

Evaluation of Recombinant and Native Timothy Pollen (rPhl p 1, 2, 5, 6, 7, 11, 12 and nPhl p 4)-Specific IgG4 Antibodies Induced by Subcutaneous Immunotherapy with Timothy Pollen Extract in Allergic Patients

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Key Words

Grass pollen · IgG4 · Immunotherapy · Recombinant allergens

Abstract

Background: Allergen immunotherapy is a widely accepted treatment for IgE-mediated allergies. The evaluation of immunotherapy-induced IgG4 antibodies based on allergen extract is questionable because the amount of allergen-extract-specific IgG4 to individual disease-eliciting allergens cannot be determined using crude allergen extracts. In this study, we examined the specific IgE and IgG4 serum binding profiles to individual *Phleum pratense* allergens in grass-pollen-sensitive patients who had received grass-pollen-specific immunotherapy (SIT). **Methods:** The study included 33 patients from North-West Italy. All suffered from seasonal rhinoconjunctivitis and/or asthma. A modified 'cluster' regimen of injections of a standardized aluminium-adsorbed *P. pratense* extract, with once-weekly visits and 10 injections for 5 weeks followed by 3 weeks of maintenance injections was instituted. Patients' sera were analyzed for specific IgE and IgG4 reactivity to individual *P. pratense* allergens (recombinant Phl p 1, Phl p 2, Phl p 5, Phl p 6,

Phl p 7, Phl p 11, Phl p 12 and native Phl p 4) and natural *P. pratense* extract using the Pharmacia CAP system.

Results: IgE reactivities to new allergen components were not detected by CAP in treated patients after 15 weeks and a cumulative dose of approximately 65 µg of the major allergen Phl p 5. Patients lacking specific IgE reactivity towards individual allergens at the start of SIT did not produce significant levels of specific serum IgG4 to serum IgE-negative allergens. On the other hand, an increase in specific IgG4 only to allergens to which patients were previously sensitized was observed. Significant increases in specific IgG4 levels to rPhl p 1 ($p < 0.05$), 2 ($p < 0.01$), 5 ($p < 0.0001$), 6 ($p < 0.0001$), 7 ($p < 0.05$), 11 ($p < 0.05$) and nPhl p 4 ($p < 0.01$) were observed after *P. pratense* extract immunotherapy. No significant rPhl p 12-specific IgG4 antibody increase was documented after treatment. **Conclusion:** These findings suggest that Phl p 12 was underrepresented in the extract used, as indicated by the low specific IgG4 response induced by this grass-pollen-specific vaccine. Thus, the simple detection of specific serum IgG4 antibodies a few weeks after the start of SIT could represent a valuable tool to estimate the presence of relevant allergens in a given immunotherapeutic allergen extract.

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Introduction

Specific immunotherapy (SIT) is the only curative therapeutic approach, particularly in patients hypersensitive to bee or wasp stings and patients with allergic rhinoconjunctivitis and/or mild to moderate asthma.

A variety of immunologic changes have been reported after SIT, including moderate reductions in mucosal recruitment of eosinophils and basophils [1]; moderate reductions in IgE antibodies and increases in allergen-spe-

cific IgG4 [2]. Particularly, a rising amount of allergen-specific IgG4 antibodies could 'block' allergens before they reach the mast cell/basophil-bound IgE and prevent degranulation [3, 4]. Previous controversial results obtained regarding the role of 'blocking' antibodies were caused by the use of natural allergen extracts for treatment and analysis [5, 6]. Most of these studies measured antibody titers against crude extract which contains a lot of antigenic proteins besides the relevant allergens. In other words, specific IgG directed to the individual disease-

Table 1. Demographic, clinical characterization of 33 timothy pollen-sensitive patients

