

# Galactose- $\alpha$ -1,3-Galactose-Specific IgE Is Associated with Anaphylaxis but Not Asthma

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**Rationale:** IgE antibodies to the mammalian oligosaccharide galactose- $\alpha$ -1,3-galactose ( $\alpha$ -gal) are common in the southeastern United States. These antibodies, which are induced by ectoparasitic ticks, can give rise to positive skin tests or serum assays with cat extract.

**Objectives:** To evaluate the relationship between IgE antibodies to  $\alpha$ -gal and asthma, and compare this with the relationship between asthma and IgE antibodies to Fel d 1 and other protein allergens.

**Methods:** Patients being investigated for recurrent anaphylaxis, angioedema, or acute urticaria underwent spirometry, exhaled nitric oxide, questionnaires, and serum IgE antibody assays. The results were compared with control subjects and cohorts from the emergency department in Virginia (n = 130), northern Sweden (n = 963), and rural Kenya (n = 131).

**Measurements and Main Results:** Patients in Virginia with high-titer IgE antibodies to  $\alpha$ -gal had normal lung function, low levels of exhaled nitric oxide, and low prevalence of asthma symptoms. Among patients in the emergency department and children in Kenya, there was no association between IgE antibodies to  $\alpha$ -gal and asthma (odds ratios, 1.04 and 0.75, respectively). In Sweden, IgE antibodies to cat were closely correlated with IgE antibodies to Fel d 1 ( $r = 0.83$ ) and to asthma ( $P < 0.001$ ).

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## AT A GLANCE COMMENTARY

### Scientific Knowledge on the Subject

There are still questions about the importance of specific and total IgE in the pathophysiology of asthma in Western countries, and also about how children in developing countries can have high total serum IgE with no relationship to allergic disease. The recent finding of IgE antibodies to the mammalian oligosaccharide galactose- $\alpha$ -1,3-galactose makes it possible to study the clinical relevance of a parasite-induced IgE response within a Western country.

### What This Study Adds to the Field

We show that the presence of IgE antibodies to galactose- $\alpha$ -1,3-galactose have no influence on the risk of asthma even though they cross-react with glycosylation on cat proteins and can make major contributions to total IgE. This is in direct contrast to IgE antibodies to protein allergens, such as Fel d 1, which are airborne in houses and strongly associated with asthma.

**Conclusions:** These results provide a model of an ectoparasite-induced specific IgE response that can increase total serum IgE without creating a risk for asthma, and further evidence that the main allergens that are causally related to asthma are those that are inhaled.

**Keywords:**  $\alpha$ -gal; red meat allergy; ticks; total serum IgE; ectoparasite

A large proportion of children and young adults with asthma have serum IgE antibodies specific to one or more common inhalant allergens, and in Western countries elevated total serum IgE (1–5). Furthermore, it is possible to make a logical argument that inhaling allergens into the lungs of a subject who is allergic is a major cause of asthma (6, 7). However, questions about the evidence for a causal relationship between allergen exposure and asthma have come from studies of different types (8–10). These included an influential paper from Arizona that reported a strong correlation between total IgE and asthma, but no correlation between asthma and specific sensitization (10). That finding was used to argue that IgE production was a non-specific response to inflammation in the lungs (11). Our view has been that inhalation of allergens by subjects who are allergic is causally related to asthma, and that the specific IgE antibodies that are important in relation to asthma can make a significant contribution to total IgE (12). However, in rural areas of developing countries where asthma is rare, most children have

elevated levels of total IgE, often as high as 1,000 IU/ml (1 IU = 2.4 ng) with no associated allergic symptoms or relationship to asthma (13–15).

Recently, we have identified a novel IgE antibody response to a mammalian oligosaccharide epitope, galactose- $\alpha$ -1,3-galactose ( $\alpha$ -gal) (16). The presence of these  $\alpha$ -gal-specific IgE antibodies, which are predominantly found in people living in the southeastern United States, has been associated with two forms of anaphylaxis: immediate-onset anaphylaxis during first exposure to intravenous cetuximab; and delayed-onset anaphylaxis after ingestion of mammalian food products (e.g., beef and pork) (16, 17). Furthermore, we have recently published evidence that IgE antibodies to  $\alpha$ -gal are induced by tick bites, predominantly those of the Lone Star tick (*Amblyomma americanum*) (18).

The oligosaccharide  $\alpha$ -gal is present on proteins derived from all nonprimate mammals, including cat and dog proteins (19). In keeping with that, most of the patients with IgE to  $\alpha$ -gal give positive intradermal skin tests and positive serum IgE antibodies to cat and dog allergens (16, 17, 20). Our preliminary experience in the clinic suggested that this IgE antibody response was not related to asthma. Thus, we have the enigma of patients who seem to be allergic to cat and dog, but in general do not report symptoms around animals and do not seem to have an increased risk of asthma. In addition, we have identified two cohorts (one from the University of Virginia emergency department (ED), the other from Kabati, Kenya) in which there was a previously unexplained predominance of IgE antibodies to cat. We report here a detailed investigation of the relevance of IgE antibodies to  $\alpha$ -gal, not only in the United States, but also in northern Sweden and rural Kenya (13, 21). The results provide direct evidence for an IgE antibody response that can increase total serum IgE but is unrelated to inhalant allergic symptoms or asthma. Some of the results of these studies have been previously reported in the form of abstracts (22, 23).

