

Value of immunoglobulin E density in predicting nasal and bronchial response to inhaled allergens in rhinitic and asthmatic subjects with multiple sensitizations

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Summary

Background In atopic subjects with multiple sensitizations to inhalant allergens the relationship between the specific serum immunoglobulin (Ig) E and the *in vivo* response to each allergen is not well established.

Objective To investigate the relationship between the specific serum IgE expressed as amount (kU/L) or density (specific IgE/total IgE percentage) with the *in vivo* response to inhaled allergens in rhinitic and asthmatic subjects with multiple sensitization.

Methods By means of Reverse Enzyme AllergoSorbent Test (REAST) the absolute values and the density of specific IgE for each sensitizing allergen was determined. Rhinitics ($n = 12$) underwent nasal and asthmatics ($n = 11$) bronchial allergen challenges with the two to three sensitizing allergens for a total of 33 nasal and 32 bronchial challenges. Correlations and degree of concordance between specific serum IgE and results of challenges were calculated.

Results IgE density significantly correlated with nasal challenge score ($r_s = 0.72$, $P < 0.001$), bronchial challenge score ($r_s = 0.56$, $P < 0.001$) and late asthmatic response ($r_p = 0.53$, $P < 0.005$). Among subjects with three sensitizations, comparison of values of IgE density with the results of challenges showed significant concordance in graduation ($\chi^2 = 11.3$, $P < 0.005$).

Conclusions In subjects with multiple sensitizations, the nasal and bronchial response to the different sensitizing allergens may be predicted, at least in part, by the IgE density. A satisfactory agreement between graduation of the IgE density to the different allergens and the *in vivo* response to the same allergens has been found within subject.

Keywords: bronchial challenge, immunoglobulin E density, late asthmatic response, nasal challenge, total immunoglobulin E

Clinical and Experimental Allergy, Vol. 29, pp. 1663–1670. Submitted 20 July 1998; revised 20 April 1999; accepted 30 April 1999.

Introduction

The majority of allergic rhinitic and asthmatic subjects show skin and RAST sensitizations to two or more allergens [1,2]. In these subjects it is often difficult to ascertain which sensitizations are clinically relevant. In subjects with only one major sensitization it has been previously shown that

the degree of the *in vivo* response may be predicted, at least in part, by an equation incorporating non-specific hyper-responsiveness and the amount of specific immunoglobulin (Ig) E [3] or the end point skin test [4]. Whether this is also true for subjects with multiple sensitizations is not well established.

In allergic subjects there is not a strong correlation between the amount of specific IgE and that of total IgE and their ratio may be quite different between subjects with many sensitizations. We reasoned that the assessment

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of the ratio of specific to total IgE (IgE density) for any sensitizing allergen might be helpful to predict the *in vivo* response in subjects with multiple sensitizations. Recently an *in vitro* method to measure specific IgE, the Reverse Enzyme Allergo Sorbent Test (REAST) has been developed [5–7] which is based on the capture of IgE with a specific antibody and the reaction with liquid biotinylated allergens. By means of REAST the amount of specific IgE can be measured and expressed as international units (kU/L) and the IgE density can be also calculated as percentage ratio between specific IgE and total IgE.

We found that, in subjects with multiple sensitizations, the nasal and bronchial response to inhaled allergens may be predicted, at least in part, by the specific IgE. The asthmatic response was better predicted by the IgE density than by the amount of specific IgE. We also found that, in polysensitized rhinitic and asthmatic subjects, graduation of IgE density is in satisfactory agreement with the graduation of the *in vivo* response to the different allergens.

Materials and methods

Subjects

We studied 23 non-smoking allergic subjects (15 females) whose mean age was 28 years, range 18–56. All subjects underwent skin prick tests (Lofarma SpA, Milan, Italy) for the most common Mediterranean allergen (grass mix, parietaria, olive, birch, mugwort, house dust mites, *Alternaria*, *Aspergillus*, dog and cat dander). To enter the study subjects had to have two or more skin responses equal to or greater than that of histamine (10 mg/mL) and to give written informed consent. Twelve subjects were suffering from isolated rhinitis, eight with seasonal and four with perennial symptoms, and the remaining 11 were prevalently suffering from asthma, three with seasonal and eight with perennial symptoms.