N	Age years	Sex	Allergies				SPT	
			season (months)	duration (years)	positive SPT	symptoms	histamine MD	<i>Phleum pratense</i> MD
1	29	F	4-6	12	g	c, r	7	7
2	22	F	5-7	9	g	c, r, a	6	6
3	22	M	4-7	9	g	c, r, a	5	8
4	31	M	5-8	6	g	c, r	6	6
5	21	F	4-7	7	g, w, d	c, r	4	6
6	17	F	4-7	9	g	c, r, a	5	5
7	17	M	5-7	5	g	c, r	6	8
8	28	M	4-7	14	g, t, w, mi	r, a	8	10
9	14	F	4-8	8	g, t, w	r, a	4	8
10	12	M	4-6	5	g	r, r	3	9
11	28	M	5-6	7	g	c, r, a	5	7
12	37	F	4-7	20	g, t, m	c, r, a	5	7
13	39	F	5-7	15	g	c, r, a	5	7
14	27	M	5-7	6	g, t, m	c, r	7	7
15	30	F	5-6	7	g, t	c, r, a	6	15
16	27	F	4-7	14	g, t, w	c, r	6	9
17	23	F	5-8	10	g, t	c, r	4	11
18	15	F	5-7	5	g, t, w	c, r	4	10
19	19	M	5-7	10	g, t, w	c, r	4	9
20	27	F	4-7	6	g, t, w	c, r	5	12
21	22	M	4-6	3	g, t, w	c, r	5	7
22	23	F	4-7	10	g, t, w	c, r	6	8
23	34	F	5-7	9	g, t	c, r, a	7	6
24	11	M	4-6	4	g	c, r, a	5	8
25	17	F	4-7	7	g, w	c, r, a	6	6
26	16	M	4-7	3	g, t, w, mi	c, r	6	9
27	30	M	4-7	16	g, t, w	c, r, a	7	14
28	32	M	5-8	7	g, t	C, r	6	7
29	55	F	5-7	25	g, t, w	a	5	6
30	37	M	4-7	10	g, t, w	c, r	6	7
31	18	M	5-7	6	g, t, w	c, r	5	13
32	22	M	5-8	9	g, t, w	c, r	5	7
33	19	M	5-8	7	g, t, w	c, r	7	9

SPT = Skin prick test; t = trees; g = grasses; w = weeds; mi = mites; d = danders; Symptoms: r = rhinitis; c = conjunctivitis; a = asthma; MD = mean diameter of the wheal reaction.

eliciting allergens cannot be determined and evaluated using crude allergen extracts.

In more recent studies, various investigators reported that: (1) immunization with purified natural and recombinant allergens induce mouse IgG1 antibodies that recognize epitopes similar to human IgE-allergen interaction and allergen-induced basophil degranulation [7]; (2) blocking antibodies induced by SIT prevent activation of CD4+ T cells by inhibiting serum IgE-facilitated allergen presentation [8]; (3) in rabbits and mice, hypoallergenic rBet v 1 fragments induce IgG antibodies that cross-

react with Bet v 1 wild-type allergen and Bet v 1 homologous allergens [9]; (4) sera containing therapy-induced allergen-specific IgG but not sera without such antibody (i.e. sera obtained before treatment or from patients who had received placebo) inhibit allergen-induced basophil histamine release [10]. Moreover, it has been reported that the ratio of IgG4 to IgG1 can serve as a valuable parameter that allows to assess the success of SIT [11]. These studies prompted us to evaluate specific IgG4 to recombinant allergens before and after SIT performed with crude allergen extracts. In our previous study we showed that the sera

