

Impaired allergy diagnostics among parasite-infected patients caused by IgE antibodies to the carbohydrate epitope galactose- α 1,3-galactose

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Background: The carbohydrate epitope galactose- α 1,3-galactose (α -Gal) is abundantly expressed on nonprimate mammalian proteins. We have recently shown that α -Gal is responsible for the IgE binding to cat IgA, a newly identified cat allergen (Fel d 5).

Objective: We sought to investigate the diagnostic relevance of IgE antibodies to Fel d 5 and α -Gal among parasite-infected patients from central Africa without cat allergy compared with patients with cat allergy from the same region.

Methods: Sera from 47 parasite-infected patients and 31 patients with cat allergy were analyzed for total IgE and IgE antibodies against cat dander extract (CDE) by using the ImmunoCAP system. Inhibition assay was performed with α -Gal on solid phase-bound CDE. The presence of IgE specific for the major cat allergen Fel d 1, Fel d 5, and α -Gal was analyzed by means of ELISA.

Results: Among the 47 parasite-infected patients, 85% had IgE antibodies against α -Gal (OD; median, 0.175; range, 0.102–1.466) and 66% against Fel d 5 (OD; median, 0.13; range, 0.103–

1.285). Twenty-four of the parasite-infected patients were sensitized to CDE, and 21 of them had IgE antibodies to Fel d 5 and α -Gal. There was no correlation between IgE levels to CDE and rFel d 1 among the parasite-infected patients but a strong correlation between CDE and Fel d 5 and α -Gal ($P < .001$). Among the group with cat allergy, only 5 patients had IgE to α -Gal, and nearly 75% ($n = 23$) had IgE to rFel d 1 (median, 7.07 kU_A/L; range, 0.51–148.5 kU_A/L). In contrast, among the patients with cat allergy, there was a correlation between IgE levels to CDE and rFel d 1 ($P < .05$) but no correlation between CDE and Fel d 5 and α -Gal.

Conclusion: IgE to α -Gal causes impaired allergy diagnostics in parasite-infected patients. Screening for IgE to rFel d 1 and other allergens without carbohydrates might identify patients with true cat sensitization/allergy in parasite-infested areas. (J Allergy Clin Immunol 2011;■■■■:■■■■–■■■■.)

Key words: Galactose- α 1,3-galactose, cat IgA, Fel d 5, cat dander extract, cat allergy, parasitic infection

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Research on allergic disorders in Africa has been limited. The small number of studies performed have focused on the prevalence of IgE-mediated disease. Those studies have demonstrated an increase during the last decade, probably depending on urbanization.¹ There are almost no data on the sensitization profiles in African patients. The few reports have revealed a different pattern in African than in European patients.² Various reasons for this have been suggested, such as different allergen exposures and genetic cause.

The relationship between parasitic infections and allergic disease has been debated. The hygiene hypothesis has suggested that parasitic infections might be protective against the development of allergic disease.^{3,4} Other studies have shown that parasitic infection might contribute to the severity of the disease.^{5,6} The discrepancies could most likely be explained by the individual parasite load and type of infection.^{6,7}

There are IgE-binding epitopes that are shared between parasites and allergens.⁸ This might explain the different sensitization profile seen in developing countries, although only a few common epitopes have been found.⁸ Several carbohydrate epitopes have been shown to elicit cross-reactivity between different allergens.⁹ The knowledge of carbohydrate or glycan epitopes in parasitic disease has mostly been focused on *Schistosoma* species.¹⁰ However, the clinical effect of these cross-reactive carbohydrate determinants is debated.

