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Antigens Drive Memory IgE Responses in Human Allergy via the Nasal Mucosa

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Key Words
Allergy · IgE memory · Recombinant allergen · Nasal mucosa · Nasal provocation

Abstract
Background: Natural allergen contact induces an increase of IgE levels and sensitivity but the mechanisms underlying the allergen-specific memory responses are poorly understood. Furthermore, it has not been studied whether allergen exposure affects the molecular reactivity profiles in patients. The aim of this study was to analyze the influence of nasal allergen encounter on the molecular profile and magnitude of memory IgE responses and on systemic sensitivity. ***Methods:*** We investigated allergen-specific IgE, IgG subclass and IgM responses to defined allergen molecules (grass pollen: Phl p 1, Phl p 2 and Phl p 5; birch pollen: Bet v 1 and Bet v 2) in allergic patients in response to natural as well as to controlled nasal and dermal allergen exposure. Changes in systemic sensitivity were monitored by skin prick testing and by basophil histamine release experiments. ***Results:*** Respiratory antigen exposure boosted IgE levels to a pre-established profile of allergen molecules without inducing significant IgM responses or new IgE specificities in allergic individuals. The importance of the route of allergen contact is demonstrated by an increase of systemic IgE levels and sensitivity after nasal exposure. In vitro sensitisation of basophils with pre- and post-seasonal serum samples suggests an allergen-induced elevation of specific IgE as a cause for the increased allergen-specific sensitivity. ***Conclusion:*** The characteristics of the allergen-driven antibody responses indicate a direct activation of an established pool of IgE memory cells with defined specificities as an underlying mechanism. Our finding that nasal allergen contact is a major factor for the boosting of memory IgE and systemic sensitivity may open new therapeutic possibilities.

Introduction

Memory antibody responses and mechanisms governing their activation or silencing are important for protective immunity (e.g. vaccination) and in a variety of immunological diseases (e.g. allergy, autoimmunity and infectious diseases) [1]. In type I allergy, a genetically determined hypersensitivity disease affecting more than 25% of the population [2], acute as well as chronic in-

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flammation is mediated by allergen-specific IgE antibodies [3]. Cross-linking of mast cell and basophil-bound IgE antibodies by allergens induces the release of inflammatory mediators and thus leads to immediate type inflammation [4]. IgE-facilitated presentation of allergens via the low- and high-affinity receptor for IgE on antigen-presenting cells can cause the activation of allergen-specific T cells and the release of pro-inflammatory cytokines associated with chronic disease [5]. The formation of allergen-specific IgE antibodies occurs in atopic individuals early in life and is induced by allergen contact [6]. This process of allergic sensitisation involves the switch from allergen-specific IgM to IgE-producing B cells and plasma cells [7, 8]. Studies performed with purified allergens and epitopes have suggested that the IgE reactivity profile in sensitised allergic individuals is constant and shows features of a typical memory (i.e. secondary) antibody response [9]. Since the serum half-life of IgE antibodies is very short [10, 11], continuous antibody production is necessary to sustain allergen-specific IgE levels responsible for allergic inflammation.

It has been suggested that long-lived plasma cells are those cells which are responsible for the maintenance of long-term antibody production [12–14]. This may also be true for allergy because it has been shown that IgE serum titres correlate with IgE+ plasma cell frequency, indicating that plasma cells may represent an important source of allergen-specific IgE antibodies [15]. Furthermore, it is known that allergy can be transferred by the transplantation of bone marrow from allergic donors to non-allergic hosts [16].

Antigen-specific memory responses may be maintained by a variety of mechanisms including persisting antigen, cross-reactivity, regulatory mechanisms as well as antigen-independent pathways (e.g. polyclonal stimulation) [1, 17–20]. For the maintenance and boosting of IgE memory, 2 mechanisms have been proposed. First, there is evidence that allergen exposure leads to an increase of allergen-specific IgE antibody levels [21, 22]. In this context it has been suggested that the respiratory tract (i.e. nasal and bronchial mucosa) which is rich in IgE-positive cells may be involved in the increase of serum IgE levels after local allergen contact [23–27]. Second, it is possible that cross-reactivity with endogenous antigens may be involved in the maintenance of IgE memory against certain allergens [28].

The analysis of the mechanisms governing IgE memory responses has been hampered by the fact that experimental animal models for allergy are mainly based on the injection of adjuvanted allergens and/or the administration of unphysiologically high allergen doses (i.e. more than 1,000-fold higher compared to natural allergen exposure) [29]. In order to study the mechanisms underlying the regulation of allergen-specific IgE memory responses directly in allergic humans we have used 2 experimental approaches with defined pollen allergens. In one set of experiments we investigated allergen-specific IgE, IgG subclass and IgM responses to defined allergen molecules in a group of allergic patients in response to seasonal allergen exposure over a period of 1 year. In a second approach, the effects of controlled allergen exposure via the nasal mucosa and skin on systemic humoral immunity and sensitivity were investigated. The results of both approaches are consistent with a model in which nasal allergen exposure leads to a strong and direct activation of IgE memory responses against a predefined allergen profile. The allergen-induced increase of specific serum IgE is linked to an aggravation of immediate allergic reactions caused by a loading of effector cells with specific serum IgE.

Material and Methods

Natural Pollen Extracts and Recombinant Allergens

Pollen from birch *(Betula verrucosa)* and timothy grass *(Phleum pratense)* were purchased from Allergon (Välinge, Sweden). Pollen allergen extracts were prepared as described [30]. Protein contents and the quality of the extracts were analysed by SDS-PAGE and Coomassie brilliant blue staining (Biorad, Richmond, Calif., USA) [31, 32].

Recombinant pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5, rPhl p 6, rBet v 1 and rBet v 2) were purchased from Biomay (Vienna, Austria).

Seasonal Allergen Exposure: Patients and Blood Samples

The development of total IgE, birch and timothy grass pollen-specific IgE and IgM, and IgE, IgG1–4 and IgM to individual recombinant pollen allergens during natural seasonal pollen exposure was studied in 15 patients (mean age 35 years) suffering from tree and/or grass pollen allergy (rhinitis, conjunctivitis or asthma during the tree and/or grass pollen season) (table 1: patients 1–15) and 15 individuals with a negative case history of pollen allergy (mean age 32 years). Serum samples were obtained at 5 time points during 1 year (February, April, June, September and January) and were stored at –20°C. At each appointment, allergic symptoms and anti-allergic medication were recorded. Pollen exposure in Vienna was recorded as described [33].