Table 2. Serological characterization of 33 timothy pollen-sensitive patients

Immunocap results, kUA/l									
total IgE, kU/l		Phl p E		Phl p 1		Phl p 2		Phl p 5	
b	a	b	a	b	a	b	a	b	a
161	133	27.6	7.4	2.6	0.9	6.3	1.9	12.8	3.8
39	32	12.2	10.1	3.4	2.9	2.7	2.0	12.0	7.2
151	182	28.2	31.9	12.4	13.6	4.5	4.7	13.8	11.3
21	24	7.4	4.6	3.3	1.9	0.8	0.4	2.4	1.6
394	302	16.1	29.3	13.8	14.7	3.0	4.7	8.3	22.4
43	41	11.9	18.8	8.4	9.2	0.5	0.5	2.3	3.0
145	110	27.8	15.8	10.8	10.8	2.9	2.0	10.5	6.3
363	n.t.	49.3	71.0	22.8	17.4	11.7	13.6	15.1	21.9
228	238	81.3	53.8	38.7	54.6	22.2	23.4	48.1	36.3
586	705	>100	>100	98.6	>100	49.1	32.9	>100	>100
95	119	24.6	22.3	11.8	9.9	11.8	7.2	8.9	7.1
122	182	9.1	14.1	3.0	5.8	1.6	3.2	3.6	6.9
n.t.	n.t.	>100	97.5	42.4	44.0	12.7	21.4	60.7	60.8
83	n.t.	19.3	24.6	7.8	11.8	4.0	6.7	4.6	8.8
785	n.t.	22.9	15.2	34.0	30.8	<0.35	<0.35	<0.35	<0.35
322	299	51.5	67.2	23.6	28.9	4.9	5.5	32.6	49.4
433	568	70.6	57.5	33.3	34.5	0.8	0.6	31.3	40.3
305	n.t.	69.7	81.8	41.8	34.8	15.0	16.8	33.9	29.8
1129	1240	>100	97.8	>100	96.5	44.1	64.3	83.0	>100
681	658	>100	>100	79.4	89.2	14.1	19.8	32.8	47.5
129	n.t.	49.4	>100	20.5	25.3	7.5	15.6	18.9	59.3
400	299	87.9	36.0	60.8	14.8	26.5	8.9	13.9	5.9
53	54	13.2	1.6	3.0	3.1	3.0	3.7	8.5	5.4
681	442	91.9	>100	38.4	42.0	21.8	23.5	37.9	25.7
201	221	40.9	42.7	26.5	25.5	9.4	11.5	18.8	27.0
357	383	>100	>100	>100	68.3	78.0	32.0	47.1	32.2
5600	3463	>100	>100	>100	>100	>100	75.9	>100	>100
562	n.t.	42.7	41.3	14.3	8.7	10.1	5.3	21.4	12.9
122	134	39.4	31.2	15.4	7.1	<0.35	<0.35	16.8	6.7
834	1086	>100	>100	39.6	46.9	50.3	56.6	>100	97.7
887	795	>100	>100	96.5	>100	<0.35	<0.35	5.9	5.8
104	145	25.2	92.0	6.1	37.8	5.1	27.6	15.9	44.4
650	1037	>100	>100	25.2	32.0	19.1	29.9	74.5	>100

Phl p E = *Phleum pratense* extract; b = before; a = after; n.t. = not tested.

of 10/11 unselected grass-pollen-allergic patients treated with an aluminium-adsorbed grass pollen extract had a significant increase in specific IgG4 to rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, and Phl p 11 [12]. In this study, we evaluated serum specific IgG4 in patients allergic to grass pollen, selected on the basis of their IgE reactivity profile. We investigated whether therapy-induced allergen-specific IgG levels could indirectly reveal the presence of relevant allergens in the extract employed, also in patients lacking serum specific IgE against individual allergens at the start of SIT.

Methods

Patients

Sera analyzed in this study were obtained from 33 patients allergic to grass pollen (tables 1, 2). These patients were subdivided into four subgroups on the basis of the following IgE reactivity profiles: group 1: rPhl p 1+ rPhl p 2+ nPhl p 4+ rPhl p 5+ rPhl p 6+ rPhl p 7– rPhl p 11+ rPhl p 12+ (n = 11); group 2: rPhl p 1+ rPhl p 2+ nPhl p 4+ rPhl p 5+ rPhl p 6+ rPhl p 7– rPhl p 11+ rPhl p 12– (n = 9); group 3: rPhl p 1+ rPhl p 2+ nPhl p 4+ rPhl p 5+ rPhl p 6+ rPhl p 7– rPhl p 11– rPhl p 12– (n = 6); group 4: patients with specific IgE to rPhl p 7 and to various *P. pratense* recombinant allergens (n = 7).