Galactose- α 1,3-galactose (α -Gal) is a carbohydrate epitope that is abundantly expressed on glycoproteins of mammalian

Abbreviations used

α -Gal: Galactose- α 1,3-galactose
CDE: Cat dander extract
HSA: Human serum albumin

origin and produced by the enzyme α -1,3-galactosyltransferase, which in Old World monkeys and primates, including human subjects, is nonfunctional.¹¹ Species not expressing the α -Gal epitope produce large amounts of IgG antibodies to α -Gal because of constant antigenic stimulation by bacteria in the normal intestinal flora.¹² In fact, approximately 1% of all IgG antibodies in human subjects are believed to be directed against the α -Gal epitope.^{12,13} These antibodies have until recently constituted a barrier to pig xenografts because they mediate hyperacute rejection.^{14,15}

We have recently shown that α -Gal is responsible for the IgE binding to cat IgA, a newly identified allergen (Fel d 5) present in cat dander extract (CDE), which was recognized by nearly 40% of cat-sensitized patients from Sweden.^{16,17} This epitope was also found on cat IgM.¹⁷ This is the first mammalian carbohydrate IgE epitope described.

IgE antibodies to α -Gal were recently reported to cause severe anaphylactic reactions in patients treated with cetuximab, a chimeric mAb approved for use in patients with colorectal cancer and squamous cell carcinoma of the head and neck.^{18,19} The allergic complication seen after the treatment often occurred shortly after the first injection, suggesting pre-existing IgE antibodies.¹⁹ In addition, IgE antibodies to α -Gal have lately been shown to be related to severe food allergy.²⁰ Patients with IgE antibodies to α -Gal were reported to experience delayed symptoms of anaphylaxis, angioedema, or urticaria after eating red meat. These complications showed great variation depending on geographic location, which led to the suggestion that the cross-reactivity could originate from locally occurring biting insects or other parasites.¹⁹

Current diagnostics of cat allergy with CDE are well established. Characterization of CDE has thus far identified 6 allergenic molecules (Fel d 1-6), but the majority of patients (95%) have been reported to have IgE antibodies directed against the major cat allergen Fel d 1.²¹⁻²³ The dominance of Fel d 1 is also emphasized by the finding that it accounts for more than 60% of the total allergenic activity of cat dander.²³ Thus IgE to Fel d 1 seems to reflect genuine cat sensitization.

The aim of this study was to investigate the diagnostic relevance of IgE antibodies to Fel d 5/ α -Gal among parasite-infected patients from central Africa without cat allergy compared with that seen among patients with cat allergy from the same region.

METHODS**Study subjects**

Sera from a group of parasite-infected patients ($n = 47$) originating from rural Zimbabwe were included. The patients were given a diagnosis by a physician as being infected with schistosomiasis ($n = 20$), geohelminth infection ($n = 16$), or a combination ($n = 11$). The parasites causing geohelminth infection were *Ascaris lumbricoides* and *Trichuris trichiura*, along with the hookworms *Necator americanus* and *Ancylostoma duodenale*. The patients did not have a case history of cat allergy.

In addition, sera from 31 patients with cat allergy originating from Harare, Zimbabwe, were enrolled. The patients with cat allergy were given diagnoses

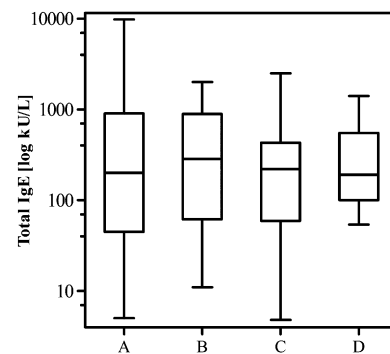


FIG 1. Total IgE levels among patients with cat allergy (A), *Schistosoma* species infection (B), geohelminth infection (C), or both *Schistosoma* species and geohelminth infection (D).

of cat allergy by a physician experienced in allergic diseases. They all had a history of allergic symptoms to cat and IgE antibody levels (>0.35 kU/L) to CDE.

Ethical consent

The study was approved by the local ethics committee of the University of Zimbabwe Medical School, Harare, Zimbabwe.