Controlled Allergen Exposure: Study Design

Nasal provocation tests and skin prick tests were done on the same day outside the pollen season. In order to allow a comparison of the effects of nasal and dermal allergen contact, patients underwent nasal and skin testing with different unrelated allergens. In 3 patients (I, IV and VIII) a second nasal provocation was

Table 1. Demographic and clinical data of patients

Patient	Sex	Age	Allergies	Symptoms
1	m	50	g, t	r
2	m	54	g, f	r, c, a
3	m	46	t, g, w, mi, a, f	r, c, d
4	f	32	t, g	r, c
5	m	29	g	r, c
6	m	33	t, g, f	r, c
7	f	26	g	r, c, a, d
8	m	28	t, g	r, a, d
9	f	27	t, g, mi, a	r, c
10	f	30	t	r, c, a
11	m	35	t, g	r, a
12	m	35	g	r, c
13	f	30	t	r
14	f	40	t, g, f	r, c
15	f	35	t, g	r, c, a
I	f	57	g, t	r, c
II	m	24	g, t, a, mi, mo, f	r, c, a
III	f	21	g, t	r, c
IV	m	32	g, a, mi	r, c, a
V	m	23	g, t, f	r, c, a
VI	f	22	g	r, c
VII	m	23	g, t	r, c
VIII	m	22	g, t, d	r, c, a

g = Grass pollen; t = tree pollen; a = animal dander; mi = mites; mo = moulds; f = foods; dr = drugs; r = rhinitis; c = conjunctivitis; a = asthma; d = dermatitis.

Table 2. Development of IgE levels and skin sensitivity to the allergens administered by the nasal and dermal route

Patient	Allergen	At the time of NP		3 weeks after NP		
		IgE kUA/l	SPT MD	IgE kUA/l	change of IgE, %	SPT MD
Nasal						
I	Phl p 5	6.9	49.0	9.9	+43.3	64.0
I	Phl p 2	1.7	36.0	2.4	+40.7	132.3
II	Bet v 2	4.4	20.3	4.5	+2.1	64.0
III	Phl p 5	14.2	42.3	18.8	+32.4	56.3
IV	Bet v 1	7.6	64.0	10.0	+31.6	156.3
IV	Phl p 2	15.7	196.0	19.4	+23.6	361.0
V	Phl p 5	7.1	64.0	9.1	+28.4	144.0
VI	Bet v 1	2.5	25.0	3.9	+56.5	20.3
VII	Bet v 1	0.4	30.3	0.6	+58.6	64.0
VIII	Bet v 1	7.1	49.0	11.5	+62.4	36.0
VIII	Phl p 5	12.0	64.0	13.3	+10.8	72.3
Mean		7.2	58.2	9.4	+30.0	106.4
Dermal						
I	Phl p 1	5.09	4.0	4.62	−9.2	20.25
II	Phl p 5	28.1	81.0	30.9	+10.0	36
III	Phl p 2	12.3	100.0	12.4	+0.8	90.25
IV	Phl p 1	15.5	n.d.	16.5	+6.5	n.d.
V	Phl p 1	3.21	9.0	3.14	−2.2	25
VI	Phl p 5	64.5	36.0	63.5	−1.6	42.25
VII	Phl p 5	7.52	169.0	8.48	+12.8	100
VIII	Phl p 1	5.23	6.3	5.19	−0.8	16
Mean		17.7	57.9	18.1	+2.3	47.1

NP = Nasal provocation; SPT = skin prick test; MD = mean area of skin reaction; n.d. = not determined.

performed with another allergen 3 weeks after the first provocation. Skin prick tests were repeated 3 weeks after the nasal provocation test. Blood samples were obtained at the time of nasal provocation and in weekly intervals thereafter for 5 weeks. The study was approved by the ethical committee of the Medical University of Vienna.

Controlled Nasal Allergen Exposure: Patients, Nasal Provocation and Blood Samples

Patients were selected according to a positive history indicative of tree and/or grass pollen allergy and positive CAP-RAST results for at least 2 of 5 selected allergens (timothy grass pollen: Phl p 1, Phl p 2 and Phl p 5; birch pollen: Bet v 1 and Bet v 2) (table 1: patients I–VIII). Four clinically and serologically non-allergic individuals were included for control purposes.

At the time of nasal provocation all patients had stable lung function, no evidence of airway infection and did not receive corticosteroids or antihistamines.

Nasal provocation was performed outside the pollen season with 1 selected allergen at a time. Purified recombinant allergens were diluted in sterile 0.9% sodium chloride solution (5, 10, 20 and 40 μg/ml) and administered using a metered pump delivering 15 μl per puff. These concentrations were selected according to pilot experiments which showed that most allergic patients have a positive reaction after provocation with 10 or 20 μg/ml of purified allergen. Patients received increasing doses of the respective allergen in 15-min intervals. The nasal reaction to provocation was monitored by anterior rhinomanometry [34]. Provocation was stopped when a nasal airflow reduction of 60% was exceeded.

Non-allergic control individuals underwent nasal provocation with the highest allergen concentration (40 μg/ml) and with 3 different recombinant allergens on the same day.

Blood samples from patients and control individuals were obtained at the time of nasal provocation and at weekly intervals for the 5 following weeks. Sera were analysed for IgE, IgG1–4 and IgM antibody levels to the allergen(s) used for provocation and to a control allergen.

Skin Prick Tests

Skin prick tests were performed with the allergens used for nasal provocation and with a control allergen (table 2) on the day of nasal provocation and 3 weeks thereafter. Allergens were di-

luted in 0.9% sodium chloride solution (20 μg/ml), and sterile 0.9% sodium chloride solution and histamine hydrochloride (ALK, Horsholm, Denmark) were used for control purposes. Twenty-microlitre aliquots of the test solutions were placed on the patients' forearms at a distance of more than 3 cm between individual application points. Skin prick tests with recombinant allergens were carried out in duplicates. Reactions were recorded 20 min after testing by transferring the ball point pen-surrounded wheal reaction with a scotch tape to paper. The maximal longitudinal (D1) and transversal (D2) diameter of the wheal was measured by 2 individual, blinded investigators and the mean diameter of the wheal was calculated according to the formula $((D1 + D2)/2)^2$.