Protocol

On a first study day subjects underwent blood drawing for specific and total IgE determination (REAST, Lofarma SpA) and methacholine (MCh) challenge. They were asked to come back to the clinic after they had refrained from taking short-acting β_2 -stimulants for at least 12 h and antihistamines for at least 5 days. Cromones had to be discontinued 1 week before the study. None of the patients had received oral or inhaled steroids in the previous month at least. On the basis of history and MCh challenge subjects were defined as rhinitics (MCh PD₂₀ > 1.2 mg) and asthmatics (MCh PD₂₀ < 1.2). Rhinitic subjects underwent nasal provocation tests and asthmatic subjects underwent bronchial provocation tests on 2 or 3 study days, depending

on the number of positive skin sensitizations that had been observed on the screening day. The interval between two consecutive challenges was at least 1 week. At the time of the study all subjects with seasonal symptoms were out of season and those with perennial symptoms were in stable conditions. No subject had suffered from viral infections of the upper respiratory tract in the month before the study.

REAST

Details of the procedure have been already published [5–7]. Briefly, the procedure is based on the following steps:

- (i) an antihuman-IgE is bound to microtitre wells and acts as IgE capture reagent;
- (ii) the serum sample is submitted to IgE-capture in two different wells, diluted 1 : 10 for the reaction with biotinylated-anti-IgE (to reveal total-IgE) and undiluted for the reaction with biotinylated allergens (to reveal specific-IgE);
- (iii) a broad range (from 0.2 to 100 kU/L) reference curve of human IgE referred to the WHO IgE IRP 75/502 is tested in parallel, and used to interpolate the sample results obtained both with anti-IgE (total IgE) and allergens (specific IgE).

Methacholine challenge

Isotonic solutions (2 and 10 mg/mL) were obtained by reconstituting lyophilized methacholine (Lofarma) in distilled water and delivered by an ampul-dosimeter device (Mefar, Brescia, Italy) driven by compressed air at a pressure of 1.5 kg/m² with 1-s actuations and 5-s intervals between breaths. Aerosols were inhaled during quiet tidal breathing. After 20 inhalations of isotonic saline as a control, doubling doses of methacholine were inhaled from 0.02 mg up to a maximum of 1.2 mg. Incremental doses were obtained using the two different methacholine solutions and varying the number of breaths. A 3-min interval was allowed before each dose increment. FEV₁ was measured 1 min after each dose by a turbine spirometer (Micro Spirometer, Micro Medical Ltd, Rochester, UK) and the best of three acceptable measurements within 100 mL was retained to create dose–response curves. The non-cumulative dose causing a 20% fall of FEV₁ from control (PD₂₀) was calculated by interpolation between two adjacent points of the dose–response curve.

After recovery from methacholine-induced bronchoconstriction, FEV₁ was monitored hourly for 8 h to detect spontaneous variations. Subjects with spontaneous variation > 10% were excluded. In asthmatics an identical MCh challenge was performed 1 week after the last allergen challenge.

Allergen challenge

Allergens for both nasal and bronchial challenge were titrated in allergenic units (AU) determined by RAST inhibition technique in comparison with in-house reference preparations containing a high titre of specific IgE (Lofarma).

Nasal challenges were performed by using allergen incorporated into lactose powder as previously described [8,9]. Lactose powder as a control and increasing concentrations of allergens (2.5, 5, 10, 20, 40, 60 and 80 AU) were delivered by means of a manual rhino-insufflator (MIAT, Milan, Italy) at 20 min intervals. Itching, sneezing, rhinorrhea, and nasal blockage were used for monitoring the nasal response to allergen inhalation. Each symptom was scored from 0 to 3 as follows: 0 = absent; 1 = mild; 2 = moderate; 3 = severe. Dosing was stopped when at least two moderate symptoms occurred and the last inhaled concentration was considered the threshold dose. The maximum score of symptoms within the first hour after the end of challenge was considered the early response. The occurrence of delayed symptoms 3–8 h after the end of challenge were also scored as above and the mean of 3–8 h scores retained for statistical analysis. Late response was considered positive when two symptoms scored 2 for at least 2 h. The reproducibility of this specific nasal provocation test was demonstrated in previous studies [8].

