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Original Article

Specific IgE density assay: A new reverse enzyme allergosorbent test-based procedure for the quantitative detection of allergen-specific IgE

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Abstract

A new method is described for the quantitative detection of IgE antibodies, based on IgE capture with a specific antibody, reaction with liquid-biotinylated allergens and biotinylated anti-IgE and immunoenzymatic development of the reaction (reverse enzyme allergosorbent test). Using a reference system based on the World Health Organization IgE International standard, this method determines total IgE in the range 2–100kU/L and specific IgE in the range 0.2–100 kU/L, from which the specific/total ratio, called 'specific IgE density', can be calculated. This procedure has been applied to the study of specific IgE in 23 sera from patients polysensitized to pollen and mite allergens: 11 with asthma and 12 with rhinitis. The sensitivity and reproducibility of the method were evaluted. Sera from asthmatic patients showed higher cumulative levels of specific IgE (mean density 57.7%) than sera from rhinitic patients (mean density 32.6%). The clinical significance of specific IgE density in patients with multiple sensitizations is discussed.

Key words: allergens, IgE density, radioallergosorbent test, reverse enzyme allergosorbent test, specific IgE, total IgE.

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Introduction

When atopic individuals are exposed to environmental antigens, pathologic overproduction of allergen-specific IgE may arise, causing allergic diseases, such as rhinitis, conjunctivitis, asthma, atopic dermatitis and anaphylaxis. The diagnosis of allergic sensitization is based on skin testing or *in vitro* detection of allergen-specific IgE in serum or plasma samples by radioallergosorbent test (RAST).[1,2] This is an allergosorbent test where allergens are chemically bound to a solid phase. This places some limits on the quantitative evaluation of results, which reflect both the quantity and the affinity of IgE antibodies.[3] Recently, a new version of the RAST, the CAP system, has been developed,[4,5] using a high-density solid phase and a reference curve based on the binding of standard IgE and an anti-IgE antibody bound to the solid phase, allowing the expression of results in International Units (kU/L) related to the World Health Organization (WHO) IgE standard.

A limitation of RAST is the interference due to IgG allergen-specific antibodies, which compete with IgE and render the test unreliable for monitoring patients in the course of immunotherapy.[6] This limitation is partly overcome in the CAP version, where the high density of allergen obtained by means of a polymeric high-surface solid phase allows the IgG–IgE competition to be minimized.

Some years after the evolution of RAST, Spanish investigators developed a new method for the detection of allergen-specific IgE, the reverse enzyme allergosorbent test (REAST). It is an enzyme immunoassay (EIA) based on the capture of IgE by a specific anti-human-IgE antibody fixed to a microplate (IgE-capture microplate) and a subsequent reaction with an allergen conjugated with an

enzyme.[7–9] Alternatively, biotinylated allergens can be used and subsequently reacted with a streptavidin enzyme and a chromogenic substrate.[10,11] The test results are read on a reference curve, obtained by incubating reference sera with known titers of IgE (in relation to the WHO international standard for IgE) with the same IgE-capture microplate and reacting with a biotinylated anti-IgE antibody. The results are then expressed in International Units (kU/L). Advantages of REAST compared to RAST are: (i) the complete avoidance of interference by allergen-specific antibodies belonging to the IgG isotype, which are common in the course of specific immunotherapy and against food allergens, which are completely removed after the IgE capture;[10] and (ii) the possibility of making the test completely automated, due to the presence of a unique solid phase and liquid (biotinylated) allergens. A preliminary study on sera from patients with monosensitization and polysensitization allowed us to determine the specificity and sensitivity of REAST compared with RAST and a skin prick test. A further preliminary study has been performed to compare REAST and CAP. An important feature of REAST is that the same IgE-capture microplate can be used to detect allergen-specific IgE and total IgE, with the same in the reference curve. Starting from this point, we have developed, and describe in the present article, a particular version of the REAST procedure directed to the simultaneous detection of specific and total IgE, which allows the calculation of the specific IgE density as a percentage of total IgE. The calculation of this parameter by conventional methods (e.g. RAST for specific IgE and paper radioimmunosorbent test (PRIST) or similar assays for total IgE) is subject to criticism, because IgE may act as antibodies in RAST and as antigens in assays for total IgE. The two reactions have different antibody–antigen affinities and time-kinetics (Falagiani and Rapisarda, unpubl. data, 1995).