Phl p 4		Phl p 6		Phl p 7		Phl p 11		Phl p 12	
b	a	b	a	b	a	b	a	b	a
4.0	1.0	20.1	5.0	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
2.4	1.7	2.0	1.7	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
15.7	15.3	3.1	2.6	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
1.6	1.4	1.5	1.0	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
6.6	10.0	1.5	7.8	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
4.1	5.5	0.9	0.7	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
13.0	6.7	2.7	0.8	<0.35	<0.35	14.3	6.6	<0.35	<0.35
11.4	10.6	15.7	16.8	<0.35	<0.35	10.6	8.9	<0.35	<0.35
23.0	31.4	9.3	5.0	<0.35	<0.35	1.6	2.6	<0.35	<0.35
33.1	9.3	18.7	5.7	<0.35	0.7	>100	>100	<0.35	<0.35
8.1	11.6	8.1	4.5	<0.35	<0.35	16.3	9.7	<0.35	<0.35
1.3	1.7	1.4	1.9	<0.35	<0.35	1.7	3.3	<0.35	<0.35
73.7	45.2	13.3	18.5	<0.35	<0.35	9.4	11.7	<0.35	<0.35
1.7	10.5	2.6	8.1	<0.35	<0.35	6.6	16.3	<0.35	<0.35
8.0	0.6	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
13.0	18.7	5.0	11.6	<0.35	<0.35	6.1	10.3	5.2	4.9
32.2	39.8	13.6	23.7	<0.35	<0.35	2.4	3.8	1.9	1.9
45.6	40.3	3.3	5.0	<0.35	<0.35	10.8	12.9	1.7	3.7
52.9	47.4	28.2	47.2	<0.35	<0.35	0.5	2.0	7.7	11.2
23.3	31.4	19.4	52.0	<0.35	<0.35	1.6	1.5	19.9	21.8
12.2	22.0	11.1	95.3	<0.35	<0.35	9.2	38.3	2.1	3.1
46.1	19.8	2.8	1.3	<0.35	<0.35	4.9	3.3	6.9	3.4
3.2	2.5	1.8	1.3	<0.35	<0.35	2.1	1.2	0.9	0.9
14.7	4.9	23.2	15.9	<0.35	<0.35	10.6	12.3	1.8	3.3
9.7	13.6	2.6	10.6	<0.35	<0.35	9.9	10.8	2.7	2.6
82.4	21.4	24.7	16.0	<0.35	<0.35	1.9	1.7	4.8	4.4
>100	97.6	>100	59.8	>100	>100	57.0	34.2	32.5	21.0
17.6	8.8	5.9	3.4	17.6	12.0	7.7	6.5	<0.35	<0.35
12.3	3.5	8.1	2.8	31.4	25.8	6.5	3.2	1.2	0.6
48.9	61.5	31.8	43.1	50.7	65.5	37.7	54.3	3.2	3.4
35.0	29.4	0.5	2.0	>100	>100	94.9	87.8	0.8	<0.35
6.5	27.4	6.4	26.6	1.3	32.9	0.8	13.7	3.0	8.2
46.0	46.2	71.5	>100	4.3	5.2	28.6	57.2	5.5	8.1

Study Design

Treatment started in November 2001. Two serum samples were taken at the start of therapy (November/December) and pre-seasonally (March) in the first year of therapy. Treatment was performed with a standardized aluminium-adsorbed *P. pratense* extract (Alutard SQ®, Alk-Abello, Hørsholm, Denmark).

Immunotherapy Protocol

A modified 'cluster' regimen of injections of Alutard SQ, with once-weekly visits for 5 weeks, followed by 3 weeks of maintenance injections was instituted (table 3). Each 1-ml maintenance injection of 100,000 standard quality units (SQU) was equivalent to 10,000 units. According to the manufacturer, the concentration of the major allergen Phl p 5 in the standardized timothy pollen extract was approximately 20.2 µg/100,000 SQU of Phl p 5.