Bronchial challenge was performed as previously described [3]. To undergo bronchial challenge baseline FEV₁ had to be >70% of predicted [10]. Double decreasing solutions from 5000 AU/mL to 8 AU/mL were extemporaneously prepared by adding redistilled water to freeze-dried allergen. Solutions were aerosolized by means of ampule-dosimeter device above described (see MCh challenge). The challenge started from the concentration of 8 AU/mL and continued with double increases of the allergen concentration until the FEV₁, measured 15 min after inhalations, fell below 80% of post-saline control, which was considered a positive early response. Each concentration was inhaled during 50 quiet breaths which corresponded with an inhaled volume of 0.5 mL. The maximum concentration of allergen was 5000 AU/mL corresponding with an inhaled dose of 2500 AU. After the end of inhalation challenge FEV₁ was measured at 30, 45 min, and then hourly for the following 8 h in order to detect the occurrence of late asthmatic response (LAR). LAR was expressed as the maximum FEV₁ decrease recorded between 3 and 8 h after the last inhalation.

Statistical analysis

Results of REAST for specific IgE have been analysed both as kU/L (amount of specific IgE) and percentage of specific IgE to total IgE (IgE density).

For both nasal and bronchial challenge the early and the late phase of the response were scored.

Dose score

To avoid the effect of different biological potency among the different allergens the inhaled dose that had provoked the nasal or the bronchial early response was scored as follows. Within each group of challenges with the same allergen a reference value = 100 was attributed to the highest provoking dose. The other lower provoking doses were expressed as percentage of this reference. Doses less than 33% of reference were scored 3, doses ranging between 33 and 66% were scored 2 and doses equal or greater than 66% were scored 1. Negative early responses were scored 0.

Late response score

In nasal challenge late-symptom scores were added to early symptom scores and dose score obtaining the nasal challenge score (NCS).

In bronchial challenge LAR was scored 0 when the FEV₁ fall was less than 10% of control, 1 when it was from 10 to 19% (mild LAR), 2 when it was from 20 to 30% (moderate LAR), and 3 when it was equal or greater than 30% (severe LAR).

In bronchial challenge the dose score was added to the LAR score obtaining the bronchial challenge score (BCS). Results of LAR expressed as 3–8 h maximum FEV₁ fall from control were also directly compared with results of REAST.

Spearman-rank (non-parametric) and/or Pearson (parametric) regression analysis were appropriately used to test the relationship between specific IgE expressed as amount or as density and NCS, BCS, and LAR. r_s and r_p indicate non-parametric and parametric regression coefficients, respectively.

The agreement between the results of IgE density and the results of nasal or bronchial challenges was also calculated as follows. In each subject with three sensitizations the sequence of the highest (**h**), middle (**m**), and lowest (**l**) value of IgE density was compared with the sequence of the highest (**h**), middle (**m**), and lowest (**l**) NCS-or BCS-score. Six combinations are possible: one combination of complete agreement (**h-h**, **m-m**, **l-l**); two combinations of partial agreement (**h-m**, **m-h**, **l-l** or **h-h**, **m-l**, **l-m**); three combinations of disagreement (**h-l**, **m-m**, **l-h** or **h-m**, **m-l**, **l-h** or **h-l**, **m-h**, **l-m**). The observed frequency of combinations of sequences was compared with the random frequency of combinations of sequences by means of the Chi-squared test. Difference of IgE between asthmatic and rhinitic subjects were tested by means of *t*-test for unpaired samples.

Results

Subjects characteristics are detailed in Table 1. Individual results of IgE density and challenge scores are given in Table 2.

Three rhinitics and one asthmatic underwent two challenges each whereas the remaining subjects underwent three challenges each, for a total of 33 nasal challenges and 32 bronchial challenges. In rhinitics a late-phase nasal response was observed on three occasions whereas in asthmatics a moderate to severe LAR occurred in 16 occasions corresponding with one moderate to severe LAR at least in all but one subject.

