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Use of a chimeric ELISA to investigate immunoglobulin E antibody responses to Der p 1 and Der p 2 in mite-allergic patients with asthma, wheezing and/or rhinitis

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Summary

Background Sensitization to indoor allergens, particularly to dust mites, is a strong risk factor for asthma in children and adults. Assessment of sensitization is carried out using *in vivo* and *in vitro* tests to detect specific IgE antibodies.

Objective To investigate IgE antibody responses to mites in patients with asthma, wheezing and/or rhinitis, using chimeric ELISA to measure specific IgE antibodies to mite allergens Der p 1 and Der p 2.

Methods Specific IgE antibodies to Der p 1 and Der p 2 were quantified by chimeric ELISA, and compared with IgE to *Dermatophagoides pteronyssinus* (Dpt) measured using the CAP system (Pharmacia). A panel of sera from 212 patients with asthma, wheezing and/or rhinitis and 11 controls was analysed.

Results There was a significant correlation between IgE to Dpt measured by CAP and IgE to Der p 1 ($r = 0.81$, $P < 0.001$), Der p 2 ($r = 0.79$, $P < 0.001$) and combined Der p 1 and Der p 2 ($r = 0.86$, $P < 0.001$). Seventy per cent of all patients had IgE to Dpt, and of those, 76.5% had IgE to Der p 1, 79.2% had IgE to Der p 2 and 83.1% had IgE to Der p 1 and Der p 2 combined. Considering the cut-off level of 2 IU/mL of IgE to either Der p 1 or Der p 2, the predictive value for a positive IgE to Dpt by CAP was greater than 95%.

Conclusions The chimeric ELISA allowed accurate quantification of IgE antibodies to Dpt allergens Der p 1 and Der p 2, and it could be useful for studying immune responses to mites in patients with asthma and/or rhinitis.

Keywords asthma, CAP system, chimeric ELISA, Der p 1, Der p 2, *Dermatophagoides pteronyssinus*, IgE antibody, mites

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Introduction

Asthma is the most common chronic disease in childhood. It has been well established that asthma is an inflammatory disease and that it is most frequently associated with immediate hypersensitivity to perennial indoor allergens [1]. Approximately 80–90% of children with asthma are sensitized to indoor allergens, indicating the presence of specific IgE [2, 3]. Studies using the ISAAC questionnaire have shown that, in Brazil, 18–27% of children aged 13 to 14 years presented asthma symptoms in the past 12 months, and 31.7% had allergic rhinitis [4, 5]. Exposure to allergens, particularly dust mites, cockroach, cat, dog and *Alternaria* is an important risk factor for sensitization and development of asthma in children and adults [1, 6–9]. We have previously reported that 84% and 55% of children with asthma and/or rhinitis in Brazil were sensitized to mites and cockroach, respectively [10].

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Detection of IgE antibodies is essential to confirm the diagnosis of allergy, identify specific sensitivities and guide treatment. Although skin testing is a major method for identifying allergen-specific IgE, it depends on consistent technique during execution and it is limited by the quality of the allergen extract used, skin reactivity and risk of anaphylaxis. In addition, some medications can suppress positivity of skin tests [11]. Alternatively, allergen-specific serum IgE can be measured by *in vitro* methods [12–19], including the classic radioallergosorbent test (RAST) originally described in 1967 [20], which requires utilization of radioactive reagents. Results of methods previously used for assaying IgE antibodies, including RAST, were expressed in arbitrary units and did not accurately determine the levels of IgE antibody. Quantitative IgE measurements can be carried out using a fully automated solid phase enzyme fluoroimmunoassay, the CAP system [21]. Calibration of the CAP system against WHO standard reagent for IgE allows expression of measurements in international units. It is currently accepted that the available methods for measuring specific IgE to common inhalant allergens provide results which correlate well with skin tests [11].