## METHODS

### Study Subjects and Populations for Comparison

Subjects over the age of 10 years presenting to allergic disease clinics in central Virginia were enrolled into one of three arms. Patients

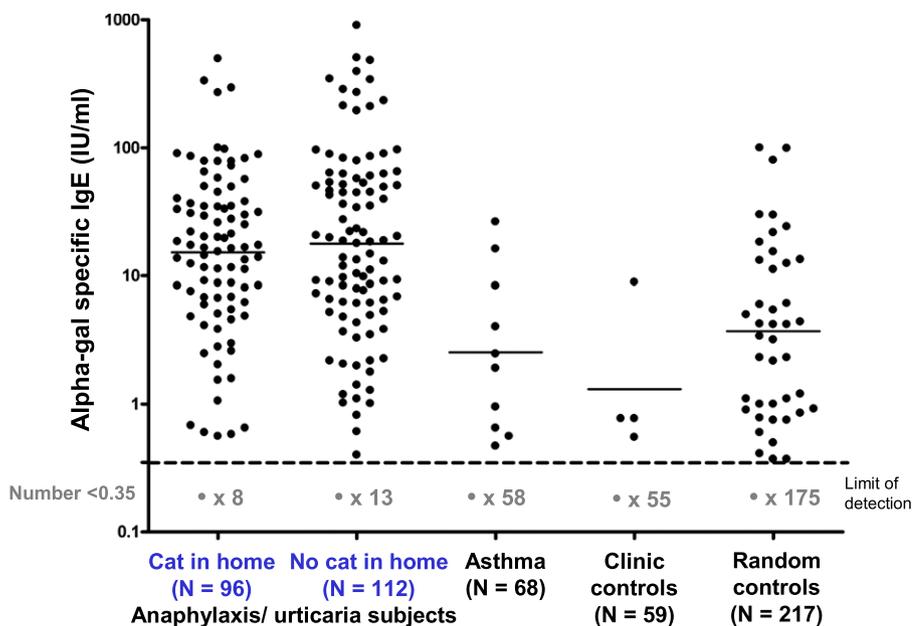
presenting with a primary complaint of otherwise idiopathic, recurrent acute urticaria, angioedema, or anaphylaxis were enrolled into the “anaphylaxis-urticaria” group (n = 208) regardless of whether their history was suspicious for delayed anaphylaxis to red meat (17). Subjects presenting to clinic with asthma, regardless of whether they may also have had symptoms of urticaria, angioedema, or anaphylaxis, were enrolled into the “asthma” group (n = 68). Subjects in the “clinic control” group (n = 59) represent patients seeking care for any other complaint; note that this group is not expected to represent a truly random control group. “Random control subjects” (n = 217) were enrolled from three southeastern states (see the online supplement). After obtaining written informed consent, subjects responded to a questionnaire (see Form I in the online supplement). Sera collected were analyzed for  $\alpha$ -gal-specific IgE and total IgE. A random selection of 78  $\alpha$ -gal-positive anaphylaxis-urticaria subjects underwent additional tests: spirometry, exhaled nitric oxide (eNO), and complete blood count. Approval for these studies was obtained locally in the area where subjects were enrolled and from the University of Virginia Human Investigation Committee.

**Asthma exacerbations and other emergency department control subjects.** Serum collected for a previous study of 130 subjects older than age 18 years presenting to the ED at the University of Virginia between 1998 and 1999 (60 with acute asthma exacerbations, 70 with any other complaint) were analyzed for total serum IgE and IgE specific to  $\alpha$ -gal, dust mite, cockroach, cat epithelium, and Fel d 1. The eNO, sinus CT, and inflammatory marker results of that study have previously been reported (24, 25).

**Assessment of subjects from geographically diverse areas.** As part of a prospective study on asthma in northern Sweden, sera were obtained from 963 subjects at age 18 years (21, 26). Those sera with positive assays for IgE antibodies to cat epithelium and dander of class 2 or higher (i.e., >0.70 IU/ml; n = 148) were assayed for IgE to  $\alpha$ -gal and Fel d 1. Similarly, IgE titers to cat epithelium,  $\alpha$ -gal, and Fel d 1 were measured using banked serum from our previous study of 131 schoolchildren (mean age, 11.5 years) living in Kabati, Kenya (13). Details on these cohorts are available online.

### ImmunoCAP IgE Assays and Absorption Experiments

Total and specific IgE antibodies were measured by using either commercially available ImmunoCAP (Phadia US, Portage, MI) or a modification of the assay with streptavidin on the solid phase (27). Absorption assays were performed with  $\alpha$ -gal or bovine thyroglobulin bound to sepharose beads using methods as previously published and as detailed in the online supplement (16, 17).



**Figure 1.** Galactose- $\alpha$ -1,3-galactose ( $\alpha$ -Gal)-specific IgE antibody titers. Numbers in gray denote the number of subjects in each group with no detectable IgE antibodies to  $\alpha$ -gal. Note that  $\alpha$ -gal IgE titers are sometimes markedly elevated. IgE titers do not differ between subjects living with or without cats. Prevalence of IgE antibodies to  $\alpha$ -gal was significantly higher ( $P < 0.001$ ) among patients with anaphylaxis, angioedema, or acute urticaria, and the mean IgE antibody titer among positives was significantly higher than among the random control subjects ( $P < 0.001$ ). Bars represent the geometric mean of the positive values for each group.