The particular version of REAST procedure described in the present paper, called REAST density, has been applied to the analysis of 23 sera from polysensitized asthmatic and rhinitic patients. The value of this test in indicating the clinical sensitivity to offending allergens, evaluated by specific provocation tests, is discussed.

METHODS

Sera

Twenty-three sera, from allergic patients sensitized to two or more allergens, were studied; 12 had rhinitis (eight with seasonal and four with perennial symptoms) and 11 suffered mainly from asthma (three with seasonal and eight with perennial symptoms). All the sera were divided into small aliquots and kept at – 20°C until used.

Reagents for REAST

The REAST reagents were from a commercial source (Realtest® Density kit; Lofarma SpA, Milan, Italy). The technical features of the components of the kit are, briefly, as follows.

1. An anti-IgE-capture solid phase, consisting of microplates (8-well polystyrene strips, flat bottomed) coated with a high-density layer of polyclonal (goat) anti-IgE antibody according to Ishikawa *et al.*[12] and submitted to overcoating with bovine serum albumin (BSA) as blocking agent.[9] The IgE-isotype specificity of the antibody had been previously checked by immunoelectrophoresis with human sera and ELISA reactivity with IgG, IgA, IgM and IgE myeloma sera.
2. The allergens, *Dermatophagoides pteronyssinus* (Der), *Parietaria judaica* (Par), *Poa pratensis* (Poa), *Betula verrucosa* (Bet), *Olea europea* (Ole) and the anti-IgE antibody (polyclonal, from goat) are biotinylated by reaction with activated biotin (ε-caproylamido-biotin-*N*-hydroxy-succinimide ester). Before use, the absence of non-specific binding is checked with negative control human sera.
3. Phosphate-buffered saline (PBS), 0.15 mol/L, pH 7.2, containing 0.05% Tween 20, is included for washing cycles.
4. Streptavidin-peroxidase is included as an enzymatic label, tetramethyl-benzidine (TMB) as a chromogenic substrate and 1 mol/L HCl as a stopping solution.
5. A reference curve of human sera is titrated in terms of total IgE, with reference to the international IgE standard (WHO IgE IRP 75/502), at the following concentrations: 0, 0.2, 0.5, 1.0, 5.0, 10, 25, 50 and 100 kU/L.

Reagents for RAST

A commercial radioimmunoassay (RIA) kit was used, consisting of polystyrene beads solid phase, polyclonal anti-IgE (from goat) labeled with [^{125}iodine] and a grass pollen-positive reference curve, (Sferikit®-specific IgE; Lofarma SpA, Milan, Italy). The RAST radioactivity was measured with a gamma counter (Cobra; Packard Instrument Company, Meriden, CT, USA).

The REAST density assay

All the reagents and samples for the assay were at room temperature.

Standards for IgE (50 μL at 0, 0.2, 0.5, 1.0, 5.0, 10, 25, 50 and 100 kU/L) and the sera under assay, undiluted (for specific IgE) and diluted 1:10 (for total IgE), were dispensed in duplicate into the anti-IgE-capture microplate and incubated for 60 min at room temperature. The liquid was aspirated and washed three times with 0.35 mL washing solution (automatic washer model 812 SW1; SLT Labinstruments, Milan, Italy). Biotinylated anti-IgE (100 μL) was dispensed into the wells containing the standard curve and the 1:10 dilution of samples (for total IgE) and 100 μL biotinylated allergens dispensed into wells containing undiluted samples (for specific IgE). These were incubated for 60 min at room temperature and then the liquid was aspirated and the wells washed three times. Streptavidin peroxidase (100 μL) was dispensed into all wells except the two blank wells and incubated for 30 min at room temperature before the liquid was aspirated from the wells and five washing cycles were done. Chromogenic substrate (100 μL) was dispensed into all wells, including the two blanks, and incubated for 20 min, avoiding exposure to bright light. Blocking solution (100 μL) was dispensed into all wells in the same sequence as for the chromogenic substrate, in order to maintain the same incubation time for all the wells. Within 60 min, the optical density (OD) was read at 405 and 450 nm, setting the instrument with the blank (ELISA reader Spectra; SLT, Labinstruments). The dual wavelength measurement mode was adopted, with 620 nm reference wavelength, to eliminate any influence caused by the microplate itself (fingerprints, scratches, dust etc.).