IgE antibodies to Der p 1 and Der p 2 have been reported to be present in 80–90% of mite-allergic patients [22–26]. Absolute amounts of specific IgE antibodies to individual allergens can be determined by chimeric ELISA. This method uses a chimeric antibody developed against the mite allergen Der p 2 to set up a control curve. This antibody consists of the heavy-chain variable domains and the light chains of murine monoclonal antibody 2B12 [27], and the heavy chain constant domains of human IgE (2B12-IgE) [28, 29]. The use of a chimeric ELISA to measure Fel d 1 specific IgE antibodies allowed accurate diagnosis of cat allergy, and showed comparable sensitivity with the IgE by CAP system [30]. A chimeric IgG1 antibody to the NP hapten has been previously used as a reference reagent for calibrating measurements of antibodies to *H. influenzae* type B [31]. However, the chimeric mouse/human IgE anti-Der p 2 antibody is the only chimeric, allergen-specific IgE antibody that has been reported [28].

The aim of the present study was to assess levels of Der p 1 and Der p 2 specific IgE antibodies in order to investigate the role of these allergens in the immunological response to mites. We have studied IgE-mediated immune responses in patients with asthma, wheezing and/or rhinitis by using the chimeric ELISA for the measurement of specific IgE to mite allergens, and have compared the results with those obtained by using a fluoroenzymeimmunoassay (CAP system).

Methods

Subjects

A panel of sera from 212 patients (196 children aged 1 month to 16 years and 16 adults) and 11 controls was analysed. One hundred and twenty-nine sera were from patients with asthma and/or rhinitis, selected from those attending the Pediatric Allergy Clinics at the School of Medicine of Ribeirão Preto, School of Medicine of São José do Rio Preto and Paulista School of Medicine, São Paulo, Brazil. Eighty-three sera were from patients participating in a study of risk factors for acute wheezing in children. These patients were seen at the emergency room of the Clinical Hospital, School of Medicine of Ribeirão Preto, and Santa Lydia Hospital, and required inhaled β_2-agonist for treatment of acute wheezing. Controls were 11 non-allergic non-asthmatic individuals with negative skin test and serum IgE to *Dermatophagoides pteronyssinus* (Dpt). The study was approved by the ethical committees of all the participating institutions. All patients or their guardians provided informed consent to participate in the study.

Specific IgE to D. pteronyssinus

Serum IgE antibodies to Dpt were determined using the CAP system (Pharmacia), according to the manufacturer's instructions.

Chimeric ELISA for Der p1 and Der p 2 specific IgE

Specific IgE antibodies to Der p 1 and Der p 2 were measured using a chimeric ELISA, as previously described [30]. Briefly, microtitre plates were coated with 1 μg/well of monoclonal antibodies 5H8 (anti-Der p 1) or anti-DpX (anti-Der p 2), overnight at 4 °C. For the 'combined' ELISA for Der p 1 and Der p 2, both antibodies were used at the same time to coat the plates, at similar concentrations. After washing, plates were blocked with 1% BSA-PBS Tween, and incubated for 1 h with 0.1 mL of dilutions of a Dpt extract (Bayer, Spokane, USA) containing 0.5 μg/mL Der p 1 and/or Der p 2. Plates were washed, sera were added at 1:10 dilution and incubated for 2 h. Biotinylated goat anti-human IgE (1:4000) was added for 1 h, followed by incubation with streptavidin-peroxidase (1:1000). The reaction was developed using 1 mmol 2, 2′-azino-bis (3-ethylbenzthiazoline-6 sulphonic acid) (Sigma, St Louis, MO, USA) and H_2O_2, and optical densities were measured at 405 nm.

The ELISA for IgE to mite allergens was quantified using a chimeric mouse Fab/human Fc epsilon antibody. This antibody was engineered from the V_H domains and light chains of a murine mAb to Der p 2 (clone 2B12) and the heavy chain domains of human IgE. Dilutions of 2B12-IgE from 0.14 to 140 ng/mL were used to construct a control curve, using anti-Der p 2 mAb (aDpX) to coat microtitre plates, and Dpt extract (10 000 AU/mL, Bayer Co., Spokane, WA, USA) as a source of Der p 2. The extract was diluted to contain 500 ng/mL Der p 2 for use in the ELISA. The bound chimeric 2B12-IgE was detected with biotinylated antihuman IgE and streptavidin-peroxidase, as described above.

Statistical analysis

The Mann–Whitney test was used to assess differences in total IgE levels. IgE antibodies to Dpt and to Der p 1 and Der p 2 were compared by linear regression analysis, using GraphPad Prism. ROC analysis was performed to obtain the cut-off level of 2 IU/mL for IgE to Der p 1 and Der p 2, using StatsDirect. Undetectable levels of specific IgE by chimeric ELISA were assigned a value of 0.4 IU/mL to allow statistical analysis.