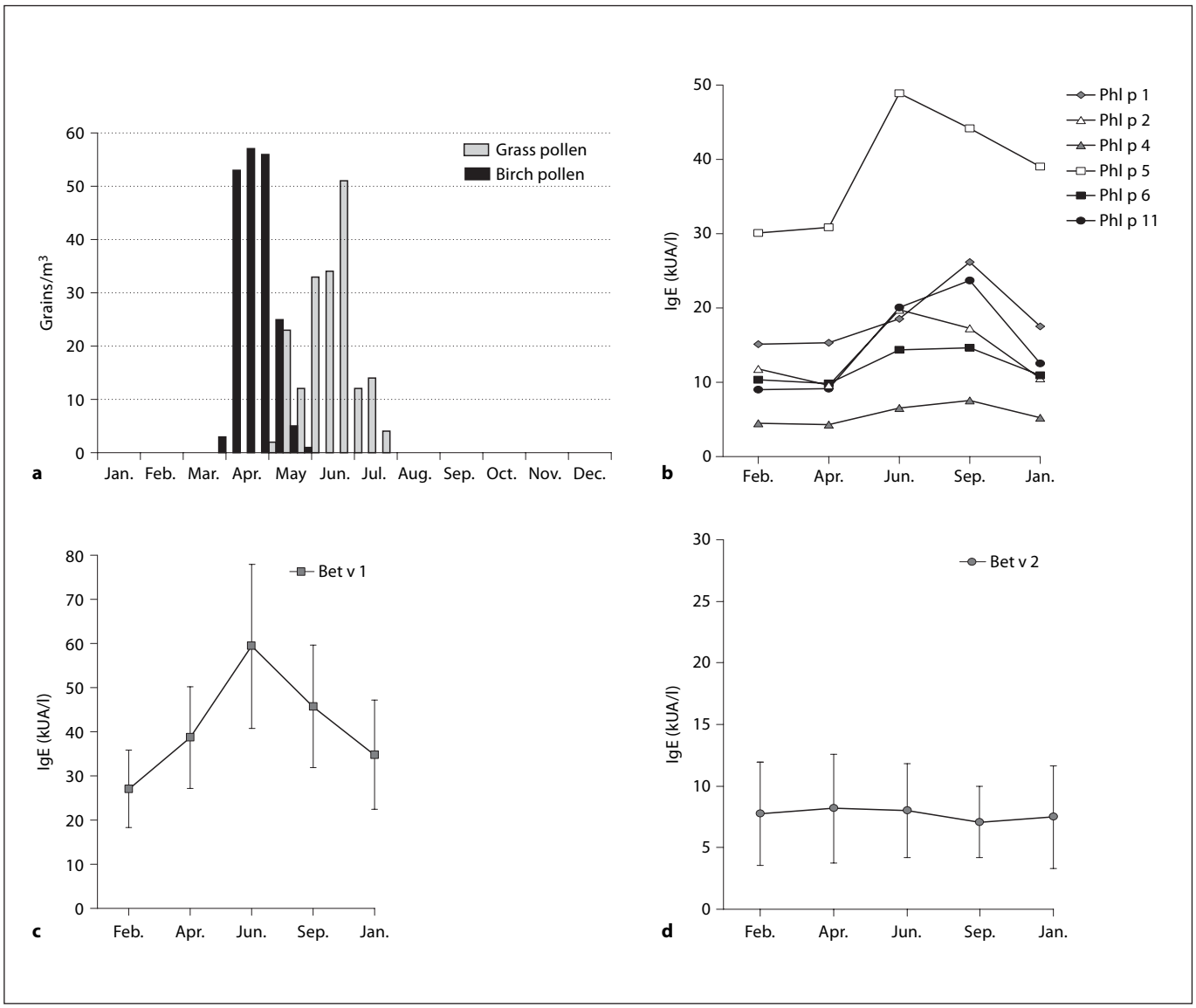
Determination of Total and Specific IgE

Total and specific IgE levels to birch pollen extract, timothy grass pollen extract and recombinant birch and timothy grass pollen allergens were determined by quantitative CAP-FEIA measurements (Pharmacia, Uppsala, Sweden).

Determination of Allergen-Specific IgG1–4 and IgM Levels

Measurement of IgG1–4 subclass levels to purified recombinant allergens were performed by ELISA as described [35]. Allergen-specific IgM levels were determined either by ELISA (controlled organ-targeted allergen exposure) or by immunoblotting (natural seasonal exposure). ELISA determinations of IgM levels were performed similarly to IgG1–4 measurements using a serum dilution of 1:20 in TBS, 0.05% vol/vol Tween 20, 1% wt/vol BSA and a monoclonal mouse anti-human IgM antibody (PharMingen, San Diego, Calif., USA).

Changes of IgM reactivity profiles to natural birch and timothy grass pollen extract as well as to recombinant timothy grass pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5 and rPhl p 6) and the major birch pollen allergen (rBet v 1) were studied by immuno-