The RAST assay

The routine RAST procedure was followed: (i) incubation of serum samples with solid phase; (ii) washing cycle; (iii) incubation with [^{125}I]-anti-IgE; (iv) washing cycle; and (v) measurement of radioactivity. The results were expressed in arbitrary RAST units, in a range from 0.35 to 17.5 on the basis of a four-class reference curve consisting of a pool of grass-positive sera.

Calculation

The mean OD of duplicates was calculated. The first part of the reference curve was plotted by inserting the OD at 450 nm of the standards 0, 0.5, 1, 5 and 25 kU/L. The 25 kU/L standard was used in each experiment to calculate the OD 450 nm/OD 405 nm ratio, used as a correction factor for subsequent standards. The second part of the reference curve was constructed by inserting the OD at 405 nm of the 50 and 100 kU/L standards, multiplied by the correction factor. Therefore, the 25 kU/L standard represents the link between the two parts of the reference curve. This unusual procedure was adopted to obtain a broad-range reference curve from 0.2 to 100 kU/L. The OD readings obtained with samples diluted 1:10 and reacted with biotinylated anti-IgE were used to calculate total IgE. The values were calculated by interpolating the OD 450 nm readings on the reference curve when it was smaller than the 25 kU/L standard (corresponding to 250 kU/L considering the 1:10 dilution), while for higher values (from 250 to 1000 kU/L) the OD 405 nm reading was multiplied by the correction factor for the interpolation. To calculate specific IgE, the OD obtained with undiluted sera reacted with biotinylated allergens were used and the calculation was as above. Because the serum sample was undiluted, the reading range was 0.2–100 kU/L of allergen-specific IgE. The density of specific IgE was calculated according to the following formula:

(specific IgE kU/L ÷ total IgE kU/L) × 100.

Sensitivity

In the present study, sensitivity is intended as the minimal detection limit of the assay[13] and was evaluated separately for total IgE and specific IgE. For total IgE, a reference standard was progressively diluted with phosphate buffer containing 2% bovine serum albumin and 0.05% NaN3 to give the following IgE concentrations: 0.05, 0.1, 0.25, 0.40 and 0.50 kU/L. For specific IgE, four allergic sera to Poa, Par, Der and Bet at known titers of specific IgE (by REAST) were diluted to obtain the following concentrations of specific IgE: 0.05, 0.1, 0.25, 0.50 and 1.0 kU/L. The diluent alone was used as a zero point. Eight replicates were run for each point. Biotinylated anti-IgE was used for total IgE and the four biotinylated allergens for specific IgE.

The smallest amount of IgE giving an OD (mean – SD) higher than the OD (mean + SD) of the zero point was calculated and adopted as a minimal detection limit.

Reproducibility

The within-run variability was determined by testing, in the same run, eight replicates of eight positive reference sera at various IgE levels. The between-run variability was established by testing the same positive reference sera in 10 different runs. Means, SD and coefficients of variation (CV) were calculated.

Statistical analysis

The results were analyzed by a simple descriptive statistical analysis, calculating means, SD, CV% and correlation coefficient (r).

RESULTS

In a first preliminary evaluation, not described in the present paper, REAST and CAP have been compared by assaying 58 allergic sera against the most common allergens. The correlation coefficients obtained were 0.85 for Der, 0.93 for Poa and 0.85 for Par (Rapisarda and Cislaghi, unpubl. data, 1998). A second preliminary evaluation has been done to compare REAST, RAST (Sferikit®; Lofarma SpA, Milan, Italy) and skin-prick testing results in 182 patients with a positive clinical history to inhalant allergens. The sensitivity and specificity of REAST was, respectively, 90.0 and 94.3% for Der, 96.4 and 96.8% for Par, 85.9 and 92.3% for Poa and 71.4 and 98.5% for Bet, while the correlation between REAST and RAST, taking into account the classes of positivity, was 0.91 for Der, 0.95 for Par, 0.94 for Poa and 0.76 for Bet.[14]

Reference curve

The use of double wavelengths has been successful in obtaining a reference curve with a measuring range from 0.2 to 100 kU/L, as shown in Fig. 1. This measuring range is comparable to that of the CAP reference curve, ranging from 0.35 to 100 kU/L. The reproducibility of the OD of the reference curves in 20 different runs was calculated on the 25 kU/L linking point and a CV% measurement of 26.6 was obtained.