IgE density significantly correlated with nasal challenge score ($P < 0.0001$, Fig. 1) with bronchial challenge score ($P < 0.001$, Fig. 2) and with the maximum late FEV₁ fall ($P < 0.005$, Fig. 3). The amount of specific IgE correlated with bronchial challenge score ($P < 0.001$) as the IgE density did, but did not correlate with the maximum late FEV₁ fall ($P = 0.16$ NS). In rhinitic subjects correlation

between the amount of specific IgE was weaker ($P < 0.005$) than that observed with the IgE density (see Table 3 for comparisons).

Reproducibility of MChPD₂₀ was within one doubling dose in all asthmatics.

In the whole group of allergic subjects eight subjects showed complete agreement between IgE density and challenge results, eight subjects showed partial agreement and three showed disagreement. The frequency of the observed combinations (8/8/3) was significantly different from the expected random frequency (3/6.5/9.5), $\chi^2 = 11.3$, $P < 0.005$ (see Table 2 for individual data). Three rhinitic and one asthmatic subjects were not included in statistical analysis because they had only two sensitizations and showed agreement between specific IgE level and challenge results.

The level of total IgE ranged 22–1130 kU/L (geometric mean 120) in rhinitics and 28–2060 U/L (geometric mean 311) in asthmatics. The difference did not reach statistical significance ($P > 0.05$). The sum of the IgE densities for the

Table 1. Subjects' characteristics

	Age (years)	Sex	Sensitization challenge	Total IgE (kU/L)	MChPD ₂₀ (μg)	
<i>Rhinitis</i>						
1	19	F	PGH	108	>1200	
2	18	F	PGB	42	>1200	
3	25	M	PBH	103	>1200	
4	22	F	HPG	114	>1200	
5	24	F	PHG	544	>1200	
6	24	M	PGB	22	>1200	
7	20	F	PB	47	>1200	
8	22	F	PHG	1130	>1200	
9	30	M	GP	73	>1200	
10	34	M	GP	72	>1200	
11	19	F	PGB	104	>1200	
12	20	F	PBG	694	>1200	
<i>Asthma</i>					<i>1st MChPD₂₀</i>	<i>2nd MChPD₂₀</i>
1	45	F	GBP	66	150	120
2	56	F	GHB	342	30	45
3	24	F	PGB	168	20	38
4	44	F	HGP	670	97	75
5	24	F	PHG	267	49	64
6	34	M	PHG	220	130	110
7	19	M	BPH	385	236	190
8	19	F	PB	1200	468	620
9	25	F	HPG	28	422	350
10	49	M	PGH	660	587	600
11	22	M	HBP	2060	427	290

PD₂₀ MCh, dose of methacholine causing a 20% FEV₁ fall from control; P, Parietaria; G, Graminaceae; B, Betullaceae; H, House dust mite. The initials of specific sensitizations are ordered from the highest to the lowest REAST value and indicate the nasal or bronchial challenges performed.

Table 2. Individual results of IgE density and challenges ordered from the highest to the lowest value of IgE density

Rhinitic subjects	Specific IgE density — nasal challenge score			Agreement in graduation
	Highest value	Middle value	Lowest value	
1	P 14 –7	G 12 –0	H 5.5 –6	Partial
2	P 20 –8	G 15 –6	B 4.4 –9	No
3	P 12 –5	B 1.4 –1	H 0.3 –0	Yes
4	H 11 –6	P 9 –8	G 1.8 –2	Partial
5	P 9 –7	H 2.4 –3	G 0.5 –0	Yes
6	P 25 –16	G 3.3 –6	B 0.5 –0	Yes
7	P 27 –11	B 3.8 –9		
8	P 9 –7	H 2.4 –3	G 0.5 –0	Yes
9	G 20 –9	P 16 –9		
10	G 83 –8	P 2.6 –2		
11	P 14 –7	G 10 –6	B 7.7 –5	Yes
12	P 15 –9	B 2.5 –0	G 1.8 –2	Partial

Asthmatic subjects	Specific IgE density — bronchial challenge score			Agreement in graduation
	Highest value	Middle value	Lowest value	
1	G 45 –6	B 34 –4	P 19 –6	Partial
2	G 29 –4	H 18 –5	B 2.2 –5	No
3	P 56 –5	G 1.8 –2	B 0.9 –4	Partial
4	H 38 –6	G 14 –5	P 12 –5	Partial
5	P 32 –6	H 31 –4	G 0.3 –2	Yes
6	P 27 –4	H 9 –6	G 8 –3	Partial
7	B 31 –6	P 10 –4	H 1 –1	Yes
8	P 24 –4	B 0.1 –2		
9	H 47 –3	P 26 –4	G 13 –4	No
10	P 41 –5	G 1.2 –3	H 0.1 –0	Yes
11	H 32 –5	B 2.2 –2	P 0.2 –3	Partial