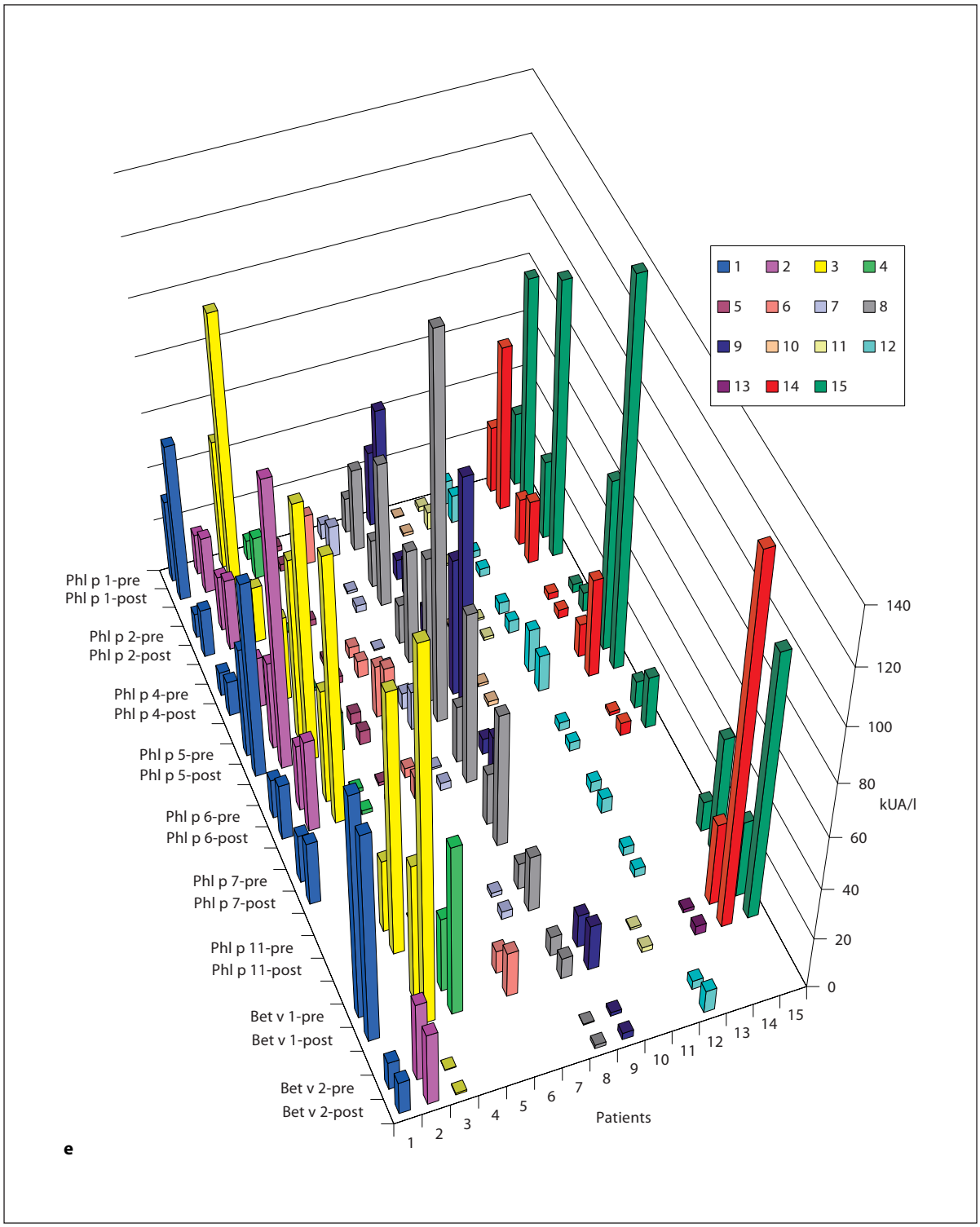


Fig. 1. Seasonal pollen exposure boosts systemic allergen-specific IgE responses. **a** The levels of birch and grass pollen exposure (10-day means of grains/m^3) in Vienna are shown for the complete year 1997. Levels of IgE antibodies specific for grass pollen allergens (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6 and Phl p 11) (**b**), a birch pollen allergen (Bet v 1) (**c**) and a cross-reactive allergen present in all pollens and plant food (Bet v 2) (**d**). IgE levels were quantified in allergic patients and are displayed as mean levels (kUA/l = kilo units of antigen/liter). **c**, **d** Data are presented as mean ± SEM. **e** Pre- and post-seasonal allergen-specific IgE levels.

blotting. A combination of rPhl p 1, rPhl p 6 and rBet v 1 (5 μg of each protein per cm gel, sheet 1), a combination of rPhl p 5 and rPhl p 2 (5 μg of each protein per cm gel, sheet 2), natural timothy grass (sheet 3) or birch pollen extract (sheet 4) (approximately 200 μg of natural protein extract per cm gel) were separated by 12.5% preparative SDS-PAGE and blotted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany).

Sera were diluted 1:10 in buffer B [50 mmol/l sodium phosphate (pH 7.5), 0.5% wt/vol BSA, 0.5% vol/vol Tween 20 and 0.05% wt/vol NaN_3] and incubated with nitrocellulose strips from each of the 4 different sheets. Bound IgM antibodies were detected by incubation of nitrocellulose strips with an alkaline phosphatase-coupled monoclonal anti-human IgM antibody (Sigma, St. Louis, Mo., USA) diluted 1:1,000 in buffer B, overnight at 4°C. The colour reaction was developed by using 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate as substrates.

Histamine Release Experiments

Granulocytes from peripheral blood and from bone marrow were isolated from a non-atopic individual as described [36, 37]. Cells were incubated with serum from a birch pollen-allergic patient (No. 3) obtained before (February) and after (June) the pollen season. Cells were washed and then incubated with increasing concentrations (0.01, 0.1, 1 and 10 μg/ml) of recombinant Bet v 1 and (for control purposes) with monoclonal anti-human IgE antibody E-124-2-8 (Immunotech, Marseille, France). Histamine released in the supernatant was measured by radioimmunoassay (Immunotech). Total histamine was determined after freeze-thawing of the cells. Results are displayed as mean values of triplicate determinations and represent the percentage of total histamine.

Statistical Analysis

Changes of IgE and IgG antibody levels induced by the nasal provocation test and seasonal allergen exposure were determined by Friedman's ANOVA and post hoc tests in case of a significance of the main effect. Comparisons of wheal areas after exposure to allergens by the nasal and the dermal route were done by Wilcoxon's matched-pairs test. In all cases $p < 0.05$ was considered significant. No correction for multiple testing was applied.

Results

Seasonal Exposure to Pollen Allergens Strongly Boosts Systemic Allergen-Specific IgE Responses

In order to investigate the effects of allergen exposure on systemic IgE responses, we quantified the IgE antibody levels to purified recombinant allergens in grass and birch pollen-allergic patients over a period of 1 year. Parallel to that, we determined the loads of birch and grass pollens for the same period (fig. 1a). In the study area, birches released pollen from the end of March to the end of May, whereas grass pollen exposure lasted from the beginning of May until the end of July (fig. 1a). The use of recombinant marker allergens with specificity for grasses (fig. 1b: rPhl p 1, rPhl p 2, rPhl p 4, rPhl p 5, rPhl p 6 and rPhl p 11) and birch (fig. 1c: rBet v 1) allowed us to dissect the effects of birch and grass pollen exposure on systemic IgE responses. In accordance with the flowering period of grasses, levels of grass pollen-specific IgE increased in June. For all grass pollen-specific marker allergens, a strong rise of IgE (rPhl p 1: +63%, $p < 0.01$; rPhl p 2: +68%, $p < 0.01$; nPhl p 4: +47%, $p < 0.01$; rPhl p 5: +85%, $p = 0.02$; rPhl p 6: +52 %, $p < 0.01$; rPhl p 11: +87%, not significant) was observed, which decreased after the grass pollen season but remained elevated after 1 year (fig. 1b). Birch pollen exposure led to a substantial increase of rBet v 1-specific IgE levels already in April and peaked in a more than 2-fold, significant increase of rBet v 1-specific IgE in June ($p = 0.01$, fig. 1c). Thereafter, the rBet v 1-specific IgE level decreased, but remained on a higher level than in the year before. The IgE level specific for profilin from birch (rBet v 2), a highly cross-reactive and ubiquitous allergen, remained largely unaltered (fig. 1d) [28, 38]. In most cases, seasonal allergen exposure increased IgE memory responses to the established allergen profile (fig. 1e), but no sensitisations to new allergens could be detected. Detailed analyses of the IgE reactivity profile of 4 patients over 1 year are presented in figure 2. These results demonstrate that seasonal allergen contact has driven an established IgE memory response within the 1-year observation period without inducing novel sensitisations.