Measurement of IgE and IgG4 Subclass

The sera were characterized in detail by determining IgE and IgG4 antibodies to rPhl p 1, 2, 5, 6, 7, 8, 11, 12 and nPhl p 4. Specific IgE/IgG4 antibodies against *P. pratense* extract were also measured by the Pharmacia CAP System (Pharmacia, Uppsala, Sweden).

Serum specific IgG4 were analyzed by a specific IgG4 fluoroenzyme immunoassay according to the manufacturer's instructions. Before the assay, all serum samples were diluted 1:100 with buffer solution and chicken serum. Briefly, the recombinant and native allergens of interest, covalently coupled to the cellulose carrier in Immuno-cap (10 µg of rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, rPhl p 12, respectively) reacted with the specific IgG4 in the

patient serum sample. After washing away nonspecific IgG4, β-galactosidase-labeled mouse antibodies against IgG4 were added to form a complex. After incubation, unbound enzyme anti-IgG4 was washed away and the bound complex was then incubated with a developing agent (4-methylumbelliferyl-β-D-galactoside). After stopping the reaction with a sodium carbonate solution, the fluorescence of the eluate was measured in Fluorocount 96 to classify the test results, fluorescence units (FU) for patient samples were compared directly with FU calibrators run in parallel. The IgG4 calibrators were traceable (via an unbroken chain of calibrations) to the international reference preparation 67/97 for human serum immunoglobulins A, G and M from the World Health Organization. The values for each of the IgG4 subclasses were established by Klein et al. [13].

Statistical Analysis

The results are expressed as means ± SD. Continued variables were analyzed by Student's t test. A p value <0.05 was considered as statistically significant.

Results

Regarding the allergen-specific IgE levels, there were no differences among the allergic subgroups, either before the onset of SIT or 15 weeks later (fig. 1–4), showing that allergen-specific IgE levels are not influenced during the early phase of treatment. IgE reactivities to new allergen components were not observed by CAP in treated patients after 15 weeks and a cumulative dose of about 65 µg of Phl p 5 administered with *P. pratense* extract.

The allergen-specific IgG4 levels of the treated subgroups had increased significantly (fig. 1–4). This clearly documents that the patients actually responded to the treatment. Interestingly, patients lacking IgE reactivity to individual allergens before the treatment did not produce specific IgG4 to the same allergens or produced negligible amounts of them. In other words, only patients with measurable specific IgE against rPhl p 7 (or other allergens) at the onset of SIT had significant levels of serum IgG4 to rPhl p 7 (fig. 4). The same was observed for other relevant allergens in single patients (fig. 1, 2). These results suggest that, at least in the early phase of SIT, one can observe an increase in specific IgG4 exclusively to those allergens to which patients were sensitized before the treatment. In this context, IgG4 responses induced by timothy pollen extract vaccine directed against rPhl p 5, rPhl p 6 (homologous of Phl p 5), rPhl p 2 and nPhl p 4 were higher, whereas rPhl p 1-specific serum IgG4 was lower, and rPhl p 11-, rPhl p 12-specific serum IgG4 were very low (fig. 5). As expected, all patients exhibited strong production of IgG4 response to natural timothy grass pollen extract, which represents the sum of epitopes and nonallergenic components to which patients' antibodies had bound.

Table 3. Alutard cluster immunotherapy up dosing and maintenance schedule

Visit	Week	Injection	Concentration SQU/ml	Volume ml
1	1	1	100	0.1
		2	1,000	0.1
		3	10,000	0.05
2	2	4	10,000	0.2
		5	10,000	0.2
3	3	6	10,000	0.5
		7	100,000	0.2
4	4	8	100,000	0.4
		9	100,000	0.4
5	5	10	100,000	0.5
		11	100,000	0.5
6	6	12	100,000	1.00
7	9	13	100,000	1.00

Injections were given at half-hour intervals and patients were observed after the last injection of each cluster. Blood samples were taken before treatment and 2 weeks after the 12th injection.