Results

A schematic representation of the chimeric ELISA is shown in Fig. 1(a). In order to validate the assay, we have performed IgE antibody measurements using sera from seven individual patients and a serum pool of mite-allergic patients (UVA 94/03), at different dilutions. The results showed that curves obtained using individual sera or mite serum pool were parallel to that of the reference preparation, for the ELISA for IgE to Der p 1, IgE to Der p 2 or IgE to combined Der p 1 and Der p 2 (Figs 1b, c, d, respectively).

We compared IgE responses to Der p 1, Der p 2, and combined Der p 1 and Der p 2, quantified by chimeric ELISA, in a group of 212 patients with asthma, wheezing and/or rhinitis. There was a significant correlation between Dpt IgE measured by CAP and IgE to Der p 1 ($r = 0.81$, $P < 0.001$), Der p 2 ($r = 0.79$, $P < 0.001$) and combined Der p 1 and Der p 2 ($r = 0.86$, $P < 0.001$) in the group of patients with detectable IgE to Dpt by CAP ($n = 149$) (Figs 2a, b, c). Of the 149 patients with IgE to Dpt by CAP, 114 (76.5%), 118 (79.2%) and 123 (83.1%) had IgE antibodies to Der p 1, Der p 2, and combined Der p 1 and Der p 2, respectively. Of the 63 patients who had undetectable IgE to Dpt by CAP, 14 (22.2%), 21 (33.3%) and 12 (19%) had IgE antibodies to Der p 1, Der p 2, and combined Der p 1 and Der p 2, respectively. However, the levels of IgE antibodies were much lower than those measured in sera from patients with

