammals. To confirm the specificity of the reaction, we showed that the binding of IgE antibodies against cat, dog, beef, and pork proteins and cetuximab was inhibited by soluble galactose-α-1,3-galactose and could be absorbed out of the serum with porcine thyroglobulin, which is glycosylated with galactose-α-1,3-galactose (Table 2 of the Supplementary Appendix).

DISCUSSION

Severe anaphylactic reactions have been reported after treatment with several different monoclonal antibodies, but the mechanism of these reactions has not been defined, and their rates have generally been less than 1%.[1-5,7,8,22] Our results show that most of the severe hypersensitivity reactions to cetuximab in the subjects we studied were associated with IgE antibodies against galactose-α-1,3-galactose that were present before treatment with cetuximab. The assay we used identified 17 of the 21 subjects whose treatment had to be discontinued after the first infusion because of a hypersensitivity reaction.

Unlike most other monoclonal antibodies, cetuximab is produced in the mouse cell line SP2/0, which expresses the gene for α-1,3-galactosyltransferase.[17,18] The evidence that IgE antibodies that are specific for the post-translational modification of a molecule can cause severe infusion reactions may have relevance for an understanding of allergic responses to other recombinant molecules.

It is now recognized that all humans have IgG antibodies specific for the oligosaccharide galactose-α-1,3-galactose, which is closely related to substances in the ABO blood group.[23-25] This oligosaccharide is one of the major barriers to the transplantation of organs from

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other mammals in humans and has prompted the development of a strain of pigs in which the gene for α-1,3-galactosyltransferase has been knocked out.[24,26]

Natural exposure to galactose-α-1,3-galactose appears to induce the production of IgE antibodies against galactose-α-1,3-galactose in some people. The presence of such IgE antibodies before treatment may put patients who receive monoclonal antibodies containing galactose-α-1,3-galactose at risk for hypersensitivity reactions. The rapid reactions to cetuximab may be explained by intravenous injection, and the presence of galactose-α-1,3-galactose on both Fab segments of the cetuximab antibody allows for the efficient cross-linking of IgE on mast cells (Fig. 2). Patients who have such antibodies do not report a rapid onset of allergic symptoms after the ingestion of beef, pork, or cow's milk. However, we have identified a series of patients with IgE antibodies against galactose-α-1,3-galactose who reported having had episodes of anaphylaxis or severe angioedema 1 to 3 hours after eating beef or pork (unpublished data). The explanation for such a delayed reaction is not clear, but a similar delay has been reported in patients with IgE antibodies against carbohydrate epitopes of plant proteins.[27,28] In addition, it has recently been reported that some patients with cat allergy have IgE antibodies that bind to a carbohydrate epitope on cat IgA.[29]

The high prevalence of hypersensitivity reactions to cetuximab in the Southeast is supported by our own data from the Tennessee group and in other recent studies.[11] The striking difference in the prevalence of the IgE antibodies against cetuximab provides an explanation for the difference in rates of clinical hypersensitivity reaction between subjects in Boston or northern California and those in Tennessee, Arkansas, or North Carolina.[6,11,30] A high prevalence of IgE antibodies against neuromuscular blocking agents in Norway was found to be associated with anaphylaxis, and the difference in incidence between Norway and Sweden was attributed to suxamethonium, an ingredient in a commonly used cough syrup in Norway.[31,32] The explanation for the regional distribution of IgE antibodies against galactose-α-1,3-galactose in the United States is not clear. Most humans have IgG antibodies against galactose-α-1,3-galactose,[24-26] but we do not know why people in one area of the country have IgE antibodies against galactose-α-1,3-galactose, whereas in other areas the incidence of such IgE antibodies is very low. The regional exposures that could be relevant include histoplasmosis, ameba, tick bites, coccidioidomycosis, nematodes, or cestodes. The effect does not appear to be a nonspecific enhancement of IgE production, since we found little or no association with IgE antibodies against allergens other than those derived from mammals.