Extracts and antigens

Gal- α 1,3-Gal- β 1,4-GlcNAc (α -Gal) coupled to human serum albumin (HSA) was commercially obtained (Dextra Laboratories, Reading, United Kingdom). rFel d 1 and cat IgA were produced as previously described.^{24,25}

Determination of total and allergen-specific IgE reactivity

ImmunoCAP. Total IgE levels and IgE antibody reactivity to CDE were analyzed by using the ImmunoCAP System (Phadia AB, Uppsala, Sweden), according to the manufacturer's instructions. The cutoff for total IgE was 2 kU/L or greater, and that for allergen-specific IgE was 0.35 kU_A/L or greater.

ImmunoCAP inhibition. The capacity of α -Gal to inhibit IgE binding to solid phase-bound CDE was performed essentially as described for the ImmunoCAP System. However, a prior step of overnight preincubation of sera diluted 1:1 with α -Gal-HSA to a final concentration of 50 μ g/mL or diluted 1:1 with PBS with 1% BSA as a control was included.

ELISA. IgE binding to rFel d 1, cat IgA, and α -Gal was evaluated by means of indirect ELISA. Each serum was analyzed in duplicates, essentially as described previously.^{24,25} Briefly, 96-well microtiter plates (Nunc, Roskilde, Denmark) were coated with 0.5 μ g of antigen blocked with BSA followed by 100 μ L of serum diluted 1:2 in PBS with 1% BSA. IgE was detected by using rabbit anti-human IgE antibodies, followed by conjugated anti-rabbit antibodies and substrate. The absorbance was measured at 405 nm (ALP) or 450 nm (HRP). A negative reference pool for cat IgA and α -Gal was prepared from 6 healthy subjects without cat allergy. IgE values to cat IgA and α -Gal were considered positive when the IgE OD responses exceeded the mean + 2 SDs of the negative reference pool (OD, ≥ 0.100).

For quantification of IgE (in kilounits of allergen per liter) to rFel d 1 by means of ELISA, a reference curve was generated as previously described.²⁶ The cutoff was set to 0.35 kU_A/L.

Statistical analysis

Statistical analysis was performed with SPSS version 14.0 software (SPSS, Inc, Chicago, Ill). The Spearman rank correlation coefficient was used for correlation analysis. The Kruskal-Wallis test was used to analyze differences

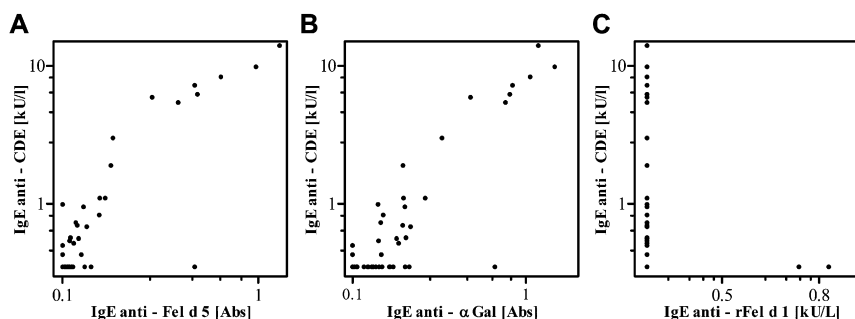


FIG 2. Parasite-infected patients ($n = 47$) showing a correlation between IgE antibody levels to CDE (y -axis) and Fel d 5 ($r = 0.75$, $P < .001$; **A**), α -Gal ($r = 0.715$, $P < .001$; **B**), and rFel d 1 ($r = -0.20$, $P > .05$; **C**; x -axis). *Abs*, Absorbance.

