

Poor association between allergen-specific serum immunoglobulin E levels, skin sensitivity and basophil degranulation: a study with recombinant birch pollen allergen Bet v 1 and an immunoglobulin E detection system measuring immunoglobulin E capable of binding to FcεRI

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Summary

Background Results from several studies indicate that the magnitude of immediate symptoms of type I allergy caused by allergen-induced cross-linking of high-affinity Fcε receptors on effector cells (mast cells and basophils) is not always associated with allergen-specific IgE levels.

Objective To investigate the association of results from intradermal skin testing, basophil histamine release and allergen-specific IgE, IgG1–4, IgA and IgM antibody levels in a clinical study performed in birch pollen-allergic patients ($n = 18$).

Methods rBet v 1-specific IgEs were measured by quantitative CAP measurements and by using purified FcεRI-derived α -chain to quantify IgE capable of binding to effector cells. Bet v 1-specific IgG subclasses, IgA and IgM levels were measured by ELISA, and basophil histamine release was determined in whole blood samples. Intradermal skin testing was performed with the end-point titration method.

Results Our study demonstrates on the molecular level that the concentrations of allergen-specific IgE antibodies capable of binding to FcεRI and biological sensitivities are not necessarily associated. A moderate association was found between cutaneous and basophil sensitivity.

Conclusion Our results highlight the quantitative discrepancies and limitations of the present diagnostic tools in allergy, even when using a single allergenic molecule. The quantity of allergen-specific serum IgE is only one component of far more complex cellular systems (i.e. basophil-based tests, skin tests) used as indirect diagnostic tests for IgE-mediated allergic sensitivity.

Keywords basophil histamine release, Bet v 1, birch pollen, IgE, intradermal skin testing, recombinant allergen, type I allergy

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Introduction

The cross-linking of effector cell (mast cells and basophils)-bound IgE antibodies by allergens is a crucial event for the induction of the immediate symptoms of type I allergy [1]. As described in the classical experiments by Prausnitz and Küstner [2], this event depends on three major factors, i.e. allergen-specific IgE antibodies, effector cells and allergens. Because the characterization of IgE antibodies and the development of diagnostic tests capable of measuring the precise amount of allergen-specific IgE antibodies, several studies have investigated the association of allergen-specific serum IgE levels and biological sensitivity to allergens in allergic patients [3–10]. It is well established that the presence of allergen-specific serum IgE is a pre-requisite for the

occurrence of an immediate type of reaction, but whether the amount of allergen-specific IgE correlates with immediate-type sensitivity to the given allergen has been a matter of great debate. To address the problem almost all of the investigations carried out in the past have used allergen extracts, i.e. mixtures of allergens and non-allergenic molecules [3–6, 9, 10]. This is the reason why these studies could not analyse the association between allergen-specific IgE levels and biological activities at molecular levels. Recent studies using purified natural and recombinant allergens to re-investigate the relation between skin sensitivity and allergen-specific IgE levels report considerable discrepancies between these parameters [7, 8].

In our study, we have used purified recombinant Bet v 1, the major birch pollen allergen, as a paradigmatic tool to further investigate the association between allergen-specific IgE levels, effector cell responses and *in vivo* sensitivity. In a population of 18 birch pollen-allergic patients, selected on well-defined clinical criteria, and out of the pollen season, we

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quantified skin sensitivity and basophil degranulation in response to defined amounts of structurally folded recombinant Bet v 1. The results of the biological and of the serological tests were compared. For the measurement of Bet v 1-specific IgE antibody levels we used two different assays: one to detect any Bet v 1-specific IgE, and the other to detect Bet v 1-specific IgE able to bind to effector cells.

Material and methods

Study population

The study was performed between January and April before the beginning of the birch pollen season in Strasbourg, France. Eighteen patients, eight women and 10 men aged between 28 and 58 years (mean age: 45.6 years), were included in the study on the basis of clinical history of birch pollinosis and positive skin prick tests to birch pollen extract. All patients had moderate to severe rhino-conjunctivitis first diagnosed at least 3 years before. Five patients had mild asthma during birch pollen season and 12 patients had oral allergy syndrome with fruits of the Rosaceae family (apple, peach, apricot and almonds) and vegetables from Solanaceae (potato, tomato) and Apiaceae family (celery, carrot). Skin prick tests with a standard panel of respiratory allergens (Stallergènes, France) consisting of house dust mites, mixtures of fungal allergens, dog and cat dander, cockroach, grass, trees (including birch, olive and ash) and weed pollens were performed to identify the sensitization profile. Patients' characteristics are displayed in Table 1.

