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The carbohydrate galactose- α -1,3-galactose is a major IgE-binding epitope on cat IgA

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To the Editor

Cross-reactive carbohydrate determinants are widely occurring IgE epitopes. Glycan-related IgE reactivity has been demonstrated in most allergen sources, especially in the plant kingdom.¹ The clinical effect of these cross-reactive carbohydrate determinants is debated.

We were recently able to show that IgE Abs to the cat IgA, present in cat-sensitized patients, are mainly directed to a glycan moiety localized on the α -chain.² In addition, we have reported that these carbohydrates are present on IgM Abs from cat, as well as on IgM from many different mammalian species, but not human immunoglobulins.³ Interestingly, IgE antibodies to cat IgM and cat IgA show a complete cross-reactivity, whereas cat IgG does not, suggesting an identical oligosaccharide on the 2 former immunoglobulin classes. Because this is the first mammalian carbohydrate IgE epitope found, it is of major interest to identify the carbohydrate structure responsible for the broad cross-reactivity.

Chung et al⁴ have recently investigated subjects with anaphylactic reactions after treatment with the drug cetuximab, a chimeric mouse–human IgG₁ mAb against the epidermal growth factor receptor, which is approved for use in colorectal cancer and squamous-cell carcinoma of the head and neck. The authors found that a carbohydrate epitope on the mouse Fab portion, galactose- α -1,3-galactose, a part of the Gal α 1,3Gal β 1,4GlcNAc-R (α -gal) epitope, was responsible for the IgE binding. Furthermore, in most subjects, the IgE antibodies against cetuximab were present in serum before therapy.

The α -gal epitope is expressed on many different glycoproteins in mammals, except for old world monkeys, apes, and human beings. Species lacking the α -gal residues produce large quantities of IgG antibodies to this epitope.⁵ Studies have demonstrated that approximately 1% of antibodies in all healthy subjects are directed to α -gal.⁶ These antibodies also react with closely related carbohydrate structures in the ABO blood group and are one of the major obstacles in xenotransplantation.

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Here we investigated whether α -gal is present on cat IgA and whether it is a major epitope responsible for IgE binding to cat IgA.

Cat IgA was purified from cat serum,³ and α -gal–human serum albumin was obtained from Dextra Laboratories, Reading, United Kingdom. To investigate the presence of α -gal on cat IgA, a monoclonal anti-Gal antibody was used in ELISA. Plates were coated with 5 μ g/mL α -gal, cat IgA, or recombinant Fel d 1,⁷ which was included as negative control. Incubation with monoclonal anti-Gal antibodies (Alexis Biochemicals, Lausen, Switzerland), diluted 1:25, was followed by antimouse–IgG-alkaline phosphatase (Dako, Glostrup, Denmark) and substrate solution (Sigma, Steinheim, Germany). We found that the anti-Gal reactivity to α -gal and cat IgA was almost identical, whereas no reactivity was detected to recombinant Fel d 1 (Table I).

Twenty sera from the United States, 9 from patients who were found to have IgE antibodies to the α -gal epitope on cetuximab by using the streptavidin CAP technique,⁸ (range, 0.79 to >100 kilo Units per Liter [kU_A/L]; median, 61.1 kU_A/L) and 11 negative controls were examined. Eight of the 9 patients showed positive IgE responses to cat dander (range, 0.61–61.8 kU_A/L; median, 17.8 kU_A/L). The US sera were tested blind. Sera from 6 Swedish patients with cat allergy with IgE responses to cat IgA (range, 0.47–13.0 kU_A/L; median, 1.5 kU_A/L)² were included.

We investigated by ELISA the correlation between IgE responses to cat IgA and α -gal, whether the cetuximab-positive sera from the United States had IgE antibodies to cat IgA, and whether this response could be blocked by α -gal. Likewise, we elucidated whether our Swedish cat IgA-positive sera had IgE antibodies to α -gal and whether the IgE reactivity to cat IgA could be inhibited by α -gal.

Direct ELISA with solid-phase bound α -gal and cat IgA was performed as described.² Nine US sera showed IgE reactivity to cat IgA (OD, range, 0.302–2.712; median, 1.548) as well as to α -gal (OD, range, 0.364–2.575; median, 1.291) and were later confirmed to be cetuximab-positive sera. The 11 negative controls exhibited low values for IgA (OD, range, 0.066–0.196; median, 0.129) and α -gal (range, 0.043–0.207; median, 0.107). All but 1 of the cat IgA-positive Swedish sera displayed IgE responses to α -gal (OD, range, 0.043–0.989; median, 0.412). The reason for the single negative result is presumably the presence of epitopes other than α -gal on cat IgA. Our previous study revealed the presence of epitopes other than carbohydrates, and 1 serum displayed even higher IgE reactivity to cat IgA after de-glycosylation.² The results in direct ELISA demonstrated a high correlation between IgE reactivity to α -gal and cat IgA ($r = 0.98$; Fig 1).

In the next step, inhibition ELISA was performed with 1 Swedish and 1 US sera by using solid-phase bound cat IgA (5 μ g/mL) and α -gal as competing antigen (3-fold dilutions from 300 μ g/mL to 0.14 μ g/mL). The responses were dose-dependent and similar for both sera (Fig 2). Complete inhibition was achieved at the highest antigen concentration (300 μ g/mL), whereas no inhibition was detected with the lowest antigen concentration.

In conclusion, we have shown that the previously described dominant IgE epitope on cat IgA² is the carbohydrate α -gal. This epitope exists on a large number of other mammalian proteins. Although we are exposed to mammalian proteins carrying the α -gal epitope both in the air (eg, cat, dog, or horse immunoglobulins) and by ingestion of foods such as beef, pork, or cow's milk,⁹ current evidence suggests that IgE responses to α -gal may be induced by infections or parasitic agents. This epitope has, in addition, been demonstrated to be responsible for potentially life-threatening anaphylactic reactions to cetuximab in patients who have specific IgE antibodies to α -gal. The results suggest that screening for IgE

antibodies to cat IgA or the α -gal epitope itself might help to identify patients who are at risk for anaphylaxis when treated with cetuximab.

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FIG 1. Reaction of IgE antibodies in sera from 6 Swedish patients with cat allergy, 9 US cetuximab-reactive patients (*closed circles*), and 11 US controls (*open circles*), tested by ELISA on wells coated with α -gal (*y-axis*) and cat IgA (*x-axis*).

FIG 2.

IgE competition ELISA using solid-phase bound cat IgA and α -gal as inhibitor performed on 1 serum with IgE antibodies to cat IgA from Sweden (*filled boxes*) and 1 serum with IgE antibodies to cetuximab from the United States (*filled circles*).

TABLE I

Comparison of monoclonal antigalactose reactivity to solid phase bound α -gal, cat IgA, and recombinant Fel d 1 (rFel d 1) by ELISA

Antigen	OD (450 nm)
α -gal	0.69
Cat IgA	0.67
rFel d 1	0.05