Allergen-Induced Elevations of IgE Levels Lead to Increased Release of Histamine from Basophils

In order to investigate whether the rise of allergen-specific serum IgE levels contributes to the aggravation of immediate inflammatory reactions, basophils from a non-allergic donor were loaded with serum samples obtained from a birch pollen-allergic patient before and after the birch pollen season. Basophils loaded with post-seasonal serum containing elevated rBet v 1-specific IgE released more histamine than basophils loaded with pre-seasonal serum when exposed to Bet v 1 (fig. 3). This effect was observed with naïve basophils from peripheral blood (fig. 3a) and from bone marrow (fig. 3b) almost in the complete range of tested allergen concentrations. Histamine release induced by anti-IgE cross-linking showed no relevant difference between pre- and post-seasonal serum samples because the patient was sensitised to multiple other allergens (fig. 3).

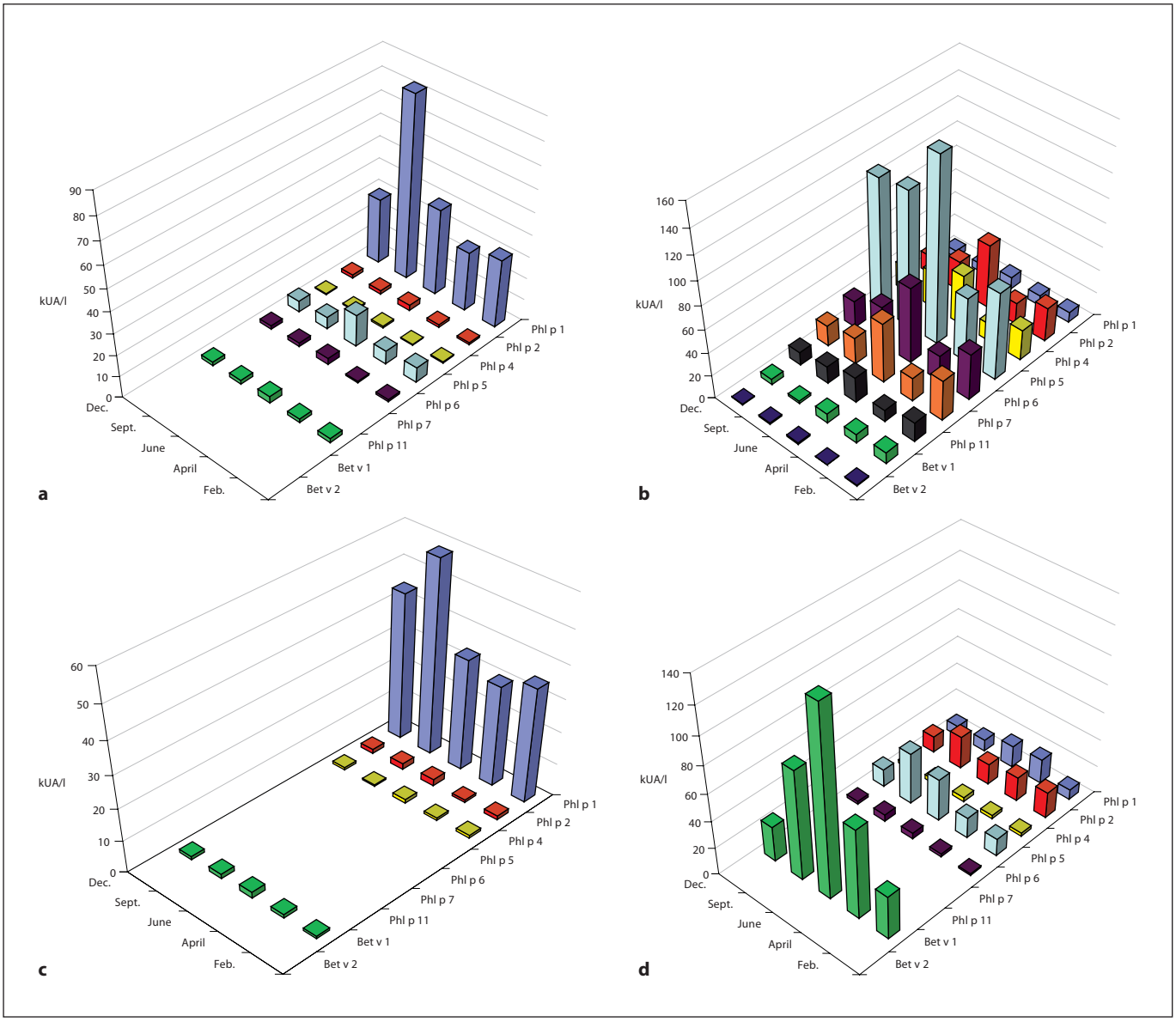


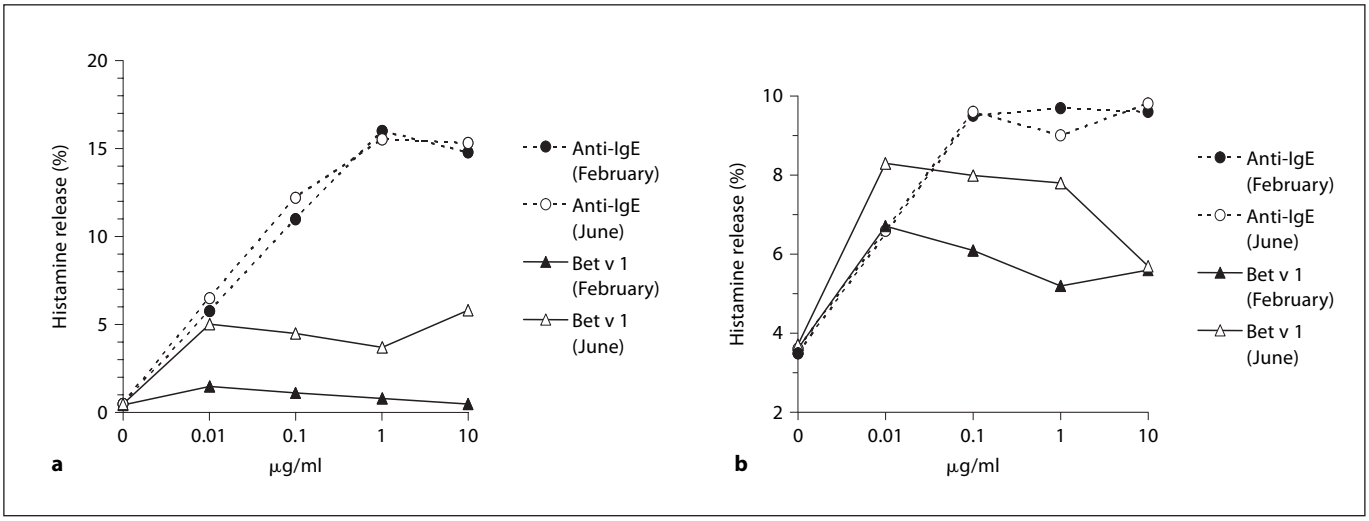
Fig. 2. Allergic individuals have constant IgE reactivity profiles which are boosted by allergen exposure. Levels of IgE antibodies specific for grass pollen (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11) and birch pollen allergens (Bet v 1, Bet v 2) measured at 5 points during 1 year (February, April, June, September, December) are displayed for 4 different pollen-allergic patients: patient 7 (**a**), patient 8 (**b**), patient 11 (**c**) and patient 14 (**d**).