The procedure to interpolate the part of the curve exceeding 25 kU/L, multiplying the OD at 405 nm by the correction factor (OD 450 nm/OD 405 nm), can be easily automated by a personal computer. In the present study, an automatic calculation procedure on a personal computer (using Microsoft Excel) has been developed and successfully employed.

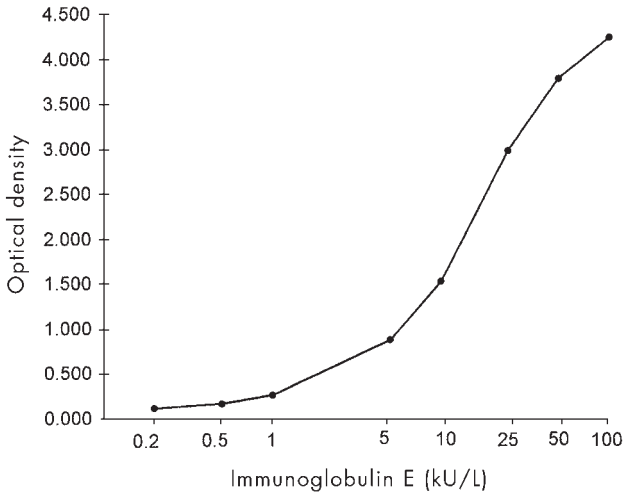

Fig. 1 The broad-range reference curve is obtained by combining the optical density (OD) readings at 450 (0.2–25 kU/L) and 405 nm (25–100 kU/L). The OD (405 nm) values have been multiplied by the OD (450 nm)/OD (405 nm) correction factor.

Minimal detection limit

The minimal detection limit of total IgE, determined with biotinylated anti-IgE and calculated on the basis of the mean – SD with respect to the mean + SD of diluent alone, was 0.1 kU/L and was considered to be the zero point (Fig. 2a). This sensitivity level means that the test could also be used for detection of total IgE in cord blood samples as a predictor for atopic disease, because 0.35 kU/L is the lowest cut-point commonly used.[15]

The minimal detection limit of specific IgE, determined with biotinylated allergens, was 0.25 kU/L for Poa and 0.1 kU/L for the other three allergens Par, Der and Bet (Fig. 2b–e). These sensitivity values are fully consistent with the clinical use of the test, being the positive thresholds of IgE measurement against different allergens ranging from 0.40 and 1.00 kU/L, as demonstrated by receiver operating characteristic (ROC) curve analysis of CAP results.[16]

Reproducibility

With regards to reproducibility of the total IgE determination, the within-run variability with three samples at different IgE levels gave a CV value of between 4.8 and 10% and the between-run CV was 5.5–9.3% (Table 1). The reproducibility of specific IgE determination was evaluated on three samples. The within-run variability gave CV values between 1.3 and 2.5% and the between-run CV values were between 11 and 12% (Table 1).

Total and specific IgE determination

The total IgE (T-IgE) values determined by REAST, the RAST values for specific IgE (S-IgE) and the REAST values for S-IgE are reported in Tables 2 and 3 for asthmatic and rhinitic patients, respectively. In the same tables, the density of S-IgE and their sums are calculated. For total IgE, the values were, as expected, spread over a wide range: from 28 to 2060 kU/L for asthmatic (mean 551, SD 603.4 kU/L) and from 22 to 1130 kU/L for rhinitic patients (mean 237.9, SD 330.4 kU/L). A comparison between RAST and REAST results cannot be done for S-IgE, because the RAST units are arbitrary and not related to the kU/L adopted for REAST. Preliminary experiments to determine the equivalence between the two systems showed that 1 RAST unit on the Sferikit reference curve corresponds to 4.65 kU/L (Rapisarda, unpubl. data, 1997). The S-IgE values detected by REAST were remarkably higher in asthmatic than in rhinitic patients (means 58.3 and 12.9, respectively). The values of density of S-IgE, expressed as a percentage ratio of total IgE, showed a great variability among patients, with a tendency to be higher in asthmatic than in rhinitic patients (means 14.1 and 8.0%, respectively). As expected, the sum of densities showed the same tendency, with means of 57.7 and 32.6%, respectively.