Study design

To analyse the possible association between allergen-specific IgE levels, skin sensitivity, and basophil degranulation, patients were bled and their skin was tested on the same day. The analyses were carried out strictly out of the birch pollen season to exclude effects because of seasonal allergen contact. Patients were not allowed to take anti-allergic or anti-inflammatory medication at least 1 week before the study was performed. None of the patients had received allergen-specific immunotherapy over the last 5 years. After informed consent was given, blood was collected for basophil histamine release and for serum sampling. Immediately thereafter, intradermal skin tests were performed using the end-point titration method [11].

Detection and quantification of allergen-specific antibodies

rBet v 1-specific IgE levels were quantified using the Pharmacia CAP system and were expressed as kU/L. In addition, ^{125}I -labelled recombinant α -chain of the high-affinity Fc ϵ RI was used for quantification of Bet v 1-specific IgE in a RAST-based assay under conditions of allergen excess. Purified α -chain was iodine labelled using the chloramine T method [12, 13]. Nitrocellulose dotted rBet v 1 (2 μg per dot) was exposed to two serum dilutions (1:5 and 1:10) of the allergic patients' sera in 50 mmol sodium phosphate pH 7.5 containing 0.5% w/v BSA, 0.5% v/v Tween 20 and 0.05% NaN_3 as described [14]. Bound

IgE antibodies were detected with ^{125}I -labelled α -chain (100 000 counts per minute (c.p.m.)/mL) by overnight incubation and quantified by gamma counting. All determinations were carried out in duplicate, and the results are expressed in means (c.p.m.) with less than 10% deviation.

Allergen-specific IgG1 to IgG4 subclass levels as well as allergen-specific IgM and IgA levels were measured by ELISA using isotype-specific monoclonal antibodies as described [15]. Results represent means of duplicate determinations and are shown as optical density values corresponding to the amount of bound antibodies.

Basophil histamine release test

The challenge of whole blood with rBet v 1 and anti-IgE as a positive control was performed in a dose-response fashion according to the method described by Tanizaki et al. [16]. Ten millilitres of venous blood was drawn into a plastic syringe containing 1 mL of heparin. Two hundred and fifty microlitres of different concentrations of rBet v 1 (from 10^{-4} to $10 \mu\text{g/mL}$) or anti-IgE (from 10^{-4} to 10^{-3} ; ϵ -specific, Dako, Glostrup, Denmark) were added to the test tubes containing 500 μL of whole blood diluted 1:4 in Tris buffer (10 mmol/L Tris, 136 mmol/L NaCl, 2.7 mmol/L KCl, 0.23 mmol/L MgCl_2 , 1.8 mmol/L CaCl_2 , 5.5 mmol/L glucose; pH 7.3). The mixed solution was incubated for 30 min at 37°C . The reaction was stopped, and the cells were separated by cold centrifugation (4°C) at 375 g for 5 min. Two hundred microlitres of the cell-free supernatant was used for histamine quantification in a radioimmunoassay with acylated histamine monoclonal antibodies (Immunotech, Marseille, France) as described previously [17]. Total histamine was measured after cell lysis by repeated thawing and freezing. All experiments were performed in duplicate.

The parameter used to describe the degree of basophil sensitivity was the lowest allergen concentration inducing 30% of total histamine release.

Intradermal testing

Threshold intradermal skin tests were performed by injection of 0.03 mL of 10-fold dilutions of rBet v 1 on the lateral part of the arm. Serial dilutions were prepared from a solution of $1000 \mu\text{g/mL}$ and the first dilution tested was $10 \mu\text{g/mL}$. The tests were read 15 min after injection. The area of weal and erythema was recorded. The test was considered positive when the induced weal area exceeded that of the weal induced by injection and the lowest concentration of allergen inducing a positive test result was used for comparison with other parameters [11].