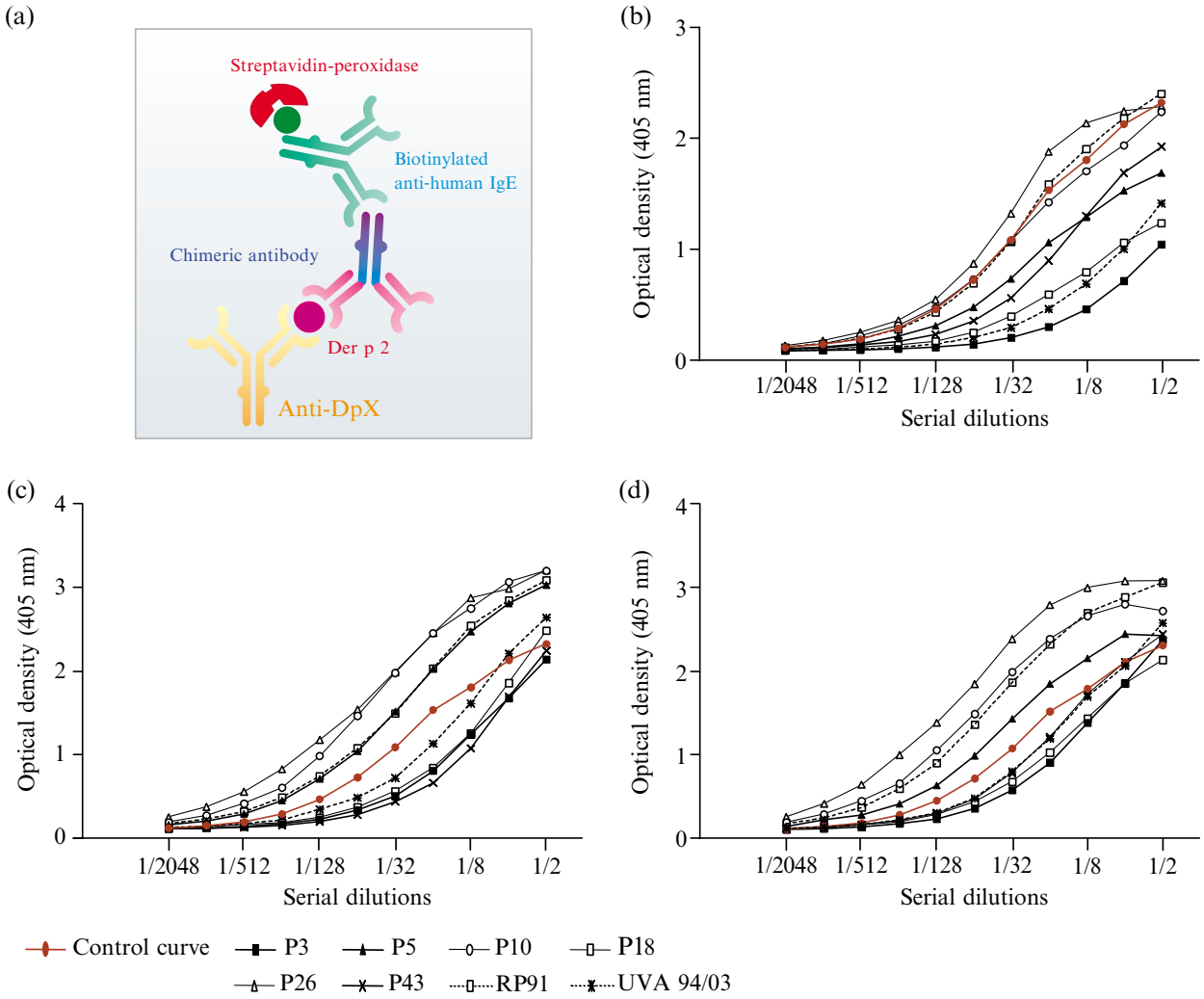

Fig. 1. Schematic representation of the chimeric ELISA (a) and assay curves constructed with the reference standard, serial twofold dilutions of sera from seven individual patients (identification numbers P3, P10, P18, P28, P26, P43 and RP91), and a serum pool from mite-allergic patients (UVA 94/03), in the ELISA for IgE to Der p 1, IgE to Der p 2 or IgE to combined Der p 1 and Der p 2 (b, c and d, respectively). Curves obtained using individual sera or mite serum pool were parallel to that of the reference preparation.

detectable IgE to Dpt, ranging from < 0.4 IU/mL to 8.8 IU/mL (Table 1).

Considering the CAP system as a reference method, we determined predictive values of the chimeric ELISA including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). As shown in Table 2, higher sensitivity, specificity, PPV and NPV were obtained using the combined Der p 1 and Der p 2 assay, as compared with Der p 1 or Der p 2 alone. In addition, the performance characteristics of using a level of 2 IU/mL of IgE to Der p 1, Der p 2 or both by chimeric ELISA, in predicting a positive measurement of IgE to Dpt by CAP, are shown (Table 2). The cut-off level of 2 IU/mL has been defined by ROC analysis [32]. Using a 2-IU/mL cut-off level, the positive predictive values for Der p 1, Der p 2 or both ranged from 95% to 98%. As expected, the sensitivities were lower, indicating that this cut-off level should only be used to predict a positive IgE to Dpt by CAP, and not as a means of excluding mite allergy.

Comparison of IgE levels to Dpt by CAP and IgE to combined Der p 1 and Der p 2 by chimeric ELISA is shown in Fig. 3. Most patients with CAP classes equal or greater than 4, considered very high levels of specific IgE, had levels of IgE to Der p 1 and Der p 2 greater than 2 IU/mL.

Discussion

In the present study, we have used a chimeric ELISA to measure IgE antibodies to the major mite allergens Der p 1 and Der p 2. In keeping with previously reported data using similar methods for measuring IgE to the major cat allergen Fel d 1 [30], our results revealed that the chimeric ELISA is suitable for studying IgE responses to mites. Using a combination of monoclonal antibodies to Der p 1 and Der p 2 as coating antibodies and a chimeric anti-Der p 2 antibody to establish a control curve, it was possible to accurately quantify levels of specific IgE to these allergens in IU/mL in a single assay. Chimeric ELISA results showed a good correlation with measurements of IgE to Dpt done by the CAP system, indicating that reactivity to these allergens is found in most mite-allergic patients.