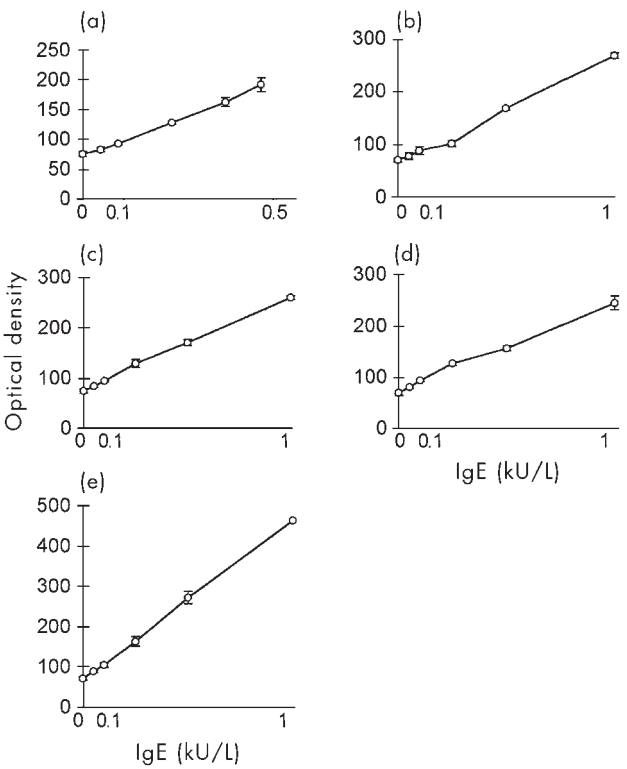

Fig. 2 Identification of the minimal detection limit of (a) total IgE and specific IgE to (b) *Poa pratensis*, (c) *Parietaria judaica*, (d) *Dermatophagoides pteronyssinus* and (e) *Betula verrucosa*.

Discussion

The REAST is a reliable procedure for detecting allergen-specific IgE antibodies.[7–11] Inhibition studies have demonstrated the efficiency of liquid biotinylated allergens in the binding of IgE antibodies and sensitivity is good compared with the conventional solid-phase RAST.[17] The new version of the REAST procedure described in the present paper provides an advantage over RAST in allowing the determination of the density of IgE antibodies, a new parameter corresponding to the ratio of specific to total IgE levels. This cannot be determined with conventional procedures, such as a combination of RAST for specific IgE and sandwich assay (PRIST or similar) for total IgE. These differ widely in terms of the affinity and kinetics of antigen–antibody reactions, the IgE being an antibody in RAST (binding the allergen on the solid phase) and an antigen in PRIST-like assays (bound by anti-IgE antibody on the solid phase). The grade of IgE-mediated allergic reaction and the severity of clinical symptoms should be more closely correlated to the percentage of IgE antibodies than to

Table 1 Reproducibility of the reverse enzyme allergosorbent test

		Within-run variability			Between-run variability		
	No. replicates	Mean (kU/L)	SD (kU/L)	CV (%)	Mean (kU/L)	SD (kU/L)	CV (%)
Total IgE							
Sample 1	10	25	1.21	4.8	15.7	1.39	8.9
Sample 2	10	57	5.92	10.4	33.3	5.18	5.5
Sample 3	10	82	8.34	10.2	82.8	7.74	9.3
Specific IgE							
Poa$^+$ sample	8	9.4	0.12	1.3	3.13	0.37	11.8
Der$^+$ sample	8	3.58	0.09	2.5	5.63	0.63	11.2

Poa, *Poa pratensis*; Der, *Dermatophagoides pteronyssinus*; CV, coefficient of variation.