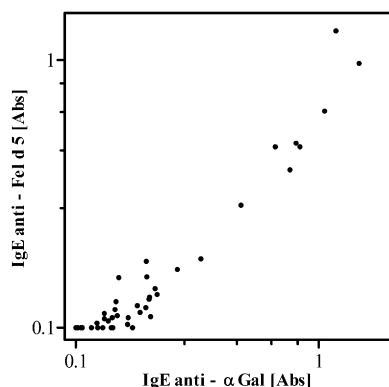


FIG 3. Parasite-infected patients ($n = 47$) showing significant correlation between IgE antibody levels to Fel d 5 (y -axis) and α -Gal (x -axis; $r = 0.94$, $P < .001$, $n = 47$). *Abs*, Absorbance.

in IgE levels between groups. A P value of less than .05 was considered significant.

RESULTS

Analysis of allergen-specific and total IgE antibodies

Total IgE. There were no differences in total IgE levels between patients with cat allergy (median, 200 kU/L; range, 5-9,800 kU/L) and parasite-infected patients (median, 220 kU/L; range, 4.8-2,500 kU/L; $P > .05$) or between the various types of parasitic infections ($P > .05$, Fig 1).

Parasite-infected patients. Of the 47 patients in the parasite-infected group, 40 (85%) had IgE antibodies against α -Gal (OD; median, 0.175; range, 0.102-1.466), and 31 (66%) had IgE antibodies to Fel d 5 (OD; median, 0.13; range, 0.103-1.285). Notably, 24 patients had IgE antibodies against CDE (median, 0.97 kU_A/L; range, 0.43-14 kU_A/L), whereas none of them displayed IgE to the major cat allergen rFel d 1. Twenty-one of the 24 CDE-sensitized patients showed IgE binding to Fel d 5 (OD; median, 0.155; range, 0.109-1.285), as well as to α -Gal (OD; median, 0.203; range, 0.141-1.466). Only 2 of 47 the parasite-infected patients had IgE to rFel d 1 (0.72 and 0.83 kU_A/L), and both had negative results for CDE.

There was a strong correlation between IgE responses to CDE on the one hand and Fel d 5 ($r_s = 0.75$, $P < .001$; Fig 2, A) and α -Gal ($r_s = 0.72$, $P < .001$; Fig 2, B) on the other. However, there

was no correlation between the IgE antibody levels to CDE and rFel d 1 (Fig 2, C). Furthermore, the IgE levels against Fel d 5 correlated significantly with the IgE levels against α -Gal ($r_s = 0.94$, $P < .001$, Fig 3).

Patients with cat allergy. Among the 31 patients with cat allergy, 23 (74%) had IgE antibodies to rFel d 1 (median, 7.07 kU_A/L; range, 0.51-148.5 kU_A/L). One patient had IgE antibodies to both Fel d 5 and rFel d 1. Two patients were monosensitized to Fel d 5 (OD; median, 0.149; range, 0.0911), and 2 were monosensitized to α -Gal (OD, median, 0.144; range, 0.408). Three patients had IgE to both Fel d 5 and α -Gal, and 1 of them had IgE to rFel d 1 as well (Fig 4).

There was no significant correlation between IgE antibody levels to CDE and Fel d 5 or between CDE and α -Gal (Fig 4, A and B). However, there was a strong correlation between IgE antibody levels to CDE and rFel d 1 ($r_s = 0.70$, $P < .001$; Fig 4, C).

ImmunoCAP inhibition assay

We investigated the nature of IgE binding to CDE by means of inhibition. α -Gal was preincubated with sera from 5 patients from the group with cat allergy (median, 1.86 kU_A/L; range, 1.18-36 kU_A/L to CDE) and 8 from the parasite-infected group (median 2.36 kU_A/L; range, 0.84-3.6 kU_A/L to CDE), and IgE responses to CDE ImmunoCAP were analyzed. No inhibition of IgE to CDE was observed in sera from patients with cat allergy, whereas the IgE reactivity to CDE was markedly inhibited (>80%) in 6 of 8 sera from the parasite-infected patients (Fig 5).