In conclusion, we have identified a mechanism underlying a hypersensitivity reaction to cetuximab, preexisting IgE antibodies against an oligosaccharide present on the recombinant molecule. Our results have implications for evaluating risks associated with antibody-based therapeutics and for understanding the relevance of IgE antibodies specific for post-translational modifications of natural and recombinant molecules.

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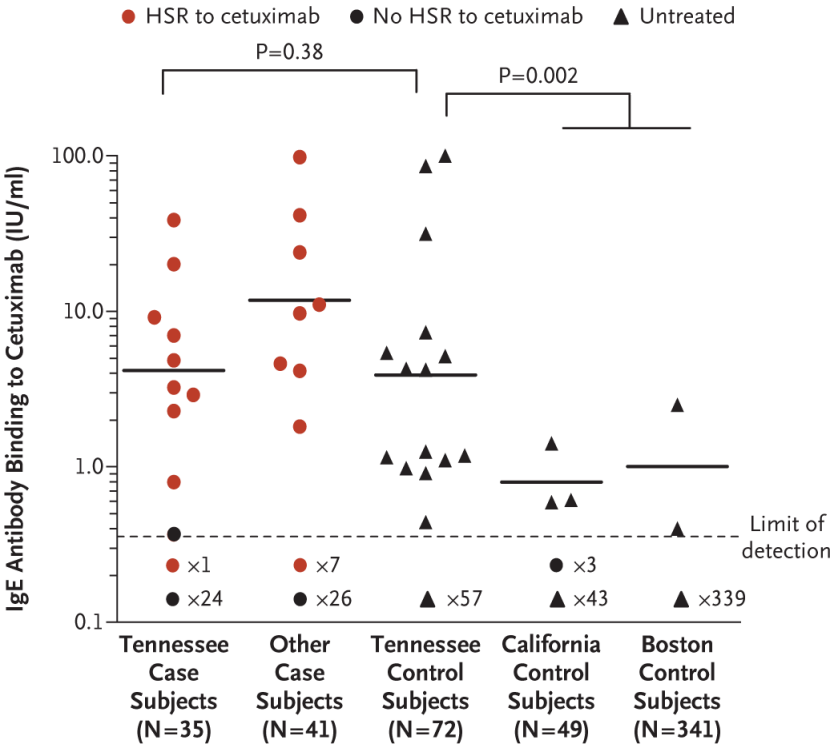

Figure 1. IgE Antibodies Binding to Cetuximab in Serum Samples from 76 Case Subjects and 462 Control Subjects

Results are shown according to whether the treating physician reported a hypersensitivity reaction (HSR) to cetuximab or no HSR reaction. Results are also shown for pretreatment serum samples from control subjects and from subjects who had not received cetuximab. The horizontal lines indicate geometric mean values for the positive results. Values with multiplication signs indicate the number of negative values for each symbol.

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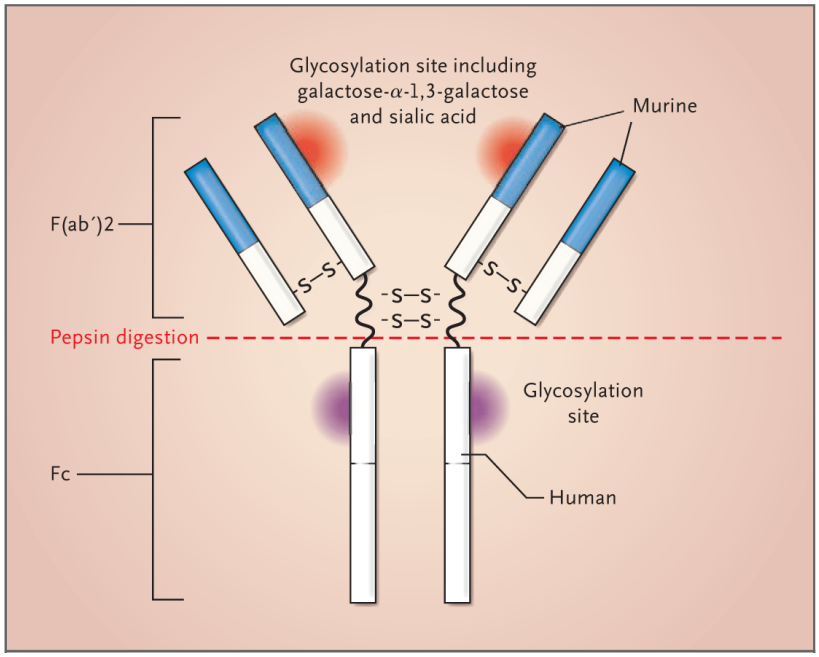