Statistical analysis of the data

Correlation between different parameters was tested by Spearman's non-parametric tests using VisualStat[®] Professional software (version 2003).

Table 1. Clinical data, results of serology, basophil histamine release and skin testing for the study population

N	Initials	Age	Symptom	Positive prick test	Food allergy	+ID test	30% HR	IgE class	Specific IgE (kU/L) (S)	Total IgE (kU/L) (T)	IgE (c.p.m.)						
											(S/T)%	1:5	1:10	IgG1	IgG2	IgG3	IgG4
1	F-T	58	R-C	m, b, o, g	a, c, p, al, n	10 ⁻³	0.3 × 10 ⁻²	3	12.1	30.6	39.5	237	167	0.24	0.15	0.181	0.131
2	S-F	33	R-C	b		0.3 × 10 ⁻¹	0.3 × 10 ⁻²	4	24.1	142	16.9	400	259	0.798	0.111	0.092	0.408
3	W-F	53	R-C	b, o	a, p, n	10 ⁻¹	10 ⁻¹	3	5.22	11.5	45.4	95	61	0.537	0.079	0.788	0.082
4	F-JJ	51	R-C	b		10 ⁻³	0.3 × 10 ⁻³	5	59.9	128	46.8	1800	856	1.066	0.106	0.072	0.198
5	G-S	49	R-C	m, b	a, p, ap	0.3 × 10	10 ⁻¹	3	17.1	33	51.8	177	112	0.187	0.074	0.065	0.075
6	S-S	50	R-C	b, o, w, c		1	0.3 × 10 ⁻²	4	41.1	168	24.5	630	318	0.287	0.081	0.081	0.103
7	B-A	39	R-C, A	b, a		0.3 × 10	0.3 × 10 ⁻¹	4	20	43	46.5	233	152	0.298	0.083	0.081	0.071
8	O-C	37	R-C	b, o, a, w	a, c, p	10	10 ⁻¹	5	79.9	231	34.6	1737	565	0.658	0.09	0.83	0.209
9	L-N	44	R-C, A	b, a	a, n	10 ⁻⁵	10 ⁻²	4	26.6	115	23.1	351	195	1.325	0.098	0.074	0.136
10	M-C	43	R-C, A	b, o		10 ⁻³	0.3 × 10 ⁻³	3	4.51	6.9	65.4	84	64	0.5	0.173	0.063	0.06
11	H-C	58	R-C	b, o, a	a	10 ⁻⁴	10 ⁻⁴	4	22.7	113	20	321	114	0.505	0.1	0.068	0.208
12	P-D	49	R-C, A	m, b, a, g	a, c, ap, p, n, ce, ca	10 ⁻²	1	3	17.4	94.5	18.4	227	116	1.043	0.075	0.065	0.129
13	H-M	41	R-C	m, b, o	a, c, p, al, n	0.3 × 10 ⁻¹	10 ⁻²	5	51.5	82.2	62.6	879	102	0.183	0.059	0.065	0.062
14	W-S	53	R-C, A	m, b	a	0.3 × 10 ⁻²	0.3 × 10 ⁻¹	4	45.5	72.3	62.9	945	130	0.236	0.161	0.075	0.889
15	B-E	28	R-C	b, g	n, al	1	10 ⁻²	3	14	90.9	15.4	117	132	0.389	0.069	0.079	0.078
16	B-M	50	R-C	b, o, g	a, p	0.3 × 10 ⁻²	10 ⁻³	3	4.74	16.5	28.7	156	126	0.14	0.064	0.068	0.065
17	W-B	46	R-C	m, b, g, o, w, c	a, ca, k	10 ⁻¹	10 ⁻¹	4	21.4	91.3	23.4	107	100	0.344	0.086	0.075	0.111
18	S-B	40	R-C	b		1	1	2	1.65	NA	NA	117	78	0.113	0.061	0.065	0.074

Symptoms: R, rhinitis; C, conjunctivitis; A, asthma.

Positive prick test: m, mites; b, birch; o, olive; g, grass; w, weeds; a, ash; c, cat.

Food allergy: a, apple; ap, apricot; c, cherry; p, peach; al, almond; n, nuts; k, kiwi; ce, celery; ca, carrot.