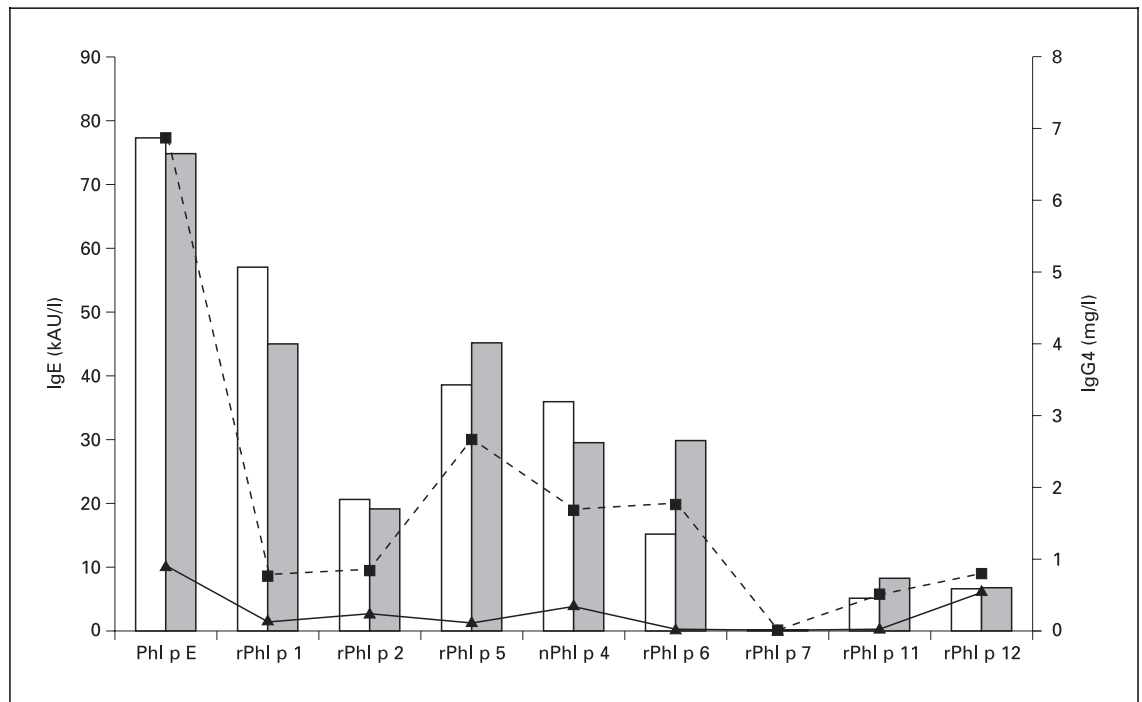


Fig. 1. Levels of IgE and IgG4 to timothy grass pollen extract (Phl p E), and recombinant and native allergens in group 1 (rPhl p 1+ rPhl p 2+ nPhl p 4+ rPhl p 5+ rPhl p 6+ rPhl p 7– rPhl p 11+ rPhl p 12+; n = 11. White bars = serum IgE before SIT; gray bars = serum IgE after SIT; ▲ = serum IgG4 before SIT; ■ = serum IgG4 after SIT. n = 11.