TABLE 1. SUBJECT CHARACTERISTICS BY GROUP

	Anaphylaxis, Angioedema, or Urticaria		Asthma	Clinic Control Subjects
	Cat*	No cat		
Number enrolled	96	112	68	59
$\alpha$ -Gal positive, n	88	99	10	4
Age range	10–77	9–83	20–74	19–74
Percent male	52%	34%	45%	38%
Physician-diagnosed asthma (%)	10 (10)	22 (20)	68 (100)	7 (12)
Total serum IgE, IU/ml <sup>†</sup>	164 <sup>‡</sup>	190 <sup>‡</sup>	166 <sup>§</sup>	28 <sup>  </sup>

Definition of abbreviation:  $\alpha$ -gal = galactose- $\alpha$ -1,3-galactose.

\*Anaphylaxis–urticaria subjects living with a cat in the home.

<sup>†</sup>Geometric mean.

<sup>‡</sup>No significant difference between those living with a cat or not.

<sup>§</sup>No significant difference between the asthma group and anaphylaxis group.

<sup>||</sup> $P < 0.001$  compared with anaphylaxis group or asthma group.

### Airborne Sample Collection and Inhibition Radioimmunoassay

Samples were collected using Ionic Breeze Quadra (The Sharper Image, New York, NY) and quantified using an inhibition radioimmunoassay specific for the  $\alpha$ -gal epitope (28, 29). The details of both procedures are available online.

### Statistical Analyses

Statistical analyses were performed with SPSS software, version 18.0 (SPSS Inc., Chicago, IL), and GraphPad Prism, version 4 (GraphPad Software, La Jolla, CA).

## RESULTS

### IgE Responses to $\alpha$ -Gal in Virginia Are Associated with Anaphylaxis and Can Contribute to Total Serum IgE but Do Not Create an Increased Risk for Asthma

Of 208 patients who presented to allergy clinics in central Virginia for evaluation of apparently idiopathic anaphylaxis, angioedema, or repeated episodes of severe urticaria, 187 were found to have serum IgE antibodies to  $\alpha$ -gal (Figure 1, Table 1). Furthermore, the titer of  $\alpha$ -gal-specific IgE was significantly higher among those in the anaphylaxis–urticaria group compared with random control subjects with IgE antibodies to  $\alpha$ -gal (geometric mean 16.5 vs. 3.7 IU/ml, respectively;  $P <$

0.001). The prevalence of a positive test for  $\alpha$ -gal (i.e.,  $\alpha$ -gal-specific IgE  $\geq 0.35$  IU/ml) among subjects in the random control group was only 19%, compared with 89% positive in the anaphylaxis–urticaria group. The anaphylaxis–urticaria group was also tested for total IgE, and there was a clear correlation between IgE to  $\alpha$ -gal and total IgE ( $r = 0.60$ ;  $P < 0.001$ ) (Figure 2). In many cases, the IgE antibodies to this oligosaccharide seemed to represent greater than 10% of the total serum IgE, and in some cases greater than 30% of the total. This was true even in sera with total IgE more than 1,000 IU/ml (Figure 2). To confirm that  $\alpha$ -gal-specific IgE antibodies were directly contributing to elevated total IgE, absorption studies were performed on eight sera, which showed that removing IgE antibodies to  $\alpha$ -gal removed an equal quantity of total IgE (Table 2). The results confirm that the unit used here for IgE antibodies is quantitatively the same as the unit used for total IgE. In keeping with our previous results, greater than 90% of the  $\alpha$ -gal-positive sera also gave positive results for IgE to cat and dog allergens (17).

In questionnaires, only a minority (13%) of subjects in the anaphylaxis–urticaria group with IgE to  $\alpha$ -gal had received a diagnosis of asthma from a physician at some point in their life. Spirometry, eNO, and peripheral blood eosinophil counts were obtained in 78 subjects who were randomly selected from the anaphylaxis–urticaria group; all 78 had IgE antibodies to  $\alpha$ -gal, 43 were living in a house with a cat, 46 had an  $\alpha$ -gal-specific IgE titer greater than or equal to 10 IU/ml, and 11 (14%) had been diagnosed with asthma by a physician (Figures 3A and 3B, Table 3). These results show that these patients do not have a prevalence of asthma greater than that of the general population, and also that neither lung function nor eNO were influenced by living with an animal. Even when those subjects with  $\alpha$ -gal titers greater than 10 IU/ml were considered separately, objective measures of asthma including eNO, FEV<sub>1</sub>, and FEV<sub>1</sub>/FVC are no different from those with  $\alpha$ -gal titers less than 10 IU/ml (Table 3). Likewise, those with high-titer IgE to  $\alpha$ -gal were no more likely to have physician-diagnosed asthma ( $\chi^2 = 0.52$ ;  $P = 0.47$ ) or require daily asthma medication ( $\chi^2 = 0.38$ ;  $P = 0.54$ ). The rate of sensitization to common nonmammalian inhalant allergens (e.g., timothy grass, *Alternaria*, and so forth) among anaphylaxis–urticaria subjects with IgE to  $\alpha$ -gal was no higher than that of the general population (*see* Table E1 in the online supplement).