Seasonal Allergen Exposure Induces Subtle Changes of Allergen-Specific IgG4 Levels but No Primary Allergen-Specific IgM Antibody Responses

IgG1–4 subclass levels to recombinant Phl p 5 and Bet v 1 are displayed in figures 4a and b. IgG1, IgG2 and IgG3 levels to these allergens were very low, both in allergic and non-allergic individuals (data not shown), and no seasonal variations could be detected for these subclasses.

Allergen-specific IgG4 reactivities were detected mainly in allergic patients. While IgG4 levels to Phl p 5 changed only slightly after seasonal exposure (fig. 4a), IgG4 levels to Bet v 1 exhibited a significant increase ($p < 0.05$, fig. 4b).

The visualisation of IgM antibody reactivities to birch and grass pollen allergens as well as to non-allergenic components in natural timothy grass and birch pollen extract


Histamine release (%)
20
15
10
5
0
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0.01
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1
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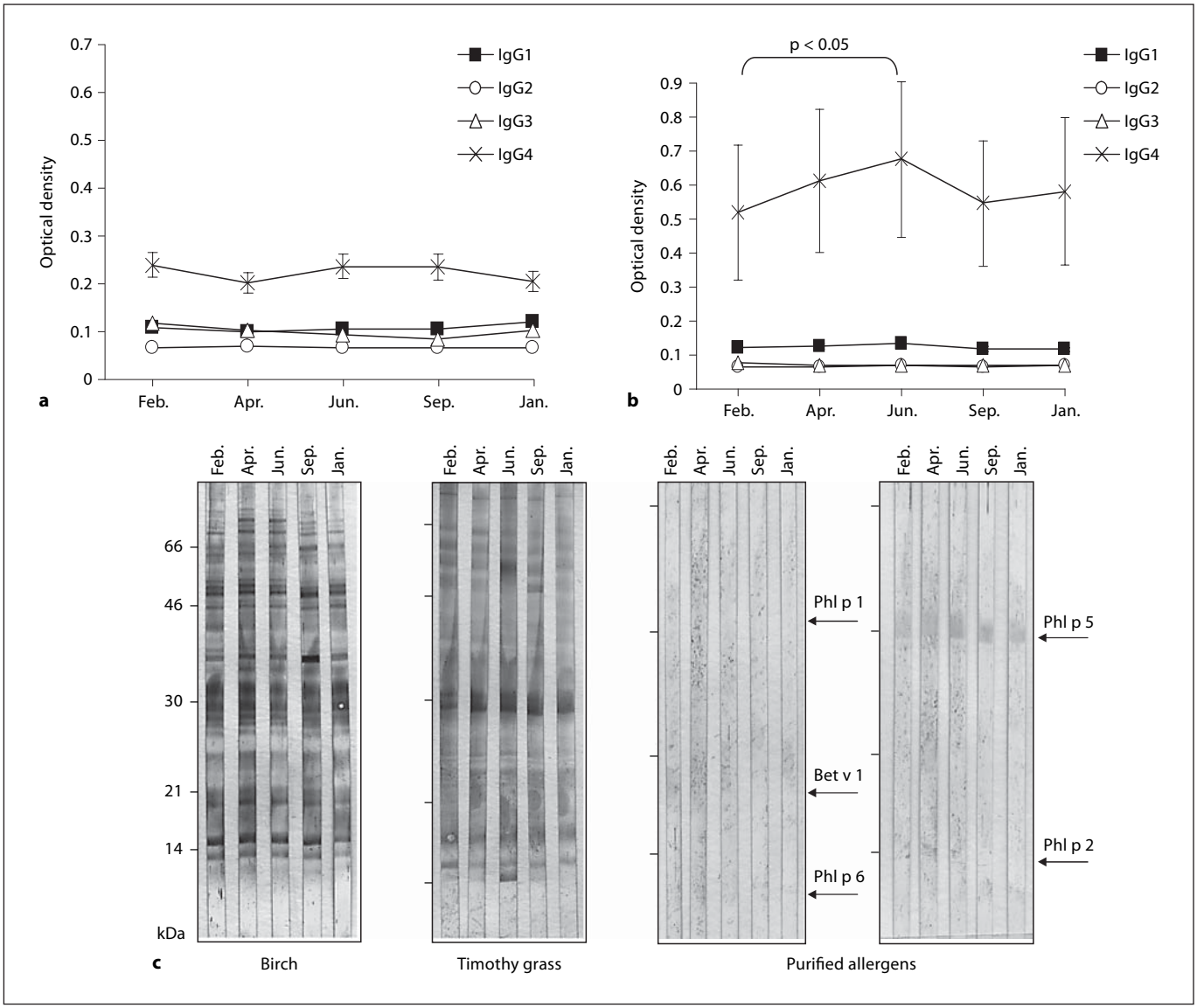
a
Anti-IgE (February)
Anti-IgE (June)
Bet v 1 (February)
Bet v 1 (June)
Histamine release (%)
10
8
6
4
2
0
0.01
0.1
1
10
μg/ml
b
Anti-IgE (February)
Anti-IgE (June)
Bet v 1 (February)
Bet v 1 (June)