Numbers in bold font are values of IgE density (Amount of specific IgE/Total IgE percentage). Numbers in courier font are the corresponding challenge scores; P, parietaria; G, graminaceae; B, betullaceae; H, house dust mite.

different allergens was significantly higher in asthmatics than in rhinitics ($58\% \pm 21$ vs $32\% \pm 19$, $P < 0.01$) (Fig. 4).

Discussion

The main finding of this study indicates that in subjects with multiple sensitizations, as with monosensitized subjects, the degree of the nasal or bronchial response to different inhaled allergens may be predicted, at least in part, by the level of serum-specific IgE. The density rather than the amount of specific IgE is a better predictor.

Comment on methodology

REAST was performed for the five most common Mediterranean allergens to which subjects were sensitized. We

cannot exclude that subjects may have specific IgE for other unknown allergens. For inhalation challenges we used different allergens with possible different biological potency *in vivo*. For this reason the provoking doses of both nasal and bronchial challenge were comparable within the same type of allergen but not between different types of allergen. We calculated a standardized provoking dose for minimizing difference due to allergen potency. Specific serum level may vary over the time and upon exposure. For this reasons our patients were studied out of season and in stable conditions.

In asthmatic subjects with single sensitization it has been previously demonstrated that both the early and the late bronchoconstriction may be predicted by using a step-wise, multiple regression analysis incorporating as independent variables the airway sensitivity to MCh or histamine

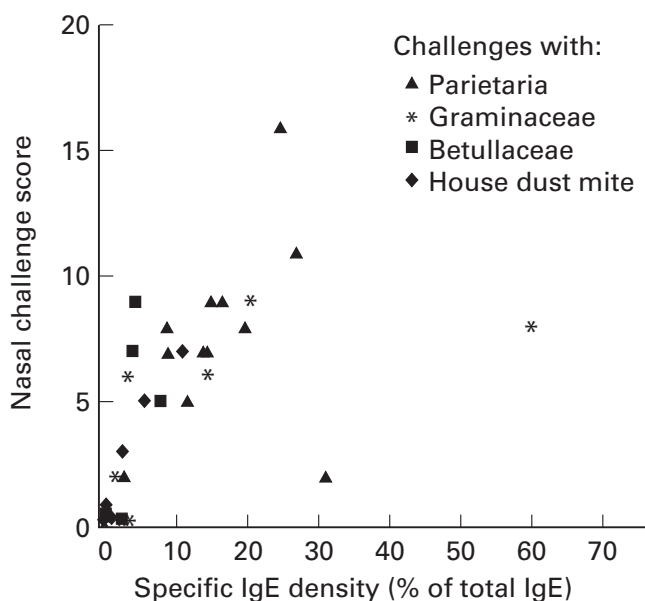


Fig. 1. Relationship between REAST values expressed as percentage of total serum IgE (IgE density) and nasal challenge score (NCS). $n = 33$, $r_s = 0.72$, $P < 0.0001$.