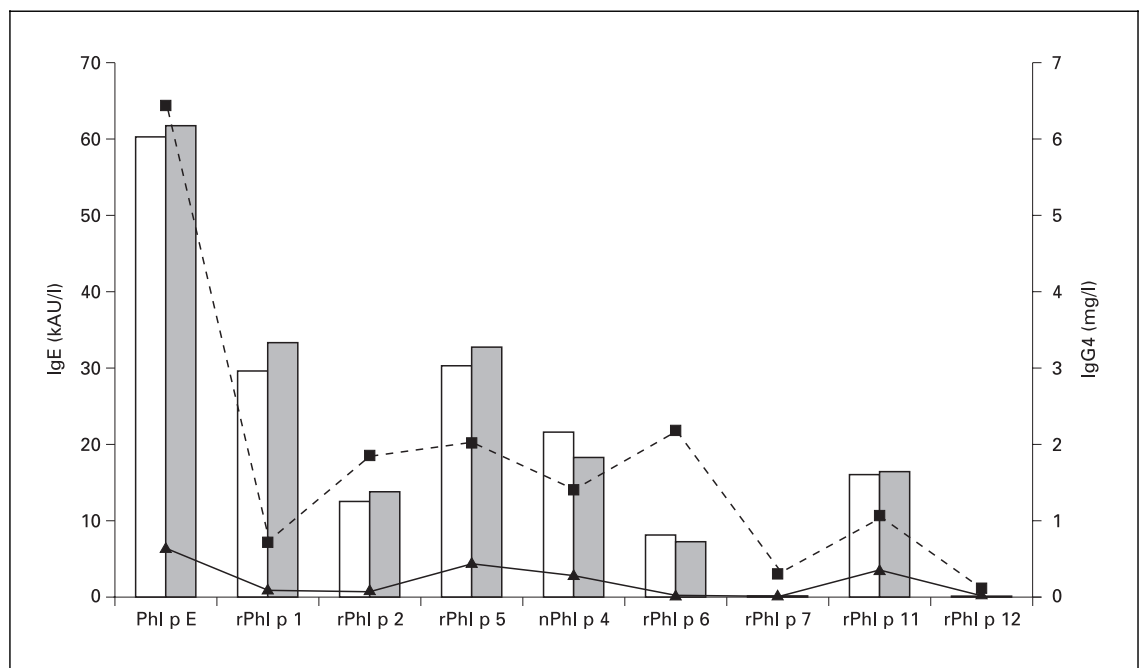


Fig. 2. Levels of IgE and IgG4 to timothy grass pollen extract (Phl p E) and recombinant and native allergens in group 2 (r Phl p 1+ rPhl p 2+ nPhl p 4+ rPhl p 5+ rPhl p 6+ rPhl p 7– rPhl p 11+ rPhl p 12–; n = 9. White bars = serum IgE before SIT; gray bars = serum IgE after SIT; ▲ = serum IgG4 before SIT; ■ = serum IgG4 after SIT.