ID test, intradermal test; HR, histamine release (values in µg/mL); c.p.m., counts per minute.

Results

Poor association between rBet v 1-specific immunoglobulin E levels and skin sensitivity to rBet v 1

To compare rBet v 1-specific IgE levels and skin sensitivity, rBet v 1-specific IgE levels were measured by CAP and correlated with the threshold concentration of rBet v 1 inducing a positive intradermal test wheal reaction. Figure 1 shows that there is no association between allergen-specific IgE levels and skin sensitivity ($r = -0.007$, $P = 0.977$). In individual patients we observed a strong discrepancy between allergen-specific IgE and skin sensitivity. For example, patient 8 exhibited high Bet v 1-specific IgE level (79.9 kU/L) but showed a positive ID reaction only at 10 µg/mL of rBet v 1 (Table 1). On the other hand, patient 10 had low rBet v 1-specific IgE (4.5 kU/L), yet with a 1000-fold greater skin sensitivity to Bet v 1 (positive ID test reaction at 1 ng/mL of rBet v 1) than patient eight. Seven patients (2, 5, 7, 9, 11, 12 and 17) with similar rBet v 1-specific IgE levels (17.1–26.6 kU/L) exhibited an extremely broad range of skin sensitivity to rBet v 1 (from 3 to 10^{-5} µg/mL) (Table 1).

Poor association between rBet v 1-specific immunoglobulin E levels and rBet v 1-related basophil sensitivity

Figure 2 (IgE vs. 30% histamine release) shows that there is also a lack of association between rBet v 1-specific IgE levels and Bet v 1-induced basophil sensitivity (Fig. 2: $r = -0.113$, $P = 0.656$). The concentration of rBet v 1 required to induce 30% histamine release varied from 10^{-3} to 1 µg/mL. For given levels of rBet v 1-specific IgE (RAST class 3: 4.51–17.1 kU/L), the concentration of rBet v 1 inducing 30% histamine release varied 1000-fold (1 – 10^{-3} µg/mL).

Association between rBet v 1-induced basophil histamine release and skin sensitivity

Figure 3 shows that the results of intradermal testing and basophil histamine release tests are better associated than the results of serological and biological tests. There is a significant

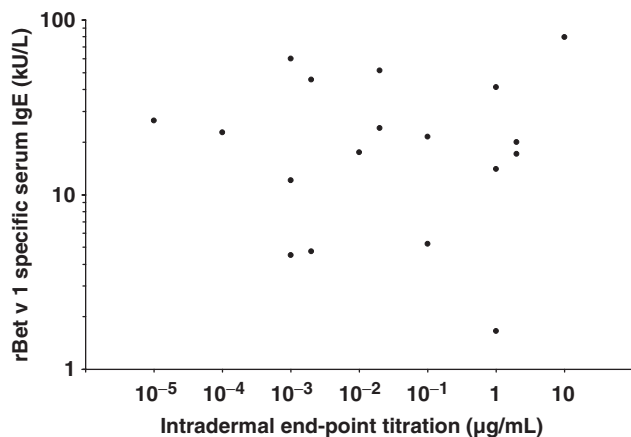


Fig. 1. Association of results from intradermal end-point titration (x-axis: allergen concentration giving the first positive reaction) and rBet v 1-specific serum IgE (y-axis: kU/L CAP System).

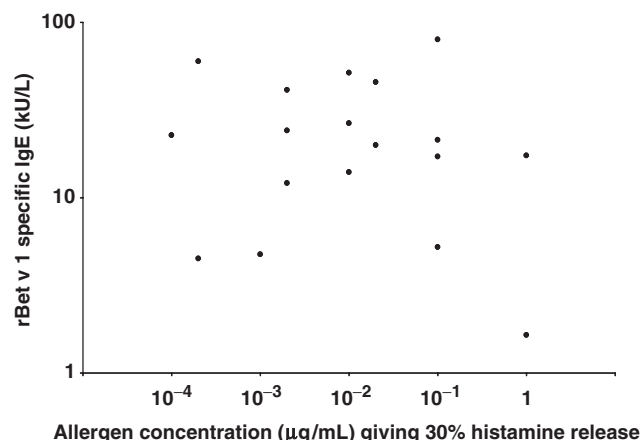


Fig. 2. Association of results from basophil histamine release (x-axis: allergen concentration giving 30% histamine release) and rBet v 1-specific serum IgE (y-axis: kU/L CAP System).