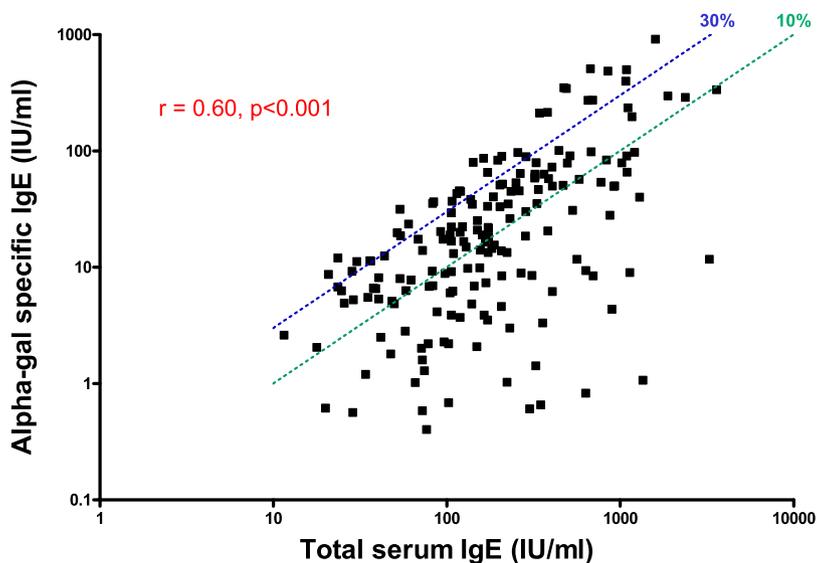


Figure 2. Total serum IgE correlates with the titer of IgE specific for galactose- $\alpha$ -1,3-galactose ( $\alpha$ -gal) among the anaphylaxis–urticaria group. Those subjects whose  $\alpha$ -gal-specific IgE antibodies make up at least 10% (or 30%) of their total serum IgE fall on or above the green (or blue) line.

**TABLE 2. ABSORPTION OF SERA USING  $\alpha$ -GAL DECREASES SPECIFIC AND TOTAL IgE**

Serum No.	Preabsorption IgE Values		Postabsorption with $\alpha$ -Gal				Postabsorption with Bovine Thyroglobulin			
	$\alpha$ -Gal	Total	IgE Values		Difference		IgE Values		Difference	
			$\alpha$ -Gal	Total	$\alpha$ -Gal	Total	$\alpha$ -Gal	Total	$\alpha$ -Gal	Total
E333	7.05	14.5	<0.35	6.28	6.70	8.22	0.92	7.11	6.13	7.39
E340	6.71	23.7	<0.35	21.5	7.54	8.40	ND	ND	ND	ND
E361	44.3	357	0.37	292	42.5	37	ND	ND	ND	ND
E368	28.6	146	3.85	126	24.8	20	3.49	131	25.1	15
E377	4.72	77.4	<0.35	69.5	4.37	7.90	0.63	71.1	4.09	6.30
E384	43.9	124	2.37	75.8	41.5	48.2	4.53	83.1	39.4	40.9
T405	434	1,050	7.02	612	427	438	40.9	666	394	384
T454	1.27	21.9	<0.35	18.3	0.85	0.90	ND	ND	ND	ND

Definition of abbreviations:  $\alpha$ -gal = galactose- $\alpha$ -1,3-galactose; ND = not done.

The similar postabsorption values, reported both in absolute values and as the decrease in  $\alpha$ -gal-specific or total IgE (IU/ml), confirm that specific IgE responses represent a large proportion of the total.

### IgE Antibodies to Fel d 1 but Not to $\alpha$ -Gal Correlate with Acute Asthma among Patients Presenting to an ED

Subjects presenting to the University of Virginia ED with acute asthma ( $n = 60$ ) or any other complaint ( $n = 70$ ) were found to have IgE antibodies to cat epithelium that were only weakly associated with asthma (odds ratio [OR], 2.15; 95% confidence interval [CI], 0.98–4.75;  $P = 0.08$ ). The sera from that study have now been assayed for IgE antibodies to the major cat allergen Fel d 1, and to  $\alpha$ -gal. The results show that IgE antibodies to Fel d 1 were significantly associated with asthma (OR, 4.7; 95% CI, 1.6–13.8;  $P = 0.005$ ), whereas IgE antibodies to  $\alpha$ -gal showed no association (OR, 1.04; 95% CI, 0.43–2.54;  $P = 0.93$ ) (Table 4). The findings for cat allergen are explained by the fact that the solid phase used for the IgE assay for cat epithelium contained Fel d 1 and proteins glycosylated with  $\alpha$ -gal. In the ED study, having IgE antibodies ( $>0.35$  IU/ml) to any one of three indoor allergens (dust mite, cockroach, or Fel d 1) was only modestly associated with asthma (OR, 1.6; 95% CI, 0.81–3.2;  $P = 0.18$ ); high-titer antibodies ( $\geq 10$  IU/ml) to one or more of the three indoor allergens carried a highly significant risk for asthma (OR, 3.5; 95% CI, 1.48–8.2;  $P = 0.0004$ ) (Table 4).