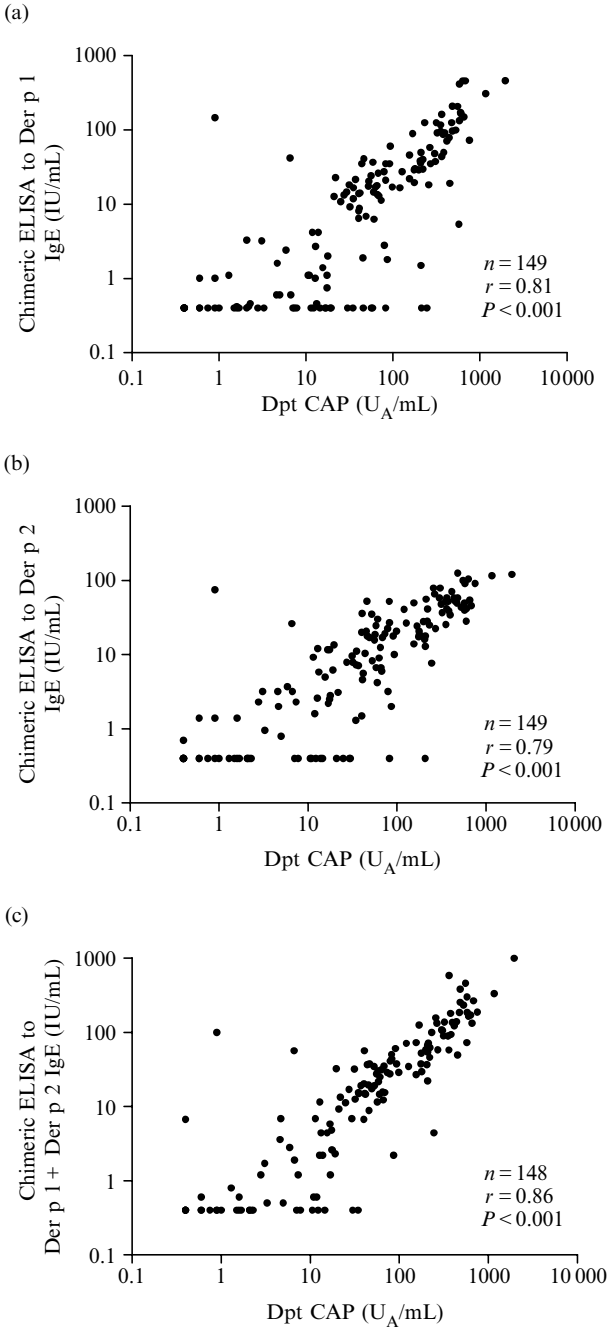

Fig. 2. Correlations of CAP system and chimeric ELISA results. A significant correlation was found of levels of IgE antibodies to *D.pteronyssinus* measured using the CAP system, as compared with IgE antibodies to Der p 1 (a), Der p 2 (b) and combined assay for Der p 1 and Der p 2 (c), quantified by chimeric ELISA. Undetectable levels of specific IgE by chimeric ELISA were assigned a value of 0.4 IU/mL to allow for statistical analysis.

In agreement with previous observations, approximately 20% of our patients had IgE to Dpt, and no detectable IgE to either Der p 1 or Der p 2. At least 13 groups of allergens from *Dermatophagoides* ssp. mites have been reported, including the major Group 1 and Group 2 allergens [9, 33]. It is possible that reactivity to other mite allergens could account for the observed positivity to *Dermatophagoides pteronyssinus.*

We have found that a proportion of patients with undetectable IgE to Dpt by CAP had IgE antibodies to Der p 1 and Der p 2 by chimeric ELISA (Table 1). One possible explanation would be that high total IgE levels could have interfered by increasing background measurements and therefore causing false-positive results. Total IgE levels were significantly higher in the group of CAP positive/chimeric ELISA positive patients, than in the group of CAP negative/chimeric ELISA positive patients (geometric means 522 kU/L and 40 kU/L, respectively, $P < 0.001$, Mann–Whitney test, data not shown). In addition, analysis of 10 sera from non-allergic children aged 3 to 6 years with current *Ascaris* infection, living in north-east Brazil, with total IgE levels ranging from 881 kU/L to 13 300 kU/L (geometric mean 2713 kU/L) revealed that 9/10 patients had undetectable levels of IgE to Der p 1 and Der p 2 by chimeric ELISA, and only one patient had a level of 1.5 IU/mL of specific IgE to these allergens (data not shown). At present, the significance of the presence of low levels of IgE antibodies to Der p 1 and Der p 2 in patients with undetectable IgE to Dpt by CAP is not clear. Taken together, our results suggest that interference of high total IgE levels within the assay seems unlikely.