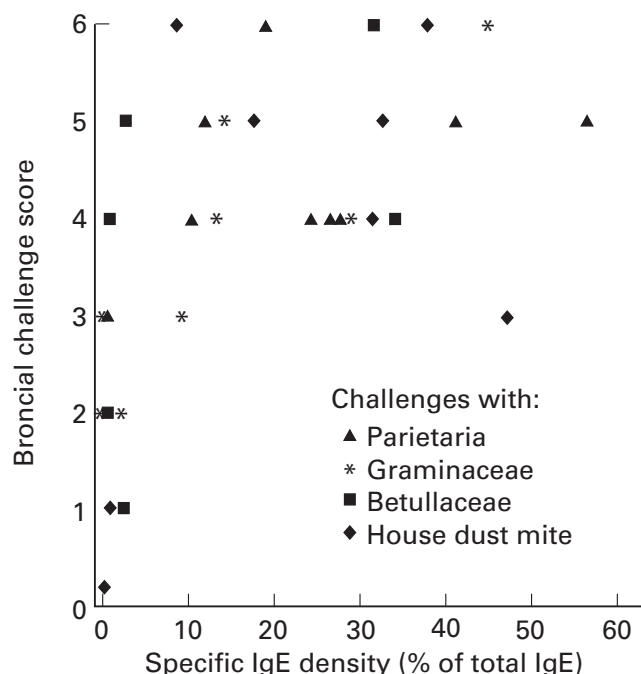


Fig. 2. Relationship between REAST values expressed as percentage of total serum IgE (IgE density) and bronchial challenge score (BCS). $n = 32$, $r_s = 0.56$, $P < 0.001$.

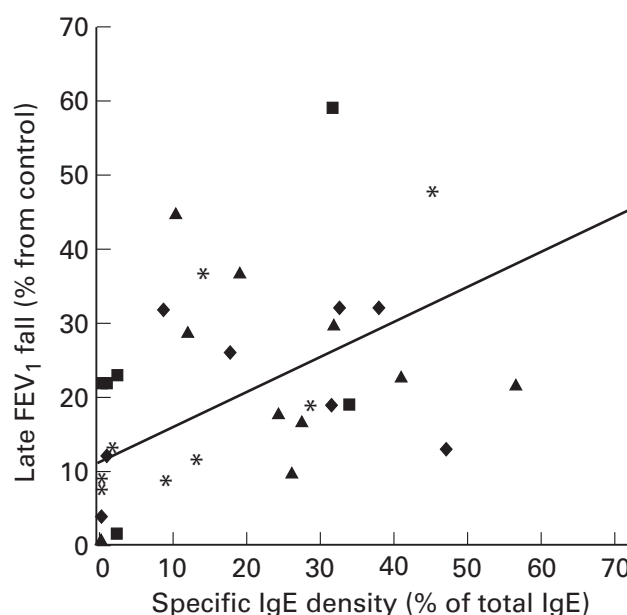


Fig. 3. Relationship between REAST values expressed as percentage of total serum IgE (IgE density) and maximum late FEV₁ fall (LAR) as percentage from control value. $n = 32$, $r_p = 0.53$, $P < 0.005$.

and the degree of allergic sensitization, the latter being expressed as serum IgE level [3] or end-point skin test [4]. We could not use this statistical approach to the present results since in each subject the same MCh PD₂₀ would have been included two or three times in the regression analysis. For this reason the weight of MCh sensitivity in determining the asthmatic response to allergens could not be taken into account between asthmatic subjects. This may explain the weaker correlations found in asthmatic than in rhinitic subjects.

In asthmatics we did not perform MCh challenge before any allergen challenge so we cannot exclude some difference of airway responsiveness on different allergen challenge days. Nevertheless, the 'within one doubling dose' reproducibility of MCh PD₂₀s obtained at the beginning and at the end of the study in all asthmatic subjects suggests that airway responsiveness was stable throughout the study.

The categorical analysis used to quantify the agreement between graduation of specific IgE and the *in vivo* response is the only possible statistical approach but tends to underestimate the variability of the 'distance' between h, m, and l values. For this reason also individual data are given in Table 2.

Comment on results

In rhinitics with multiple sensitizations we found that half of the response to nasal challenge can be predicted by

Table 3. Relationship between challenge scores, late asthmatic response and specific IgE

	Amount of specific IgE	IgE density
Nasal challenge score	$r_s = 0.48$; $P = 0.005$	$r_s = 0.72$; $P = 0.001$
Bronchial challenge score	$r_s = 0.58$; $P = 0.001$	$r_s = 0.56$; $P = 0.001$
Late asthmatic response	$r_p = 0.25$; $P = 0.16$ (NS)	$r_p = 0.53$; $P = 0.001$

r_s , coefficient of non-parametric correlation (Spearman); r_p , coefficient of parametric correlation (Pearson); NS, not significant.