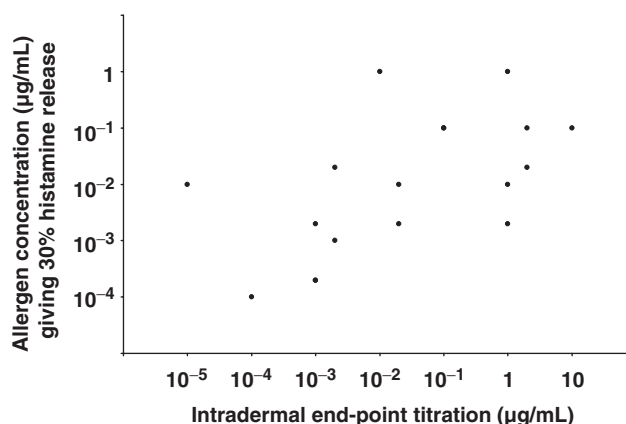


Fig. 3. Association of results from intradermal end-point titration (x-axis: allergen concentration giving the first positive reaction) and results from basophil histamine release (y-axis: allergen concentration giving 30% histamine release).

trend between the concentrations of rBet v 1 eliciting 30% histamine release and intradermal wheal reactions ($r = 0.614$; $P = 0.007$). Patients with extremely bad association between rBet v 1-specific IgE levels and results of biological testing (e.g. patients 8 and 10) showed better association when intradermal testing results were compared with basophil histamine release (Table 1).

Results of other tests performed in order to explain the discrepancies between serological and biological tests are given below.

Measurements of α -chain-bound rBet v 1-specific immunoglobulin E

In order to investigate whether the presence of biologically inactive IgE (e.g. α -chain-bound IgE) in serum might be responsible for the discrepancy between allergen-specific IgE levels and cellular as well as *in vivo* sensitivity, we used labelled α -chain for quantification of allergen-specific IgE (Table 1). We observed a good correlation between the levels of rBet v 1-specific IgE as determined by CAP measurements and by using labelled α -chains [for 1:5 dilution $r = 0.888$;

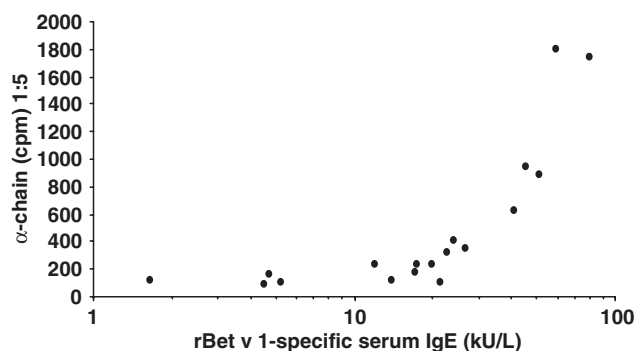


Fig. 4. Association of Bet v 1-specific IgE determined by CAP (x-axis: kU/L) and of rBet v 1-specific IgE determined with labelled α -chain (y-axis: counts per minute (c.p.m.); 1:5 serum dilution).

$P = 0.000$ (Fig. 4) and for 1:10 dilution $r = 0.626$; $P = 0.005$ (data not shown)]. We observed no improvement of the correlation between rBet v 1-specific IgE levels determined with labelled α -chain and results of skin sensitivity (for 1:5 dilution: $r = -0.108$; $P = 0.670$; for 1:10 dilution: $r = -0.013$; $P = 0.958$) or between rBet v 1-specific IgE levels determined with labelled α -chain and histamine release tests (for 1:5 dilution: $r = -0.229$; $P = 0.360$ and for 1:10 dilution: $r = -0.305$; $P = 0.219$).