Table 1. Analysis of IgE antibodies to Der p 1, Der p 2 and combined Der p 1 and Der p 2 using chimeric ELISA and IgE to *D.pteronyssinus* by CAP system

CAP system	Chimeric ELISA		
	Der p 1	Der p 2	Der p 1 + Der p 2
CAP positive (class $\geq$ 1)			
n	149	149	148
Positive sera (%)	76.5%	79.2%	83.1%
Range (IU/mL)	0.4–462.5	0.4–125.8	0.4–996.7
CAP negative (class = 0)			
n	63	63	63
Positive sera (%)	22.2%	33.3%	19%
Range (IU/mL)	0.4–8.8	0.4–5.6	0.4–4.6

Table 2. Performance characteristics of using the assay detection limit of 0.4 IU/mL and the cut-off level of 2 IU/mL* of IgE to Der p 1, Der p 2 or both by chimeric ELISA, in predicting a positive measurement of IgE to Dpt by CAP

	Sensitivity	Specificity	PPV	NPV
Der p 1				
$\geq$ 0.4	0.77 (0.69–0.83)	0.78 (0.66–0.87)	0.89 (0.82–0.94)	0.58 (0.47–0.69)
$\geq$ 2	0.63 (0.55–0.71)	0.92 (0.82–0.97)	0.95 (0.87–0.98)	0.51 (0.43–0.61)
Der p 2				
$\geq$ 0.4	0.79 (0.72–0.85)	0.67 (0.53–0.78)	0.85 (0.78–0.90)	0.58 (0.47–0.69)
$\geq$ 2	0.73 (0.65–0.80)	0.92 (0.82–0.97)	0.96 (0.90–0.99)	0.59 (0.49–0.69)
Der p 1 + 2				
$\geq$ 0.4	0.83 (0.76–0.89)	0.81 (0.69–0.90)	0.91 (0.85–0.95)	0.67 (0.55–0.78)
$\geq$ 2	0.75 (0.67–0.82)	0.95 (0.82–0.99)	0.98 (0.94–0.99)	0.5 (0.38–0.62)

PPV = positive predictive value; NPV = negative predictive value. Ninety-five per cent confidence limits are indicated in parenthesis. *Determined by ROC analysis [32].

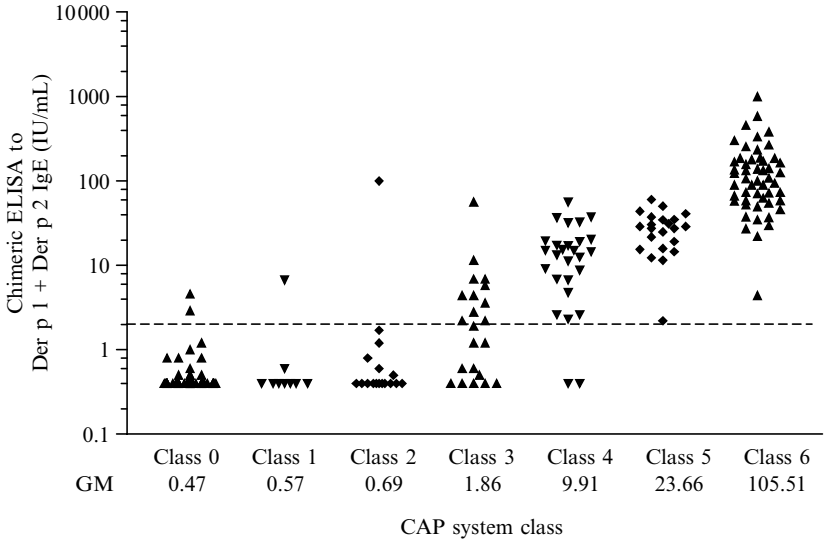

Fig. 3. Comparison of IgE levels to Dpt by CAP and IgE to combined Der p 1 and Der p 2 by chimeric ELISA. Most patients with CAP classes equal or greater than 4, considered very high levels of specific IgE, had levels of IgE to Der p 1 and Der p 2 greater than 2 IU/mL. The dotted line indicates the cut-off level of 2 IU/mL of IgE to Der p 1 and Der p 2.