specific IgE determined by REAST, mainly when IgE is expressed as density rather than amount. In asthmatics one third of LAR can be predicted by IgE density but not by their amount. Altogether these results suggest that in polysensitized subjects the amount of specific IgE seems less important than the IgE density in determining the *in vivo* response to that allergen. We observed that, comparing couples of asthmatics with similar MCh Pd₂₀ and similar bronchial response to a given allergen, the amount of specific IgE to that allergen may vary by more than 10-fold. For example asthmatics $n=4$ and $n=6$ of Table 1 had similar MCh Pd₂₀ (97 and 130 μ g, respectively), similar early (30 and 36%) and late (32% for both) asthmatic response to house dust mite (provocative score = 6 for both) but highly different amount of specific IgE (254 and

19 kU/L, respectively). This difference was less when specific IgE were expressed as density (38 and 9%, respectively). In this context LAR, which is considered a good model of natural asthma, was found correlated with the IgE density but not with the amount of specific IgE. If the severity of LAR has clinical relevance IgE density should also have clinical relevance in asthma. The finding that the amount of circulating IgE is a poor index for predicting the response to allergen exposure among subjects with multiple sensitizations suggests a phenomenon of saturation of the IgE receptors and overproduction of IgE. If this occurs IgEs with different specificity might compete each other for binding to the receptors which are still free, making the IgE density a better predictor of airway response to allergen.

However, our results indicate that in asthmatic subjects with multiple sensitization the response to allergen exposure is less predictable than in monosensitized subjects [3,4]. The different degree of airway sensitivity between subjects can be invoked as a cause of the unexplained variance among subjects. Nevertheless, it is difficult to explain the unpredicted variance within subject. Indeed we observed eight partial agreements between REAST and nasal or bronchial response and three disagreements. This means that in three subjects the most severe response to allergen inhalation corresponded with the lowest level of serum-specific IgE. Unknown factors of the IgE kinetics at the mucosa level might alter the expected response to the sensitizing allergens in some polysensitized subjects.

Interestingly we found that asthmatic subjects had the sum of specific IgE densities significantly higher than that of rhinitic subjects. However, the limited number of subjects does not allow us to state that this difference contributes to the development of asthma.

Conclusion

IgE density as calculated by REAST procedure, thanks to the simultaneous determinations of total and specific IgE, is a new model for expressing specific IgE values that improves the correlation with *in vivo* results. In rhinitis

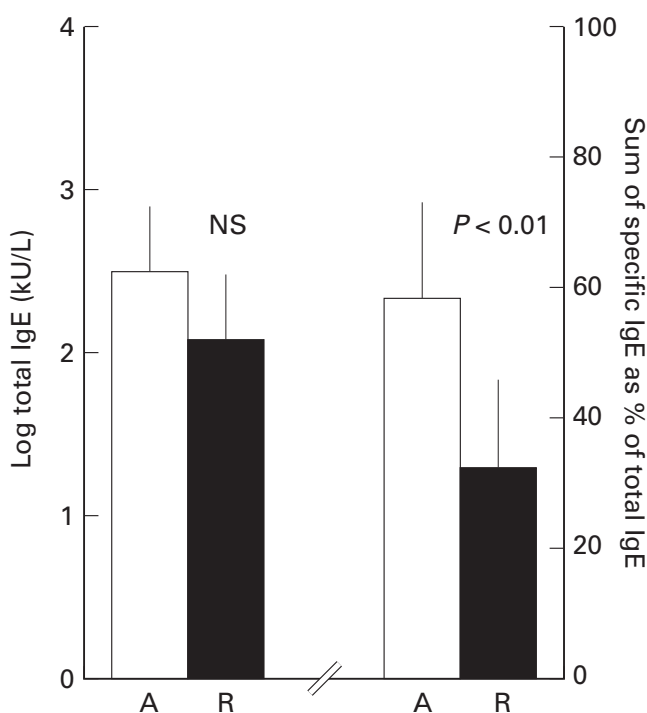


Fig. 4. Total IgE (left y-axis, geometric mean $\pm$ geometric SD) and sum of IgE densities (right y-axis, mean $\pm$ SD) in asthmatic (A) and rhinitic (R) subjects.

subjects with multiple sensitizations, IgE density appears in satisfactory agreement with the nasal response to the inhaled allergens, allowing a graduation of the intensity of multiple sensitizations. In asthmatic subjects the confounding effect of non-specific airway responsiveness blunts the predicting value of this parameter.

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