### Studies in Cohorts with or without Endemic Helminth or Ectoparasite Infection Reveal Geographic Disparities in the Relationship between IgE Antibodies to Cat and IgE Antibodies to Fel d 1

To further examine the contrast between IgE antibodies to a carbohydrate epitope and IgE to a protein allergen, such as Fel d 1, we compared results from a rural area of Kenya where parasites are ubiquitous with those from Norrbotten, Sweden, an area in the north of Sweden where helminths and ectoparasites are uncommon (13, 21, 26, 30). The results show dramatically different patterns of sensitization to cat-related allergens for the two regions (Figure 4). In northern Sweden, IgE antibodies to cat correlated quantitatively with IgE antibodies to Fel d 1 ( $r = 0.83$ ;  $P < 0.001$ ) (Figures 4A and 4C). Moreover, the quantity of IgE antibodies to Fel d 1 showed a highly significant direct relationship to asthma as judged by symptoms or medication use ( $\chi^2$  test for trend = 82,  $P < 0.001$ , and  $\chi^2 = 67$ ,  $P < 0.001$ , respectively). The sera from Kenya also had a high prevalence of IgE antibodies to cat allergens; however, it is now clear that these cat antibodies are largely explained by IgE responses to  $\alpha$ -gal, rather than Fel d 1 (Figures 4B and 4D) (17, 18). Questionnaire data regarding baseline asthma symptoms and also changes in FEV<sub>1</sub> after a

6-minute exercise challenge were collected on the Kenyan children. These data were compared with cat-specific IgE, Fel d 1-specific IgE, and  $\alpha$ -gal-specific IgE (see Table E2). The ORs comparing  $\alpha$ -gal IgE titers with exercise-induced bronchospasm, or symptoms or medication requirements, were 0.40 (95% CI, 0.14–1.20) and 0.75 (95% CI, 0.12–4.67), respectively.

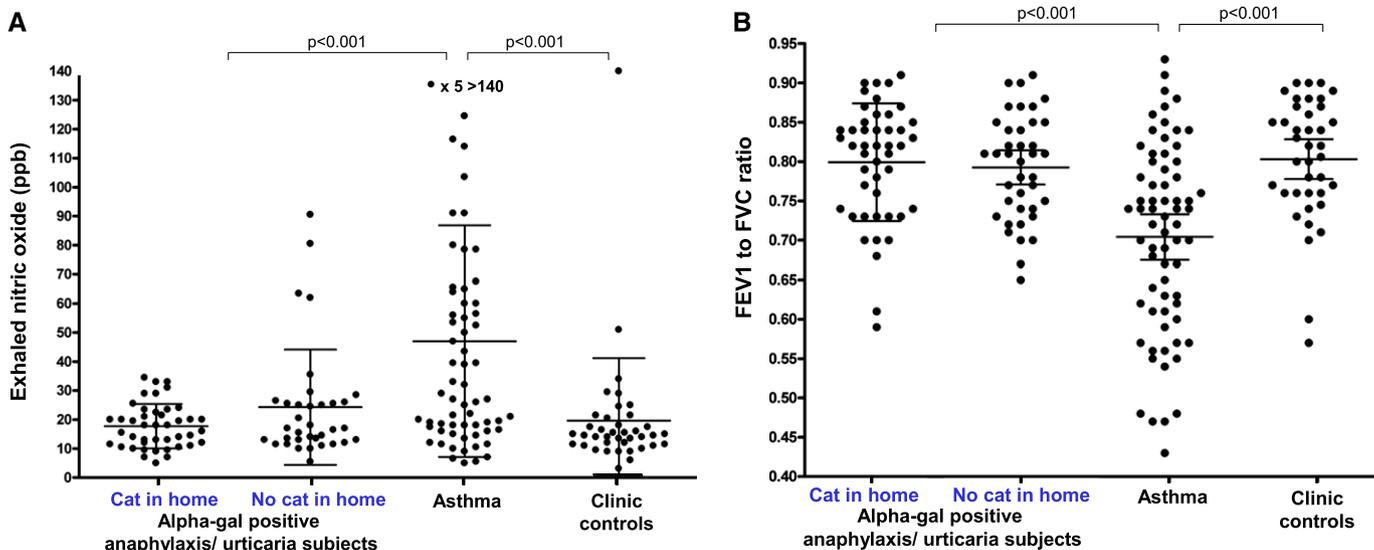
### $\alpha$ -Gal Is Not Airborne in Homes

It is well established that Fel d 1 is airborne in homes with a cat, and estimates of daily exposure range as high as 1  $\mu$ g (28, 29). Using a sensitive radioimmunoassay for  $\alpha$ -gal, we assayed this antigen in samples obtained from homes with or without cats or dogs (17). The  $\alpha$ -gal epitope was not detectable in airborne samples from these homes (Table 5). As expected, Fel d 1 and Can f 1 were present in significant quantities in the same samples.