Table 2 Test results with 11 sera from asthmatic patients

Patient No.	REAST T-IgE	Allergens	RAST S-IgE	REAST S-IgE	S-IgE density	S-IgE densities (sum)
1	66	Der	0.0	0.0	0.0	98.3
		Par	10.6	12.5	18.9	
		Poa	13.2	30.0	45.4	
		Bet	2.6	22.5	34.0	
2	342	Der	9.0	60.5	17.6	51.7
		Par	0.0	0.0	0.0	
		Poa	28.1	98.3	28.7	
		Bet	0.9	7.5	2.2	
		Ole	1.5	9.0	2.6	
3	168	Grass	0.6	3.0	1.8	62.1
		Ole	1.3	1.5	0.9	
		Par	24.8	92.5	56.5	
		Bet	1.3	4.7	2.9	
4	670	Der	18.4	253.7	37.8	66.0
		Par	25.1	80.0	11.9	
		Poa	23.2	35.0	14.1	
		Bet	2.1	15.0	2.2	
5	267	Der	5.7	84.0	31.4	70.0
		Par	26.4	85.0	31.8	
		Poa	0.9	0.9	0.3	
		Ole	0.0	17.5	6.5	
6	220	Der	2.8	19.0	8.6	45.6
		Par	24.8	60.8	27.5	
		Poa	10.5	20.0	9.0	
		Bet	0.7	1.3	0.5	
7	385	Der	0.5	3.5	0.9	43.5
		Par	2.6	40.0	10.3	
		Poa	4.2	3.3	0.8	
		Bet	9.2	121.5	31.5	
8	1200	Der	11.1	114.0	9.5	33.9
		Par	25.0	292.5	24.3	
		Poa	0.0	0.0	0.0	
		Bet	0.0	1.0	0.0	
9	28	Der	0.0	13.0	47.1	86.5
		Par	3.2	7.3	26.4	
		Poa	1.3	3.6	13.0	
		Bet	0.0	0.0	0.0	
10	660	Der	0.5	0.7	0.1	41.6
		Par	19.7	271.0	41.0	
		Poa	12.1	0.8	0.1	
		Bet	2.3	2.7	0.4	
11	2060	Der	17.1	670.0	32.5	35.60
		Par	11.8	3.9	0.2	
		Bet	7.7	14.8	0.7	
		Ole	7.7	45.5	2.2	
Mean	551		8.2	58.3	14.1	57.7
SD	603.4		9.1	116.9	16.0	21.0

Der, *Dermatophagoides pteronyssinus*; Par, *Parietaria judaica*; Poa, *Poa pratensis*; Bet, *Betula verrucosa*; Ole, *Olea europea*; REAST, reverse enzyme allergosorbent test; RAST, radioallergosorbent test; T-IgE, total immunoglobulin E; S-IgE, specific immunoglobulin E; S-IgE density, S-IgE as a percentage of T-IgE by REAST.

Table 3 Test results with 12 sera from rhinitic patients

Patient No.	REAST T-IgE	Allergens	RAST S-IgE	REAST S-IgE	S-IgE density	S-IgE densities (sum)
12	108	Der	ND	5.7	5.3	32.1
		Par	ND	15.0	13.8	
		Poa	ND	13.0	12.0	
		Bet	ND	1.1	1.0	
13	42	Der	ND	0.0	0.0	38.8
		Par	ND	8.3	19.8	
		Poa	ND	6.2	14.8	
		Bet	ND	1.8	4.3	
14	103	Der	0.0	0.4	0.4	15.3
		Par	7.3	12.0	11.7	
		Poa	2.2	1.9	1.9	
		Bet	0.3	1.5	1.4	
15	114	Der	4.0	12.2	10.7	22.7
		Par	11.1	10.0	8.8	
		Poa	1.6	3.7	3.3	
		Bet	0.0	0.0	0.0	
16	544	Par	22.2	145.6	26.8	33.3
		Poa	3.2	0.0	0.0	
		Bet	7.5	20.6	3.8	
		Ole	2.1	15.1	2.8	
17	22	Der	0.0	0.0	0.0	28.0
		Par	3.2	5.4	24.7	
		Poa	0.2	0.7	3.3	
		Bet	0.0	0.0	0.0	
18	47	Der	0.0	0.0	0.0	32.3
		Par	4.5	14.6	31.1	
		Poa	0.4	0.6	1.3	
		Bet	0.0	0.0	0.0	
19	1130	Der	2.5	18.0	1.6	11.0
		Par	22.4	100.0	8.9	
		Poa	5.2	5.5	0.5	
		Bet	0.0	0.6	0.0	
20	73	Der	0.2	0.0	0.0	39.8
		Par	10.4	12.0	16.3	
		Poa	8.0	15.0	20.4	
		Bet	0.3	1.1	1.4	
		Ole	0.0	1.2	1.6	
21	72	Par	3.1	1.9	2.6	88.0
		Poa	19.3	60.0	82.8	
		Bet	1.0	1.1	1.5	
		Ole	0.0	0.8	1.1	
22	104	Der	0.0	0.0	0.0	32.7
		Par	14.2	15.0	14.4	
		Poa	1.9	10.9	10.5	
		Bet	1.3	8.1	7.8	
23	496	Par	19.8	72.4	14.6	17.7
		Poa	11.8	12.2	2.5	
		Bet	0.4	0.0	0.0	
		Ole	1.3	3.1	0.6	
Mean	237.9		4.7	12.9	8.0	32.6
SD	330.4		6.6	27.0	13.5	19.7