3


Optical density
0.7
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IgG1
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Optical density
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66
46
30
21
14
kDa
c
Birch
Timothy grass
Phl p 1
Bet v 1
Phl p 6
Phl p 5
Phl p 2
Purified allergens

4

by IgM immunoblotting revealed that all allergic and non-allergic individuals had IgM antibodies to several pollen components (data not shown). Detectable IgM antibody levels to allergens (rPhl p 1, rPhl p 2, rPhl p 5, rPhl p 6 and rBet v 1) were observed in non-allergic but rarely in allergic individuals. Neither in allergic nor in non-allergic individuals, seasonal pollen exposure induced relevant changes of IgM antibody reactivity profiles (fig. 4c). As exemplified for a non-allergic subject, distinct alterations of IgM reactivities were only occasionally observed to non-allergenic components present in birch and timothy grass pollen (e.g. increased IgM reactivity to a 35-kDa antigen in birch pollen in September and to a 14-kDa timothy grass pollen antigen in June; fig. 4c).

Allergen Contact via the Nasal but Not the Dermal Route Increases Allergen-Specific Systemic IgE Levels and Allergen-Specific Skin Sensitivity

Figure 5 displays the changes of allergen-specific IgE levels in 8 allergic individuals (patients I–VIII) who had been exposed to a single intranasal allergen application. Relevant increases of systemic allergen-specific IgE levels became detectable in certain patients (fig. 5: V: Phl p 5; VIII: Bet v 1) already 1 week after allergen exposure. In all patients, single intranasal allergen exposure induced a rise of allergen-specific serum IgE levels between 2 and 5 weeks after allergen exposure (fig. 5, $p < 0.01$). Single exposure to another, immunologically unrelated allergen in the same allergic individual 3 weeks after the first intranasal allergen exposure caused a similar increase of allergen-specific serum IgE levels (fig. 5: I: Phl p 2; IV: Phl p 2; VIII: Phl p 5). No relevant increases of allergen-specific IgG levels were found in those patients who had exhibited increases of allergen-specific IgE production (data not shown).

The impact of increases of allergen-specific serum IgE on immediate type inflammatory reactions in a target organ (i.e. skin) of atopy which is different from that where the allergen has been administered (i.e. nose) is illustrated in table 2. The rise of allergen-specific serum IgE levels induced by intranasal allergen application after 3 weeks (table 2: patients I–VIII: mean increase 30%) caused a statistically significant increase of cutaneous sensitivity (table 2: mean wheal area at the day of nasal provocation: 58.2 mm^2; mean wheal area 3 weeks later: 106.4 mm^2, $p < 0.05$).

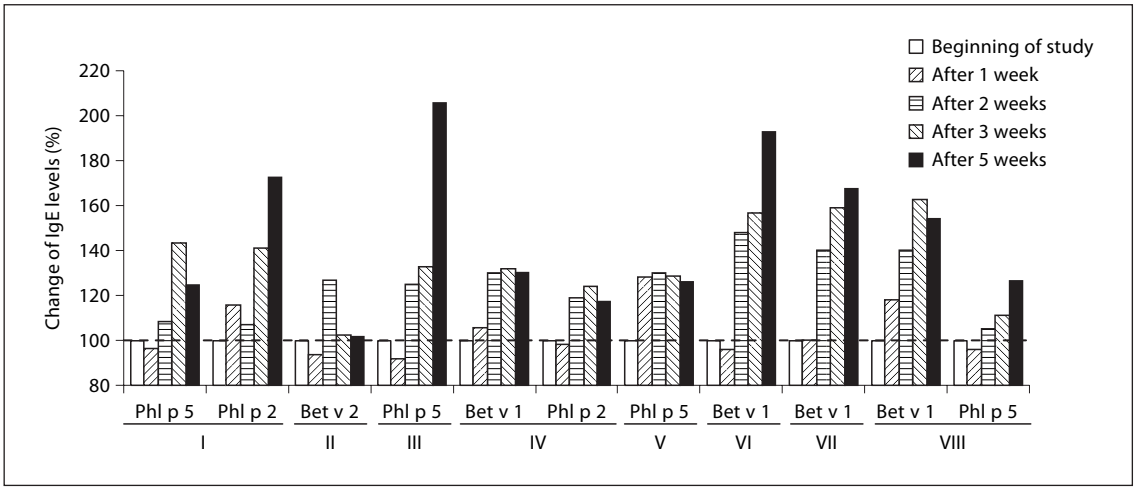

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Fig. 3. Increases of allergen-specific IgE levels cause increased allergen-specific histamine release from basophils. Basophil granulocytes from peripheral blood (**a**) and bone marrow (**b**) of a non-allergic individual were loaded with serum IgE obtained from a birch pollen-allergic patient before and after the birch pollen season. The percentage of histamine release is shown for different concentrations of Bet v 1 and anti-IgE.

Fig. 4. IgG1–4 antibody levels to Bet v 1 and Phl p 5 recorded during 1 year. Mean IgG1–4 levels specific for Phl p 5 (**a**) and Bet v 1 (**b**) of reactive individuals are displayed as optical density and SEM is displayed. IgM reactivities to nitrocellulose-blotted birch and grass pollen antigens (allergen extracts vs. purified allergens) are shown for a non-allergic individual for the same 1-year period (**c**). Molecular weights are displayed in kilodaltons.

Fig. 5. Controlled intranasal allergen application leads to increases of allergen-specific systemic IgE antibody levels. The percentage of the change of allergen-specific IgE levels in sera obtained at different times before and after intranasal allergen provocation is shown for different patients (I–VIII). In some patients, 2 different allergens were applied at an interval of 3 weeks.