DISCUSSION

We here report that the majority of the 47 parasite-infected patients (85%) without reported symptoms of cat allergy from Zimbabwe have IgE antibodies against α -Gal, and 66% have IgE antibodies against Fel d 5. However, half of these patients were sensitized to CDE, and 21 of these 24 patients had IgE to Fel d 5, as well as to α -Gal. Furthermore, none of the CDE-sensitized patients had IgE to the major cat allergen rFel d 1. On the contrary, among the patients with cat allergy recruited from the same region, nearly 75% had IgE against rFel d 1, and less than 20% had IgE against Fel d 5.

The results support that the α -Gal moiety on Fel d 5 is responsible for IgE anti- α -Gal reactivity to cat in parasite-infected patients. The strong correlation between the IgE antibody levels to CDE and α -Gal and Fel d 5, but not rFel d 1, shows

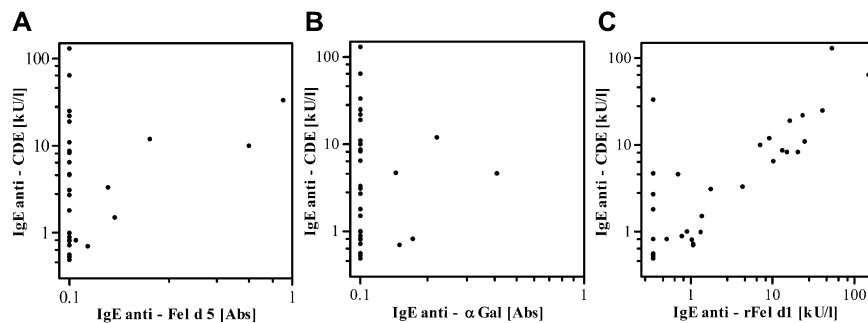


FIG 4. Patients with cat allergy ($n = 31$) showing correlation between IgE antibody levels to CDE (y -axis) and Fel d 5 ($r = 0.10$, $P > .05$; **A**), α -Gal ($r = -0.070$, $P > .05$; **B**), and rFel d 1 ($r = 0.70$, $P < .001$; **C**; x -axis). Abs, Absorbance.

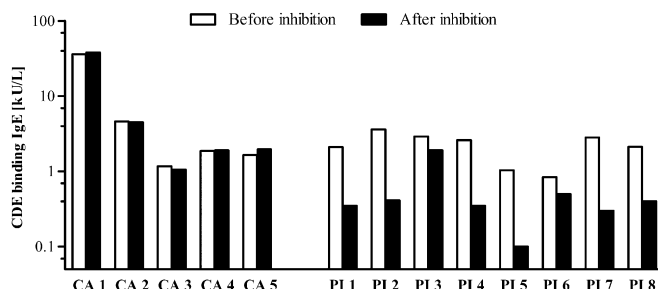


FIG 5. Competition of IgE responses to CDE among 5 patients with cat allergy (CA) and 8 parasite-infected patients (PI) by using ELISA. y -axis, OD before and after preincubation with α -Gal.

that Fel d 5 is responsible for the IgE binding to CDE in this group. Even though Fel d 5 contains α -Gal, the number of epitopes is lower in comparison with α -Gal-HSA. This explains the discrepancy in IgE binding to α -Gal and Fel d 5 among the parasite-infected patients, where a lower number of patients had positive results to Fel d 5 (66%) compared with α -Gal (85%).

Adsorption experiments with α -Gal were performed to confirm that the noted IgE reactivity to CDE was due to α -Gal. Preincubation of sera with α -Gal inhibited the IgE binding to CDE substantially among the 8 examined parasite-infected patients. In contrast, the IgE reactivity to CDE remained unchanged in sera from patients with cat allergy. This indicates that the α -Gal residue on Fel d 5¹⁶ is the common epitope causing the IgE responses to cat dander in the parasite-infected patients but not among the examined patients with cat allergy. In the latter group other allergens in cat dander than Fel d 5 are probably responsible for the IgE binding. Thus the common α -Gal epitope might cause false-positive IgE reactivity to cat dander.