Measurements of rBet v 1-specific immunoglobulin G subclasses, immunoglobulin A and immunoglobulin M

It has been described that Bet v 1-allergic patients' sera contain Bet v 1-specific IgG antibodies that may interfere with IgE binding to Bet v 1 or recognize epitopes on the Bet v 1 molecule other than IgE and hence have no effect on IgE binding to Bet v 1 [18, 19]. Therefore we determined the levels of rBet v 1-specific IgG (IgG1–IgG4; Table 1). The patients exhibited varying rBet v 1-specific IgG1–IgG4 subclass responses with most pronounced responses in the IgG1 and IgG4 subclasses. No significant levels of rBet v 1-specific IgA and IgM antibodies were detected in the sera, excluding the possibility that these antibody classes may influence IgE recognition of Bet v 1 (data not shown).

Evaluation of Bet v 1-specific immunoglobulin E as a percentage of total immunoglobulin E

If Bet v 1-specific IgE only accounts for a low percentage of total IgE, poor histamine release and skin reactivity might be explained by the fact that basophils and mast cells are primarily occupied by IgE directed against other allergens. We therefore determined the total IgE values and calculated the percentage of Bet v 1-specific IgE. The patients in our study had relatively low total IgE values (< 168 kU/L) and we found no association between a low percentage of Bet v 1-specific IgE and poor biological responses. For example, in patient 11, who showed high sensitivity, Bet v 1-specific IgE only accounted for 20% of the total IgE. On the other hand, patient 13 was less sensitive, although 62.6% of the total IgE was directed against Bet v 1 (Table 1).

Discussion

The question of whether allergen-specific IgE antibody levels, effector cell sensitivities, and clinical sensitivity correlate remains a matter of controversy. Several studies have shown a significant correlation of allergen-specific serum IgE antibodies with allergen-induced immediate type reactions even when using a complex mixture of various allergenic and non-allergenic components, which may make it difficult to compare skin tests and RAST [3–6, 9, 10]. Recently, other studies using purified allergens [7] and recombinant allergens [8] have demonstrated considerable discrepancies between antibody levels and biological sensitivity.

We have performed a clinical study using a defined purified and structurally folded allergen (i.e. the major birch pollen allergen, Bet v 1) to investigate the relation between specific IgE, basophil degranulation, and skin sensitivity at a molecular level. We found good agreement between the three methodologies and clinical relevance of birch sensitivity; however, strong discrepancies were noted between the levels of allergen-specific IgE, the basophil sensitivity and *in vivo* sensitivity (i.e. skin sensitivity as determined by end-point titration). In certain patients, we observed very low specific IgE levels but high sensitivity in basophil degranulation and skin tests and vice versa. A review of the literature reveals the scarcity of studies comparing skin tests, basophil histamine release and specific IgE levels. The few available studies showed greatly varying results and were performed with crude allergen extracts. For example, Norman et al. [9] found that the three tests were in good agreement with each other in the diagnosis of ragweed hayfever. Lichtenstein et al. [10] found a quantitatively significant relationship between skin tests and histamine release. However, no measurement of specific IgE was performed in this study.

The response of sensitized leucocytes and mast cells to antigen can depend on a great variety of factors.

One possibility for low sensitivity and poor release of histamine would be that only a small proportion of the total serum IgE accounts for allergen-specific IgE. We have therefore determined the total IgE levels and calculated the percentage of allergen-specific IgE. However, we did not find an association between low percentages of allergen-specific IgE responses and poor biological activity, which leads us to exclude this possibility, at least for our study population. The possibility that a low percentage of specific IgE out of the total IgE may be responsible for poor biological responses towards the given allergen may be of greater importance in polysensitized subjects [9, 20, 21].

In order to address the possibility that the discrepancy between allergen-specific IgE levels and biological test results could be because of the presence of allergen-reactive IgE antibodies that cannot bind to mast cells and basophils and therefore fail to induce the release of biological mediators, we also performed IgE measurements with purified recombinant α -chain of the high-affinity Fc ϵ R for IgE. This was based on the assumption that the α -chain will detect biologically active IgE that can bind to Fc ϵ RI on effector cells and induce mediator release. Furthermore, the presence of soluble Fc ϵ RI in sera may bind to IgE and generate a biologically inactive form of IgE. The results of allergen-specific IgE measurements performed with purified α -chain also showed the lack

of association between the magnitude of IgE levels and biological sensitivity.