Der, *Dermatophagoides pteronyssinus*; Par, *Parietaria judaica*; *Poa, Poa pratensis*; Bet, *Betula verrucosa*; Ole, *Olea europea*; REAST, reverse enzyme allergosorbent test; RAST, radioallergosorbent test; T-IgE, total immunoglobulin E; S-IgE, specific immunoglobulin E; S-IgE density, S-IgE as a percentage of T-IgE by REAST; ND, not determined.

their absolute amount in serum. Immunoglobulin E binds through the Cε3 domain to mast cells and basophils carrying the high-affinity receptor FcεRI and the reaction with multivalent antigens elicits the allergic reaction by triggering intracellular events leading to the activation of these cells and the release of mediators. The affinity of IgE for the receptor FcεRI is so high (K_d of approximately 10^{-10} mol/L) according to Metzger *et al.*[18] that we can consider circulating antibodies to be the result of overproduction with respect to the whole receptor capacity of these cells, which concentrate IgE on their surface. Therefore, it is reasonable to postulate that the releasability of IgE-bearing cells should be correlated more to the density of allergen-specific IgE antibodies on their surface than to the absolute amount of such antibodies in peripheral blood. From this angle, the S-IgE density seems to be a promising indicator of the clinical relevance of a given allergen. The clinical relevance of the new parameter S-IgE density was assessed in a clinical study, where organ sensitivity was studied with nasal and bronchial specific challenges, and proved to be positively correlated to S-IgE density (Crimi *et al.*, unpubl. obs., 1997). In fact, rhinitic patients have shown that specific IgE density is significantly correlated with nasal challenge scores ($r = 0.72$, $P < 0.0001$), while the levels of specific IgE expressed as absolute values (kU/L) are correlated to nasal challenge scores to a lesser extent ($r = 0.48$, $P < 0.005$). In asthmatic patients, the level of specific IgE was significantly correlated with the maximum late forced expiratory volume in 1 s (FEV_1) decrease only when it was expressed as specific IgE density ($r = 0.53$, $P < 0.005$) and not when it was expressed as an absolute value ($r = 0.25$, $P = 0.16$). These authors have concluded that, in the majority of patients with multiple sensitizations, the S-IgE density determined by REAST appears to be in satisfactory agreement with the responses to the inhaled allergens.

In terms of the technical aspects of the test, the standard curve ($r = 0.90$), the sensitivity (0.1 kU/L) and the reproducibility, both within-run (CV% 1.3–10) and between-run (CV% 5.5–12), were considered satisfactory. The T-IgE values determined by REAST have been compared with the values obtained by PRIST, with $r = 0.987$ (Feligioni, unpubl. data, 1997). The biotinylation procedure of allergens was highly reproducible and could be easily extended to all allergens of clinical interest. In our laboratory at Lofarma SpA, the REAST procedure has now been developed for 270 allergenic sources from pollens, mites, moulds, animal epithelia, insect venoms, latex and, after conjugation to a protein carrier (human serum albumin), some antibiotics.

The difference between quantitative REAST (S-IgE) and density is explained by the mode of calculation. In the density procedure, the T-IgE value goes into the calculation and IgE antibodies are expressed as a percentage. As a consequence, different samples having the same absolute value of IgE antibodies can give a wide range of IgE densities. The sum of S-IgE density values in the patient's serum always gives a figure below 100%, the highest value observed being 98.3% (patient 1, asthmatic group). This limit was observed in the present study because the allergens tested did not cross-react, whereas in further studies in progress with cross-reacting allergens (e.g. hazel, birch, alder) the sum of S-IgE densities often widely exceeds 100%. This is due to the fact that, because of cross-reactions, the same IgE antibodies recognize several allergenic sources. In the case of strictly cross-reacting allergens (e.g. *D. pteronyssinus* and *D. farinae*, grass pollens), the use of mixtures prepared by pooling the biotinylated allergens is suggested.

In conclusion, IgE density is proposed as a new method to evaluate the content of allergen-specific IgE antibodies in serum samples, based on the simultaneous detection of total and specific IgE by REAST. This new procedure promises to offer consistent advantages for better evaluation of the clinical role of different allergens, particularly in patients with multiple sensitizations.

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