### DISCUSSION

The results presented here provide extensive evidence that a specific IgE antibody response to an oligosaccharide common to all nonprimate mammals does not create a risk for asthma. Through examination of patients in Virginia with IgE to  $\alpha$ -gal, patients presenting with asthma to the ED in Charlottesville, or children in a Kenyan village, it is clear that having this antibody is not related to lung inflammation or symptoms. Given that sensitization to cat allergens has been consistently associated with asthma, it was a surprise to find a large number of patients presenting with recurrent anaphylaxis or urticaria who had positive skin tests and serum assays to cat. As judged by symptoms, eNO, and lung function, few of these patients had asthma. This conclusion was supported by further investigation of two cohorts in which we had previously noticed a high prevalence of IgE antibodies to cat but no increased risk for asthma: the Charlottesville ED and Kenyan school children (13, 25). In each case, the apparently anomalous results for cat were explained by the finding of IgE antibodies to the oligosaccharide  $\alpha$ -gal.

Allergens including house dust mite (Der p 1) and cat (Fel d 1) that clearly correlate with risk for asthma are proteins with a complex surface and multiple epitopes that favor binding of high-affinity IgE antibodies (31, 32); such protein allergens are able to induce positive skin tests with concentrations of purified proteins in the range of  $10^{-3}$  to  $10^{-5}$   $\mu$ g/ml. In contrast, our own experience and a recent study reported positive skin tests in patients with IgE to  $\alpha$ -gal using 5–50  $\mu$ g/ml of



**Figure 3.** Objective measures of asthma support our clinical observation that galactose- $\alpha$ -1,3-galactose( $\alpha$ -gal)-positive anaphylaxis-urticaria subjects are no more likely to have asthma than the clinic control group, and that living with cats has no impact. Arithmetic means with standard deviation are displayed. (A) Exhaled nitric oxide among  $\alpha$ -gal-positive anaphylaxis-urticaria subjects is significantly lower than that of the asthma group and no different from the clinic control group. (B) The ratio of FEV<sub>1</sub> to FVC among  $\alpha$ -gal-positive anaphylaxis-urticaria subjects is significantly higher than the asthma group and no different from the clinic control group.

cetuximab (33). The blunted skin test response using this mammalian oligosaccharide is consistent with responses to skin testing in patients with IgE antibodies to plant oligosaccharides (34, 35). Given the small quantities of protein that actually enter the large and medium airways, it makes sense that an allergen that requires micrograms to produce a positive skin test would not give rise to inflammation in the lungs. The evidence reported here for the association between IgE antibodies to Fel d 1 and asthma in northern Sweden is in keeping with a model where allergen entering the lungs of a sensitized subject is able to cross-link IgE receptors on mast cells and trigger inflammation in the lungs. The role of Fel d 1 in asthma may also involve T-cell epitope-mediated recruitment of T cells that contribute to the pathogenesis of lung inflammation (36–38). With regard to  $\alpha$ -gal, the specificity of the T cells associated with this potential mechanism is not known. However, exposure to this epitope either as a food or inhalant is unlikely to occur on the same carrier as was involved in the initial tick-induced IgE antibody response. The lack of a relevant carrier could also contribute to the absence of a pulmonary response in these patients.

It has been consistently reported that total serum IgE is elevated among patients with asthma, but mixed opinions about the significance of this observation persist (5, 10–12). The IgE

antibody response to  $\alpha$ -gal may provide further insight in that it not only correlates strongly with total serum IgE, but also that this IgE response can represent a large proportion of the total IgE. In addition, we have documented specific IgE to  $\alpha$ -gal and total IgE rising in parallel after tick bites (18). Although it is well recognized that helminth infection can induce IgE antibodies, only limited studies on the specificity of these IgE antibodies or the quantitative contribution of these antibodies to total serum IgE have been reported (39). As with other immunoglobulin isotypes and with other IgE responses, the specific IgE responses do not explain the total (40). The important conclusion here is that this IgE antibody response to an oligosaccharide epitope is a “cause” of elevated total IgE in the United States and is nonetheless unrelated to asthma.

The results here explain the previously enigmatic finding of IgE antibodies to cat in Kenya and South Africa (13, 15). Several studies in Africa have reported the presence of sensitization to mite allergens that was not related to asthma (13–15, 41). However, those IgE antibody responses, where measured, were low in titer (13, 15, 41). Recently, we have reported that a major effect of affluence among school children in Kumasi, Ghana, was increased titer of IgE antibodies to mite (41). The evidence reported here about the influence of high-titer IgE

**TABLE 3. SPIROMETRY, EXHALED NITRIC OXIDE, AND PERIPHERAL EOSINOPHIL COUNT**

	$\alpha$ -Gal-Positive Anaphylaxis-Urticaria Subjects					
	Total	Cat	No Cat	$\alpha$ -Gal IgE $\geq 10$ IU/ml*	Asthma	Clinic Control Subjects
Number	78	43	35	46	68	41
Physician-diagnosed asthma (%)	11 (14)	6 (14)	5 (14)	4 (9)	68 (100)	5 (12)
FEV <sub>1</sub> to FVC ratio	0.79 (0.77–0.81)	0.80 (0.78–0.82)	0.79 (0.77–0.81)	0.79 (0.76–0.82)	0.70 (0.68–0.73) <sup>†</sup>	0.80 (0.78–0.83)
% predicted FEV <sub>1</sub>	91 (88–94)	91 (88–95)	92 (87–97)	92 (87–97)	74 (69–79) <sup>†</sup>	95 (91–99)
Exhaled NO, ppb	23 (19–28)	18 (15–20)	24 (17–31)	20 (16–24)	51 (38–64) <sup>†</sup>	20 (13–27)
Eosinophils per $\mu$ l	209 (167–251)	205 (166–243)	213 (165–260)	192 (160–223)	352 (280–424) <sup>‡</sup>	183 (107–260)

*Definition of abbreviations:*  $\alpha$ -gal = galactose- $\alpha$ -1,3-galactose; NO = nitric oxide; ppb = parts per billion.