Recently, an ELISA method for measuring IgE to Der p 2 has been reported, which was shown to be sensitive and specific [26]. In adult patients with asthma, IgE to Der p 2 was detected in 70.8% of patients with positive skin tests to *D. pteronyssinus*. Although this method includes similar steps to those assays we have used, the results were obtained in ELISA units/mL. In addition, IgE binding to recombinant allergens coupled to solid phase in the CAP system has been evaluated. Results for mite and *Aspergillus* allergen Asp f 1 have indicated that measurements of IgE to few recombinant allergens may be sufficient for *in vitro* diagnosis of allergy [34, 35].

In conclusion, we have established that the chimeric ELISA allowed for accurate quantification of IgE antibodies to mite allergens Der p 1 and Der p 2. The chimeric ELISA enables investigators to easily measure IgE to specific mite (Der p 1, Der p 2) or other allergens in large clinical or epidemiological studies. Levels of IgE to major allergens could be used in research studies to monitor effects of environmental control measures or allergen immunotherapy on specific IgE levels over time. Methods that use unpurified natural allergen extracts, including CAP, would not provide comparable detailed analysis. More extensive studies, involving different groups of patients, will be necessary to establish whether the chimeric ELISA system will ultimately be useful for diagnostic purposes. In particular, levels greater than 2 UI/mL of IgE to Der p 1 and Der p 2 (established by ROC analysis) in our study were associated with specificity of 95% for the presence of IgE to Dpt, suggesting that this assay could be useful for diagnosis of mite allergy.

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References

1 Busse WW, Lemanske RF. Asthma. N Engl J Med 2001; 344:350–62.
2 Gern JE, Lemanske RF, Busse WW. Early life origins of asthma. J Clin Invest 1999; 104:837–43.
3 Platts-Mills TA, Rakes G, Heymann PW. The relevance of allergen exposure to the development of asthma in childhood. J Allergy Clin Immunol 2000; 105:S503–8.
4 The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic conjunctivitis, and atopic eczema. ISAAC. Lancet 1998; 351:1225–32.
5 Vanna AT, Arruda LK, Naspitz CK, Solé D. International study of asthma and allergies in childhood: validation of the rhinitis symptom questionnaire and prevalence of rhinitis in schoolchildren in São Paulo, Brazil. Pediatr Allergy Immunol 2001; 12:95–101.
6 Nelson HS, Szefler SJ, Jacobs J, Huss K, Shapiro G, Sternberg AL. The relationships among environmental allergen sensitization, allergen exposure, pulmonary function, and bronchial hyperresponssiveness in the Childhood Asthma Management Program. J Allergy Clin Immunol 1999; 104:775–85.
7 Eggleston AP, Arruda LK. Ecology and elimination of cockroaches and allergens in the home. J Allergy Clin Immunol 2001; 107: S422–9.
8 Sporik R, Platts-Mills TA. Allergen exposure and the development of asthma. Thorax 2001, 56:II58–63.
9 Platts-Mills TAE, Vervloet D, Thomas WR, Aalberse R, Chapman MD. Indoor allergens and asthma: Report of the Third International Workshop. J Allergy Clin Immunol 1997; 100: S2–24.
10 Santos ABR, Chapman MD, Aalberse RC et al. Cockroach allergens and asthma in Brazil: identification of tropomyosin as a major allergen with potential cross-reactivity with mite and shrimp allergens. J Allergy Clin Immunol 1999; 104:329–37.
11 Yunginger JW, Ahlstedt S, Eggleston PA et al. Quantitative IgE antibody assays in allergic diseases. J Allergy Clin Immunol 2000; 105:1077–84.
12 Hamilton RG. Laboratory analysis in the diagnosis of human allergic disease. *Methods: a Companion to Meth Enzymol* 1997; 13:25–32.
13 Plebani M, Bernardi D, Basso D, Borghesan F, Faggian D. Measurement of specific immunoglobulin E. intermethod comparison and standardization. Clin Chem 1998; 44:1974–9.