Figure 2. Structure of Cetuximab

The amino acid sequence of cetuximab has potential glycosylation sites at Asn43 of the light chain and at Asn88 and Asn299 of the heavy chain. The sugars on the Fab portion include galactose-*α*-1,3-galactose and the sialic acid *N*-glycolylneuraminic acid. In contrast, the glycosylation site at Asn43 is not glycosylated, and glycosylation of the Fc portion of the heavy chain includes only oligosaccharides that are commonly present on human proteins.[17,18] S–S denotes a disulfide bond.

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Table 1

Characteristics of the Study Groups.*

Variable	Group 1 Tennessee Case Subjects (N = 35)	Group 1 Other Case Subjects (N = 41)	Group 2 Tennessee Control Subjects (N = 72)	Group 3 California Control Subjects (N = 49)	Group 4 Boston Control Subjects (N = 341)	Total All Groups (N = 538)
Age (yr)						
Median	58	63	58	58	NA	58
Range	43–93	41–81	32–82	36–97		32–97
Sex						
Male:female ratio	22:13	22:19	40:32	37:12	0:341	121:417
Male (%)	63	54	56	76	0	22
Race or ethnic group (no.)†						
White	33	35	65	23	236	392
Black	2	5	7	2	54	70
Other	0	1	0	2	32	35
Unknown	0	0	0	22	19	41
Tobacco use (no.)						
Total						197
Current	18	14	16	0	NA	48
Former	3	11	25	0	NA	39
Never	13	16	31	0	NA	60
Unknown	1	0	0	49	341	391
Tumor site (no.)			NA		NA	
Total						125
Head and neck	18	5		49		72
Colorectal	17	35		0		52
Lung	0	1		0		1
Clinical stage (no.)			NA	NA	NA	
Total						76
I or II	9	4				13
III	8	6				14
IV	14	25				39
Unknown	4	6				10
Hypersensitivity reaction (no.)			NA	NA	NA	
Total						26
Rated by investigators						
Low-grade	6	7				13
High-grade	4	8				12
Late response	1	0				1
Rated retrospectively‡						
Low-grade	0	2				2
High-grade	11	13				24

*NA denotes not available.

†Race or ethnic group was reported by the subjects.

‡Retrospective scoring of the severity of hypersensitivity reactions was performed by blinded analysis of case reports.

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Table 2

IgE Antibodies against Cetuximab and the Fragments of the Molecule for 12 Subjects Who Had a Severe Hypersensitivity Reaction.*

Type of Reaction and Subject No.	Total IgE	Cetuximab†	Fragment of Cetuximab Molecule†		Rituximab†	Depletion with Anti-IgE Antibody‡	
			F(ab′)2	Fc		Total IgE Remaining	IgE Antibodies Remaining†
	international units per milliliter					*percent*	
Hypersensitivity reaction							
Anaphylaxis related to cetuximab							
1§	3161.0	41.6	40.9	0.35	0.35	1.3	1.0
2	887.0	38.8	52.3	ND	0.35	8.9	12.0
3§	374.0	20.2	26.0	0.35	0.35	3.4	3.2
4§	348.0	11.1	13.2	0.35	0.35	9.1	5.2
5	58.5	4.9	5.7	0.35	0.35	29.0	21.0
6§	22.2	4.2	6.6	0.35	0.35	15.0	8.4
Recurrent anaphylaxis unrelated to cetuximab¶							
7	1081.0	131.0	158.0	2.90	1.75	4.9	4.0
8	243.0	69.2	86.8	1.20	0.35	0.9	4.0
9	242.0	55.1	99.6	0.35	0.35	9.6	6.7
10	188.0	43.5	45.8	0.35	0.35	11.1	5.9
11	538.0	81.1	100.0	0.35	0.35	1.4	5.7
12	63.6	13.0	17.9	0.57	0.35	11.0	4.9
Mean	315.0‖	26.7	33.4	1.26**	NA	5.7	5.3
No hypersensitivity reaction	17.4††	0	0	0	0	NA	NA

*NA denotes not applicable, and ND not determined.