Values are the arithmetic mean followed by the 95% confidence interval in parentheses.

\*Subjects with IgE antibody titers to  $\alpha$ -gal greater than or equal to 10 IU/ml.

<sup>†</sup> $P < 0.001$  compared with  $\alpha$ -gal-positive group or clinic control group.

<sup>‡</sup> $P < 0.01$  compared with  $\alpha$ -gal-positive group or clinic control group.

**TABLE 4. IgE ANTIBODIES TO  $\alpha$ -GAL, FEL D 1, AND INDOOR ALLERGENS AMONG SUBJECTS (AGE 18–50) PRESENTING TO AN EMERGENCY DEPARTMENT IN VIRGINIA**

	All ED Subjects	Asthma ED Subjects	Control ED Subjects	Odds Ratio
Number enrolled	130	60	70	
IgE to $\alpha$ -gal (IU/ml)				
>0.35	24	12	12	1.04 (0.43–2.5); $P = 0.93$
>3.5	7	3	4	0.76 (0.16–3.6); $P = 0.73$
>10	4	1	3	0.33 (0.03–3.3); $P = 0.35$
IgE to Fel d 1 (IU/ml)				
>0.35	22	17	5	4.7 (1.6–13.8); $P = 0.005$
>3.5	8	7	1	8.4 (0.99–70); $P = 0.05$
>10	5	5	0	
IgE to indoor allergens: dust mite, cockroach, or Fel d 1 (IU/ml)				
>0.35	60	41	29	1.6 (0.81–3.2); $P = 0.18$
>3.5	46	32	14	2.9 (1.4–6.2); $P = 0.006$
>10	35	26	9	3.5 (1.5–8.2); $P = 0.004$

Definition of abbreviations:  $\alpha$ -gal = galactose- $\alpha$ -1,3-galactose; ED = emergency department.

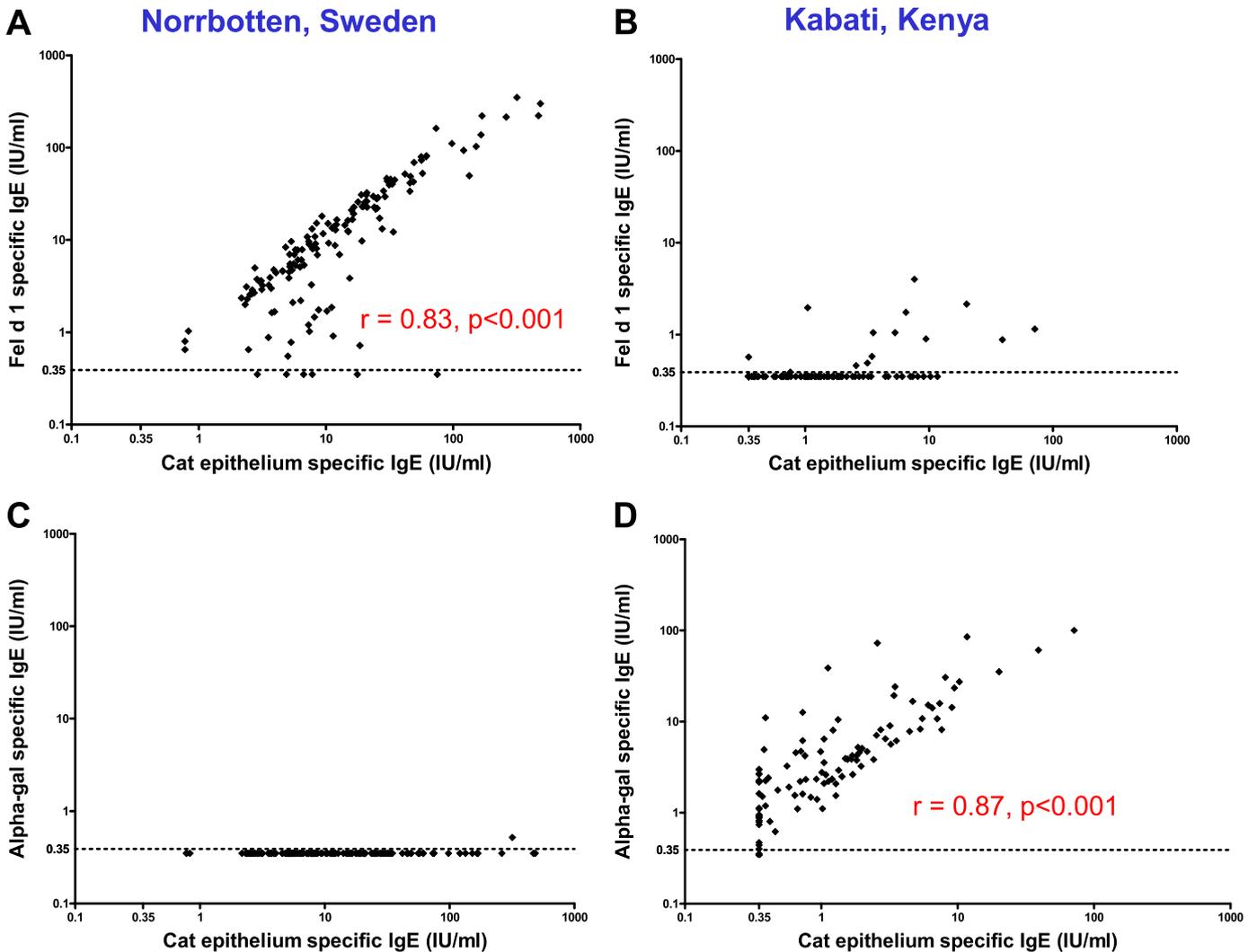
All subjects with IgE titers greater than 0.35 are included in the >0.35 group.