Another possibility that cannot be excluded at this time is that allergen-specific antibodies of a class other than IgE interfered with the binding for allergens. Although we found no association between the presence of allergen-specific IgG antibodies in sera and reduced biological sensitivity, we must be aware that not all of the allergen-specific IgG antibodies detected by ELISA in the sera will block the binding of IgE to Bet v 1 [18, 19].

There are several other factors that may be responsible for the discrepancy between allergen-specific IgE levels and biological responses but they cannot be addressed even in a system using purified allergens. They include interindividual differences in basophil and mast cell sensitivities because of variability in IgE-receptor cell surface density, a parameter that is regulated by serum IgE levels [20, 22, 23, 24]. Different cell sensitivities have been demonstrated by variable shifts of the dose-response curves (measured by 50% or 30% sensitivity) in case of similar total and antigen-specific IgE serum concentrations [20, 25]. Furthermore, it has been shown that persons with equivalent numbers of IgE molecules on basophils may release 0–100% of their histamine content [20]. The same has been observed for cutaneous mast cells [26, 27]. In addition, it has been shown that early signal events occur involving syk-kinase and IP₃ products, which are not linked to the level of specific IgE or basophil sensitivity [25, 28, 29].

Recent evidence indicates that mast cells may also be influenced via Toll-like receptors [30]. However, the rBet v 1 preparation used for our experiments did not contain endotoxins.

Finally, it is possible that the presence of IgE antibodies with varying affinities or binding specificities for epitopes inducing varying anaphylactic activity may have influenced serological and biological test results.

In conclusion, our study demonstrates on a molecular level that allergen-specific serum IgE levels are not necessarily related to the biological sensitivity as determined by cellular and *in vivo* tests. A moderate association was, however, found between the cutaneous tests and the basophil histamine release tests. Our study thus supports the interest of *in vitro* tests; however, we must acknowledge that at the moment they are only clinically relevant in case of corresponding symptoms.

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References

- Kawakami T, Galli SJ. Regulation of mast-cell and basophil function and survival by IgE. *Nat Rev Immunol* 2002; 2:773–86.
- Prausnitz C, Küstner J. Studien über die Überempfindlichkeit. *Centralbe F Bact 1 Abt Orig* 1921; 86:160–8.
- Stenius B, Wide L, Seymour WM, Holford-Strevens V, Pepys J. Clinical significance of specific IgE to common allergens. I. Relationship of specific IgE against *Dermatophagoides* spp. and grass pollen to skin and nasal tests and history. *Clin Allergy* 1971; 1:37–55.
- Bryant DH, Burns MW, Lazarus L. The correlation between skin tests, bronchial provocation tests and the serum level of IgE specific to common allergens in patients with asthma. *Clin Allergy* 1975; 5:145–57.
- Pauli G, Bessot JC, Thierry R, Lamensans A. Correlation between skin tests, inhalation tests and specific IgE in a study of 120 subjects allergic to house dust and *Dermatophagoides pteronyssinus*. *Clin Allergy* 1977; 7:337–46.
- Bousquet J, Lebel B, Dhivert H, Bataille Y, Martinot B, Michel FB. Nasal challenge with pollen grains, skin-prick tests and specific IgE in patients with grass pollen allergy. *Clin Allergy* 1987; 17: 529–36.
- Witteaman 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.
- Niederberger V, Stübner P, Spitzauer S et al. Skin test results but not serology reflects immediate type respiratory sensitivity: a study performed with recombinant allergen molecules. *J Invest Dermatol* 2001; 117:848–51.
- Norman PS, Lichtenstein LM, Ishizaka K. Diagnostic tests in ragweed hay fever. A comparison of direct skin tests, IgE antibody measurements, and basophil histamine release. *J Allergy Clin Immunol* 1973; 52:210–24.
- Lichtenstein LM, Norman PS, Winkenwerder WL. The quantitative relationship between skin testing and leukocyte histamine release with antigen E, group I antigen, crude grass, and ragweed extracts. *J Allergy Clin Immunol* 1971; 47:103 (A37).
- Grammer LC, Shaughnessy MA, Shaughnessy JJ, Patterson R. Brief report: evaluation of polymerized ragweed extracts by intradermal end point titration, RAST inhibition, and parallel line bioassay. *J Allergy Clin Immunol* 1985; 76:123–7.
- Sambrook J, Fritsch EF, Maniatis T. Molecular cloning. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1989.
- Laffer S, Hoggom E, Roux KH et al. A molecular model of type I allergy: identification and characterization of a nonanaphylactic anti-human IgE antibody fragment that blocks the IgE–FcεRI interaction and reacts with receptor-bound IgE. *J Allergy Clin Immunol* 2001; 108:409–16.
- Valenta R, Duchene M, Ebner C et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992; 175: 377–85.
- Vrtala S, Susani M, Sperr WR et al. Immunologic characterization of purified recombinant timothy grass pollen (*Phleum pratense*) allergens (Phl p 1, Phl p 2, Phl p 5). *J Allergy Clin Immunol* 1996; 97:781–7.
- Tanizaki Y, Komagoe H, Morinaga H, Kitani H, Goda Y, Kimura I. Allergen- and anti-IgE-induced histamine release from whole blood. *Int Arch Allergy Appl Immunol* 1984; 73:141–5.
- Morel AM, Delaage MA. Immunoanalysis of histamine through a novel chemical derivatization. *J Allergy Clin Immunol* 1988; 82:646–54.
- Visco V, Dolecek C, Denepoux S et al. Human IgG monoclonal antibodies that modulate the binding of specific IgE to birch pollen Bet v 1. *J Immunol* 1996; 157:956–62.
- Denepoux S, Eibensteiner PB, Steinberger P et al. Molecular characterization of human IgG monoclonal antibodies specific for the major birch pollen allergen Bet v 1. Anti-allergen IgG can enhance the anaphylactic reaction. *FEBS Lett* 2000; 465: 39–46.
- Conroy MC, Adkinson NF Jr, Lichtenstein LM. Measurement of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. *J Immunol* 1977; 118:1317–21.
- MacGlashan DW Jr, Peters SP, Warner J, Lichtenstein LM. Characteristics of human basophil sulfidopeptide leukotriene