†The assay was performed with biotinylated antigen on streptavidin ImmunoCAP.

‡Omalizumab (monoclonal anti-IgE antibody) that was bound to protein A agarose beads was incubated overnight at 4°C at a 1:5 bead-to-serum volume ratio.

§The subject had grade 4 anaphylaxis.

¶Subjects presented to the University of Virginia Allergic Disease Clinic with recurrent angioedema or anaphylaxis and had a positive test for IgE cetuximab-binding antibodies. Subjects 7 through 10 had severe episodes of anaphylaxis, and Subjects 11 and 12 had severe episodes of angioedema.

‖The IgE value is the geometric mean for 12 cetuximab-treated subjects who had a hypersensitivity reaction (95% CI, 130.0 to 760.0).

**The mean is for results from 3 of 10 subjects.

††The IgE value is the geometric mean for 11 cetuximab-treated subjects who did not have a hypersensitivity reaction (95% CI, 6.8 to 45.0).

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Table 3

Specificity of the IgE Antibodies That Cross-React with Cetuximab.*

Type of Reaction and Subject No.	Type of Cetuximab†		Galactose-α-1,3-Galactose‡§	Mouse IgG‡	Mammalian Allergens			
	SP2/0‡	CHO‡			Cat	Dog	Fel d 1‡	Beef
	international units per milliliter							
Hypersensitivity reaction								
Anaphylaxis related to cetuximab								
1	41.6	0.35	13.8	0.35	3.16	2.60	0.35	3.02
2	38.8	0.35	35.2	0.35	13.20	12.30	0.35	12.46
3	20.2	0.35	12.6	0.35	9.34	9.77	0.35	6.92
4	11.1	0.35	2.9	0.35	1.94	1.86	0.35	1.82
5	4.9	0.35	2.0	0.35	0.35	0.35	0.35	0.35
6	4.2	0.35	2.7	0.35	1.54	1.50	0.35	1.66
Recurrent anaphylaxis unrelated to cetuximab¶								
7	131.0	1.89	38.9	1.75	41.50	34.50	3.64	32.90
8	69.2	0.35	42.1	1.19	27.70	32.00	0.35	26.20
9	55.1	0.35	32.2	0.43	22.20	25.10	0.35	13.80
10	43.5	0.35	32.3	0.35	37.30	29.40	0.35	3.48
11	81.1	0.35	100.0	0.35	14.30	14.70	0.35	8.75
12	13.0	0.35	9.0	0.78	8.86	8.84	0.35	8.90
Mean	27.7	NA	25.5	ND	10.30	9.93	NA	7.16
No hypersensitivity reaction‖	0	0	0	0	0	0	0	0

*NA denotes not applicable, and ND not determined.

†Cetuximab is produced by expressing clone C225 in the mouse myeloma cell line SP2/0. A variant of cetuximab is produced in Chinese hamster ovary (CHO) cell lines. Since CHO cells do not produce α-1,3-galactosyltransferase, they have a pattern of glycosylation that differs from that of SP2/0.

‡The assay was performed with biotinylated antigen on streptavidin ImmunoCAP.

§The assay was performed with galactose-α-1,3-galactose-β;-1,4-*N*-| acetylglucosamine-β;-spacer-biotin, which binds to streptavidin ImmunoCAP.

¶Subjects presented to the University of Virginia Allergic Disease clinic with recurrent angioedema or anaphylaxis and had a positive test for IgE antibodies against cetuximab. Subjects 7 through 10 had severe episodes of anaphylaxis, and Subjects 11 and 12 had severe episodes of angioedema.

‖For statistical analysis of the positive and negative relationships with other allergens, additional data are available in the Supplementary Appendix, available with the full text of this article at www.nejm.org.