The importance of the route of allergen exposure for the boosting of IgE memory responses is shown in table 2. Exposure of the same allergic individuals to an immunologically unrelated allergen by skin prick testing on the same day when nasal allergen exposure occurred did not induce a relevant increase of allergen-specific serum IgE levels to this allergen (table 2: mean IgE levels on the day of exposure: 17.7 kUA/l; mean IgE level 3 weeks later: 18.1 kUA/l). Allergen exposure by skin prick testing also did not lead to increased skin sensitivity (table 2).

Discussion

The results of our study demonstrate that the IgE memory response underlying established human allergy is directed against a preformed and constant profile of allergens. This IgE memory response is driven by the disease-eliciting allergens via the respiratory mucosa. In an experimental model of seasonal allergen exposure in patients, we found that exogenous pollen allergens induced a strong increase of allergen-specific serum IgE levels a few weeks after exposure. Only IgE levels to the highly cross-reactive allergen profilin, which shares sequence and structural similarity with human profilin and profilins from a variety of eukaryotic organisms, remained constant [28, 38]. The analysis of the allergen-specific IgE, IgG subclass and IgM responses over a period of 1 year showed that the dramatic rise of allergen-induced serum IgE levels was not accompanied by relevant changes of allergen-specific IgM responses. De novo induction and alterations of IgM responses were only detected to non-allergenic components in birch and grass pollen but not to allergens. Only subtle changes of allergen-specific IgG4 levels, an antibody subclass which is in part regulated via mechanisms similar to those driving IgE responses, could be detected. The lack of relevant increases of allergen-specific IgM supports the assumption that in established human allergy, allergens directly activate a pool of IgE memory cells without involving primary sensitisation events that would have required the activation and switching of allergen-specific IgM-producing cells. Another feature of the IgE memory response was that its reactivity profile did not change after the seasonal boost (fig. 2). Thus, it seems that the event of allergic sensitisation takes place early in childhood and leads to the establishment of a defined IgE memory response [6]. In each allergic individual, this IgE memory response is directed against a defined allergen/epitope spectrum, remains constant during natural disease and is only boosted by contact with the very same allergens against which sensitisation has occurred.

To analyse the importance of the route and mode of allergen exposure for the boosting of the IgE memory response we utilised an experimental model of controlled organ-targeted allergen administration (i.e. strict nasal or skin application). A single intranasal allergen application to an allergic individual induced strong rises of allergen-specific IgE responses, whereas simultaneous administration of an immunologically unrelated allergen to the skin did not induce relevant changes of serum IgE levels. According to several studies on natural allergen exposure and allergen contents in pollen and dust it may be assumed that allergic patients can be exposed to microgram amounts of allergens [39–41]. The cumulative dose of allergens administered in the course of nasal provocation may thus be comparable to that of natural exposure.

It is difficult to estimate whether the doses of allergens reaching the immune system after nasal and skin application were comparable. It may be argued that only small amounts of allergen penetrate the skin in the course of skin prick testing, but we recently also found that not more than 3% of the applied allergens penetrate an intact respiratory cell layer [42]. Since both modes of allergen exposure (i.e. via the nasal mucosa as well as the skin) induced immediate reactions, we have no reason to assume that the differences in the induction of systemic IgE responses after nasal versus skin exposure are due to different amounts of allergen taken up via the 2 routes.

The nasal as well as the bronchial mucosa are rich in IgE+ B cells [43]. We thus assume that nasal allergen contact directly activated IgE memory B cells in the nasal mucosa which then may have undergone differentiation into IgE+ plasma cells secreting increased levels of allergen-specific IgE antibodies. This assumption is in agreement with data showing that the levels of IgE in allergic patients correlate with the numbers of IgE-producing plasma cells in the peripheral blood [15]. Although it has been suggested that the nasal mucosa may be a site for a de novo class switch of B cells to IgE production [26], we think that this mechanism is not the major cause for the allergen-induced rises of IgE production observed in our study for two reasons. First, we were not able to detect any relevant de novo allergen-specific IgM or IgG responses after allergen provocation although we monitored humoral immune responses after nasal provocation in weekly intervals. Second, we were able to detect rises of allergen-specific IgE as soon as 1 week after allergen exposure leaving little time for the formation of a de novo IgE response from a pri-

mary immune response. We would therefore rather advocate that allergens directly activated IgE memory B cells via their antigen receptor in the nasal mucosa.

Plasma cells have already been identified earlier as a major source of IgE in the peripheral blood of allergic patients and we therefore assume that these are the major source for allergen-specific IgE [15]. Furthermore, we have shown that the peripheral blood of allergic patients, especially after seasonal allergen exposure, contains IgE+ cells which secrete high levels of allergen-specific IgE antibodies in an IL-4-, IL-13- and allergen-independent manner [44, 45].

The importance of allergen-induced rises of IgE memory antibody responses for the clinical course of the disease is demonstrated by the fact that serum IgE elevations were associated with an increased sensitivity to allergens. Using in vitro basophil sensitisation experiments with sera obtained before and after allergen exposure, we have shown that increased sensitivity and augmented immediate-type inflammatory reaction are due to a more efficient loading of mast cells with allergen-specific IgE antibodies and hence can be triggered by the corresponding allergen to release increased amounts of inflammatory mediators.

The memory IgE response may therefore represent an important target for therapeutic strategies. There is evidence that the IgE memory response is not influenced by IL-4, IL-13 and their antagonists and hence may be difficult to control by any cytokine-based treatment [46].

However, our results indicate that it may be possible to develop novel therapeutic strategies which are based on the blocking of the allergen-induced activation of IgE memory cells in the respiratory mucosa, either by the induction of allergen-specific blocking antibodies by vaccination or by the administration of blocking antibodies or other inhibitory compounds that antagonise the recognition of allergens by IgE.

This assumption is supported by our findings that (1) allergen-specific immunotherapy induces allergen-specific IgG antibodies which block the IgE recognition of allergens [47, 48], (2) patients forming such antibodies experienced reduced rises of IgE antibodies after allergen exposure [47, 48], and that (3) these allergen-specific blocking IgG antibodies could be detected in nasal secretions [49]. It is thus conceivable that nasal IgG antibodies which interfere with the IgE allergen interaction can inhibit the activation of IgE memory cells by allergens.

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