- release: releasability defined as the ability of the basophil to respond to dimeric cross-links. *J Immunol* 1986; 136: 2231–9.
- 22 Malveaux FJ, Conroy MC, Adkinson NF Jr, Lichtenstein LM. IgE receptors on human basophils. Relationship to serum IgE concentration. *J Clin Invest* 1978; 62:176–81.
- 23 Dembo M, Goldstein B. Theory of equilibrium binding of symmetric bivalent haptens to cell surface antibody: application to histamine release from basophils. *J Immunol* 1978; 121: 345–53.
- 24 MacGlashan DW Jr, Lichtenstein LM, McKenzie-White J et al. Upregulation of FcεpsilonRI on human basophils by IgE antibody is mediated by interaction of IgE with FcεpsilonRI. *J Allergy Clin Immunol* 1999; 104:492–8.
- 25 MacGlashan DW Jr. Releasability of human basophils: cellular sensitivity and maximal histamine release are independent variables. *J Allergy Clin Immunol* 1993; 91:605–15.
- 26 Petersen LJ, Molsbech H, Skov PS. Allergen-induced histamine release in intact human skin in vivo assessed by skin microdialysis technique: characterization of factors influencing histamine releasability. *J Allergy Clin Immunol* 1996; 97:672–9.
- 27 Bordinon V, Parmiani S. Reproducibility and use of low concentration skin prick test. *Invest Allergol Clin Immunol* 2000; 10:78–82.
- 28 Miura K, Lavens-Phillips S, MacGlashan W Jr. Localizing a control region in the pathway to LTC₄ secretion following stimulation of human basophils with anti-IgE antibody. *J Immunol* 2001; 167:7027.
- 29 MacGlashan DW Jr. Two regions of down-regulation in the IgE-mediated signaling pathway in human basophils. *J Immunol* 2003; 170:4914–25.
- 30 Marshall JS, McCurdy JD, Olynch T. Toll-like receptor-mediated activation of mast cell: implications for allergic disease? *Int Arch Allergy Immunol* 2003; 132:87–97.