All values greater than 3.5 are in the >3.5 group.

Odds ratio (95% confidence interval) is that for asthma compared with control subjects.

antibody on asthma in Virginia and Sweden may help to explain the finding that low-level sensitization to mite in developing countries shows little relationship to allergic symptoms or asthma.

In a recent publication, we reported that the IgE antibody response to  $\alpha$ -gal is primarily induced by tick bites, specifically those of the species *A. americanum* (the Lone Star tick)



**Figure 4.** The meaning of a positive IgE antibody assay to cat epithelium varies by geographic location. (A and C) In northern Sweden, IgE to cat epithelium is tightly correlated with having IgE antibodies to Fel d 1, not galactose- $\alpha$ -1,3-galactose ( $\alpha$ -gal). (B and D) The opposite is true in rural Kenya, where IgE to cat epithelium is correlated with having IgE antibodies to  $\alpha$ -gal, not Fel d 1.

**TABLE 5.  $\alpha$ -GAL IS UNDETECTABLE IN AIRBORNE SAMPLES INCLUDING HOMES WITH RESIDENT CATS AND DOGS**

Category	Sample ID	Collection Time (d)	Disturbance Sample	$\alpha$ -Gal* (ng/ml)	Fel d 1 (ng/ml)	Can f 1 (ng/ml)
Houses with a cat	1	1	No	<50	66	<50
	2	1	No	<50	782	<50
	3	1	No	<50	336	<50
	4	1	No	<50	133	<50
	5	1	No	<50	<50	<50
	6	1	No	<50	2,349	271
Houses with a dog	7	1	No	<50	<50	1,373
	8	1	Yes	<50	<50	334
	9	1	Yes	<50	<50	889
Houses with cat and dog	13	1	No	<50	8,267	62
	14	1	No	<50	142	<50
	15	4	No	<50	1,801	2341
	16	4	No	<50	3,156	11,978
Dorm rooms	n = 9	1	No	<50	<50	<50
Homes: no cat or dog	n = 6	1	No	<50	<50 <sup>†</sup>	<50

\*Expressed as the quantity of Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-BSA.

<sup>†</sup>One single-family home without a cat currently in residence had 114 ng/ml of airborne Fel d 1 detected.

in the southeastern United States (18). This is an example of a parasite-induced IgE response that is common in a developed country. In contrast, we cannot make a convincing case about the cause of IgE responses to  $\alpha$ -gal in Africa where children are exposed to many different parasites, including nematodes, cestodes, and a range of ectoparasites including ticks (13, 42). Previous studies have demonstrated that specific IgE antibodies that are pertinent to asthma can be correlated with total serum IgE (12, 43); here we have documented a specific IgE antibody response that is quantitatively related to total serum IgE, but not associated with asthma. Asthma is a heterogeneous disease state with multiple known factors influencing its development. Similarly, total serum IgE is now recognized to vary among individuals based on genetics alone (44). Our findings, taken together with those of genetic studies, suggest that consideration of specific sensitizations is more pertinent to understanding risk for asthma than is total serum IgE.

The results for the 18 year olds in Sweden provide a negative control for the effects of helminths or ticks, because these parasites are very rare in the inland area of Norrbotten. In addition, those results emphasize that IgE antibody responses to  $\alpha$ -gal are not induced by the oral route (e.g., eating red meat) or by any other form of contact with a domestic animal (e.g., living in a house with a cat). Thus, the Swedish data emphasize the importance of the form and route of exposure. In Virginia, this means exposure through the skin to the salivary proteins of an arthropod.

The primary objective of this report was to investigate the clinical relevance of IgE antibody responses to  $\alpha$ -gal. However, the results address several related questions. First, it is clear that presenting an antigen through the skin can induce high-titer IgE antibody responses in humans and that these responses can give rise to allergic symptoms after exposure by a different route (oral exposure to red meat, in this case). Second, the results provide a model for parasite-related IgE responses that can contribute to high serum IgE without having an effect on respiratory allergic symptoms. Third, within the present and other recent studies, there is a consistent relationship between the titer of IgE antibodies to inhaled allergens and the risk of asthma (12, 43). These findings clearly do not exclude the possibility of a noninhaled antigen associating with asthma. The observations that  $\alpha$ -gal is not airborne and having IgE antibodies to  $\alpha$ -gal does not increase the risk for

asthma, supports the view that those allergens most relevant to asthma pathogenesis are those that are inhaled.

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