14 Witteman AM, Stapel SO, Perdok GJ et al. The relationship between RAST and skin test results in patients with asthma or rhinitis: a quantitative study with purified major allergens. J Allergy Clin Immunol 1996; 97:16–25.
15 Wood RA, Phipatanakul W, Hamilton RG, Eggleston PA. A comparison of skin prick tests, intradermal skin tests, and RASTs in the diagnosis of cat allergy. J Allergy Clin Immunol 1999; 103:773–9.
16 Kadooka Y, Idota T, Gunji H et al. A method for measuring specific IgE in sera by direct ELISA without interference by IgG competition or IgG autoantibodies to IgE. Int Arch Allergy Immunol 2000; 122:264–9.
17 Lasley MV, Shapiro GG. Testing for allergy. Pediatrics Rev 2000; 21:39–43.
18 Martins TB, Litwin CM, Jaskowski TD, Hill HR. Comparison of an inhalant allergy immunoassay with allergen specific IgE determinations in human serum. J Clin Laboratory Anal 1998; 12:213–7.
19 Corey JP, Mamikoglu B, Akbar I, Houser SM, Gungor A. ImmunoCAP and HYTEC enzyme immunoassay in the detection of allergen-specific IgE compared with serial skin end-point titration by receiver operating characteristic analysis. Otolaryngol Head Neck Surg 2000; 122:64–70.
20 Wide L, Bennich H, Johansson SGO. Diagnosis of allergy by an in-vitro test for allergen antibodies. Lancet 1967; 2:1105–7.
21 Bousquet J, Chanez P, Chanal I, Michel FB. Comparison between RAST and Pharmacia CAP system: a new automated specific IgE assay. J Allergy Clin Immunol 1990; 85:1039–43.
22 Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TA. Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (*Dermatophagoides* ssp) J Allergy Clin Immunol 1989; 83:1055–67.
23 Tovey ER, Chapman MD, Platts-Mills TA. Mite faeces are a major source of house dust allergens. Nature 1981; 289:592–3.
24 Mueller GA, Benjamin DC, Rule GS. Tertiary structure of the major house dust mite allergen Der p 2: sequential and structural homologies. Biochemistry 1998; 37:12707–13.
25 O'Brien RM, Thomas WR. Immune reactivity to Der p I and Der p II in house dust-sensitive patients attending paediatric and adult allergy clinics. Clin Exp Allergy 1994; 24:737–42.
26 Silva DAO, Gervásio AM, Sopelete MC et al. A sensitive reverse ELISA for the measurement of specific IgE to Der p 2, a major *Dermatophagoides pteronyssinus* allergen. Ann Allergy Asthma Immunol 2001; 86:545–50.
27 Ovsyannikova IG, Vailes LD, Li Y, Heymann PW, Chapman MD. Monoclonal antibodies to group II *Dermatophagoides* ssp. allergens: murine immune response, epitope analysis, and development of a two-site ELISA. J Allergy Clin Immunol 1994; 94:537–46.
28 Schuurman J, Lourens TE, Perdok GJ, Parren PW, Aalberse RC. Mouse/human chimeric IgE antibodies directed to the house dust mite allergen Der p 2. Int Arch Allergy Immunol 1995; 107:465–6.
29 Schuurman J, Perdok GJ, Lourens TE, Parren PW, Chapman MD, Aalberse RC. Production of a mouse/human chimeric IgE monoclonal antibody to the house mite allergen Der p 2 and its use for the absolute quantification of allergen-specific IgE. J Allergy Clin Immunol 1997; 99:545–50.
30 Ichikawa K, Iwasaki E, Baba M, Chapman MD. High prevalence of sensitization to cat allergen among Japanese children with asthma, living without cats. Clin Exp Allergy 1999; 29:754–61.
31 Halmilton RG. Application of engineered chimeric antibodies to the calibration of human antibody standards. Ann Biol Clin (Paris) 1991; 49:242–8.
32 Metz CE. Basic principles of ROC analysis. Seminars Nucl Med 1978; 8:283–98.
33 Arlian LG, Platts-Mills TAE. The biology of dust mite and the remediation of mite allergens in allergic disease. J Allergy Clin Immunol 2001; 107:S406–13.
34 Johansson E, Eriksson TLJ, Olsson S et al. Evaluation of specific IgE to the recombinant group 2 mite allergens Lep d 2 and Tyr p 2 in the CAP system. Int Arch Allergy Immunol 1999; 120:43–9.
35 Crameri R, Lidholm J, Grönlund H, Stüber D, Blaser K, Menz G. Automated specific IgE assay with recombinant allergens: evaluation of the recombinant *Aspergillus fumigatus* allergen I in the Pharmacia CAP system. Clin Exp Allergy 1996; 26:1411–9.