Sensitization to Fel d 1 is reported in 80% to 95% of cat dander-sensitized European subjects,^{21,22} and Fel d 1 is considered to be a marker of genuine cat sensitization. This is in line with our findings showing that nearly 75% of the patients with cat allergy from Africa had IgE antibodies to rFel d 1. Only 6 (19%) patients in this group had IgE against Fel d 5 compared with 66% among the parasite-infected patients, indicating that Fel d 5 is not a good marker for cat allergy. Yet 3 of the 6 Fel d 5-sensitized patients among the patients with cat allergy were likely genuinely sensitized to protein epitopes on Fel d 5 because they had no IgE antibodies to α -Gal. The highest IgE level to Fel d 5 was also found in one of these 3 patients with cat allergy, and this patient also lacked IgE antibodies to rFel d

1 (Fig 4, A and B). On the other hand, 2 patients with cat allergy had IgE antibodies to α -Gal and not to Fel d 5, indicating that they, in addition to cat allergy, could have a parasitic infection. We also noted that 2 of the parasite-infected patients had IgE to rFel d 1 but lacked IgE to CDE. The difference in results probably reflects a too low amount of Fel d 1 on the CDE ImmunoCAP, which was previously demonstrated.²⁷

Perzanowski et al²⁸ have reported the finding of IgE antibodies to CDE among children from a rural village in Kenya without positive skin test responses. The children did not have high exposure to cat by case history, they lacked IgG to cat, and the apparent IgE antibodies could not be inhibited by CDE. In line with these findings, Calvert and Burney²⁹ recently showed that there was no significant relationship between IgE and positive skin prick test responses to cat among children participating in a cross-sectional survey from South Africa. More than 30% (224/703) of the children who had negative skin prick test responses to CDE had IgE antibodies to cat. The findings were in contrast to the results from IgE analyses to mites, timothy grass, and *Aspergillus* species, where the authors found a strong association with positive skin prick test responses. These observed “false-positive” responses to CDE could be due to IgE antibodies to the α -Gal moiety on Fel d 5.

The clinical relevance of IgE antibodies to α -Gal is still unclear. The studies by Commins and Platts-Mills^{20,30} have shown that they are associated with severe allergic reactions. However, Jacquet et al³¹ have lately reported on a patient with IgE antibodies to beef and pork and low IgE levels to α -Gal, in whom an allergic reaction was not observed after each meal containing meat. With respect to the parasite-infected patients studied, they did not report any severe allergic reactions. Thus the question remains why IgE antibodies to α -Gal cause symptoms in some cases and not in others. Nevertheless, our study supports the fact that parasitic infections might induce IgE antibodies to α -Gal. Previous studies have put forward tick bites as a possible cause.^{31,32} However, the mechanisms by which tick bites or parasites induce α -Gal-specific IgE still needs to be elucidated.

There are differences beyond parasitic infection between the populations of rural Zimbabwe versus the population with cat allergy in urban Harare. The rural population is exposed to livestock and infections other than parasites compared with the inhabitants of Harare. The socioeconomic level of the rural population is lower, and important lifestyle factors, such as smoking and eating habits, differ,^{6,28} all of which have an effect on the incidence of illnesses, including allergic disease.

In conclusion, we show that IgE reactivity to the carbohydrate epitope α -Gal causes impaired allergy diagnostics to cat allergy. This epitope is found on Fel d 5, as well as on parasites. One way to decipher the problem of carbohydrate cross-reactivity is to use single recombinant allergens, including rFel d 1, Fel d 5, and α -Gal, in the diagnosis of cat allergy. Whether component-resolved diagnosis will provide more correct information of other allergens without carbohydrates in parasite-infested areas remains to be investigated.

Clinical implications: IgE antibodies to the carbohydrate epitope α -Gal cause impaired allergy diagnostics.

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