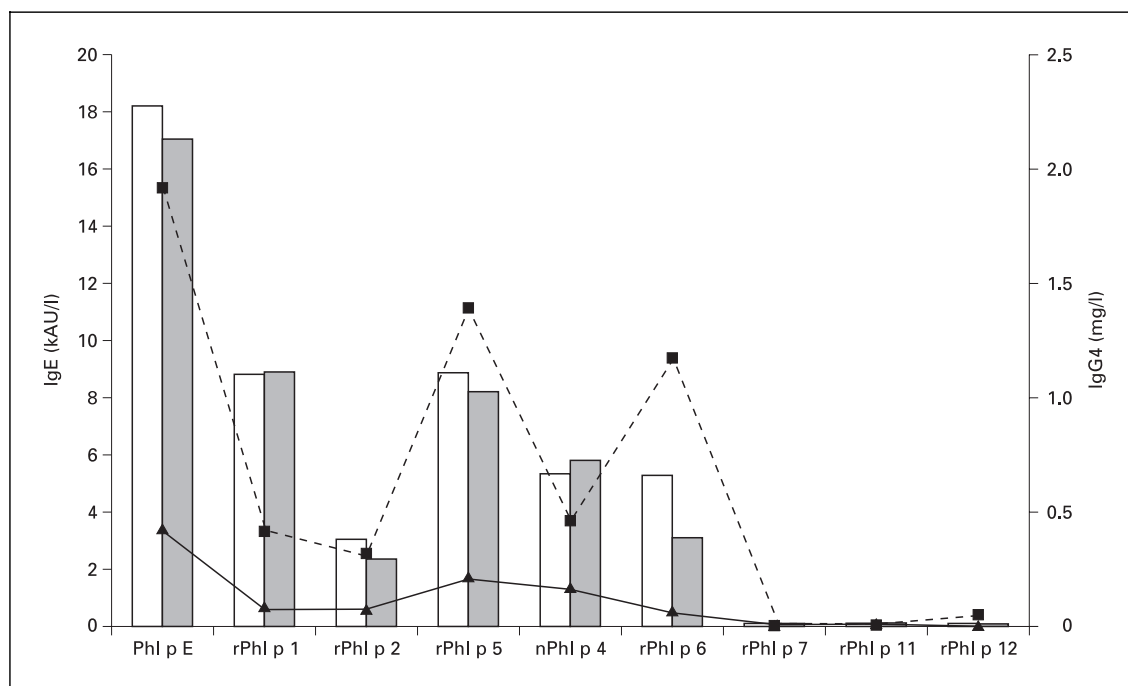


Fig. 3. Levels of IgE and IgG4 to timothy grass pollen extract (Phl p E) and recombinant and native allergens in group 3: rPhl p 1+ rPhl p 2+ nPhl p 4+ rPhl p 5+ rPhl p 6+ rPhl p 7– rPhl p 11– rPhl p 12– (n = 6). White bars = serum IgE before SIT; gray bars = serum IgE after SIT; ▲ = serum IgG4 before SIT; ■ = serum IgG4 after SIT.

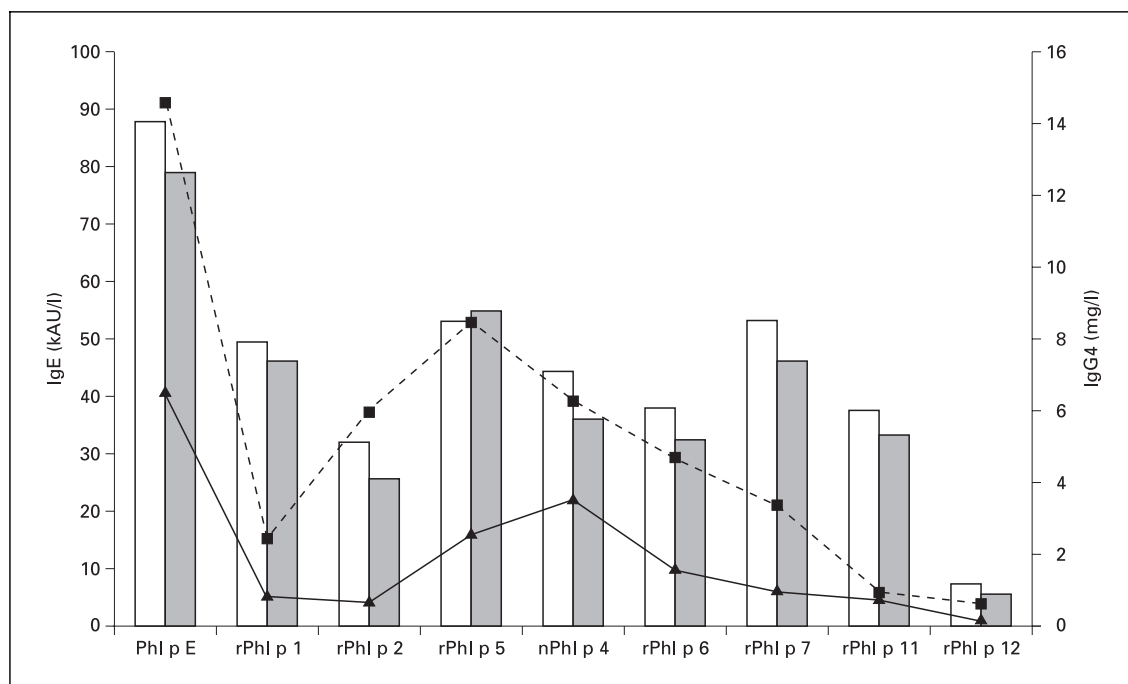


Fig. 4. Levels of IgE and IgG4 totimothy grass pollen extract and recombinant and native allergens in group 4 (patients with specific IgE to rPhl p 7 and various *P. pratense* recombinant allergens; (n = 7). White bars = serum IgE before SIT; gray bars = serum IgE after SIT; ▲ = serum IgG4 before SIT; ■ = serum IgG4 after SIT.

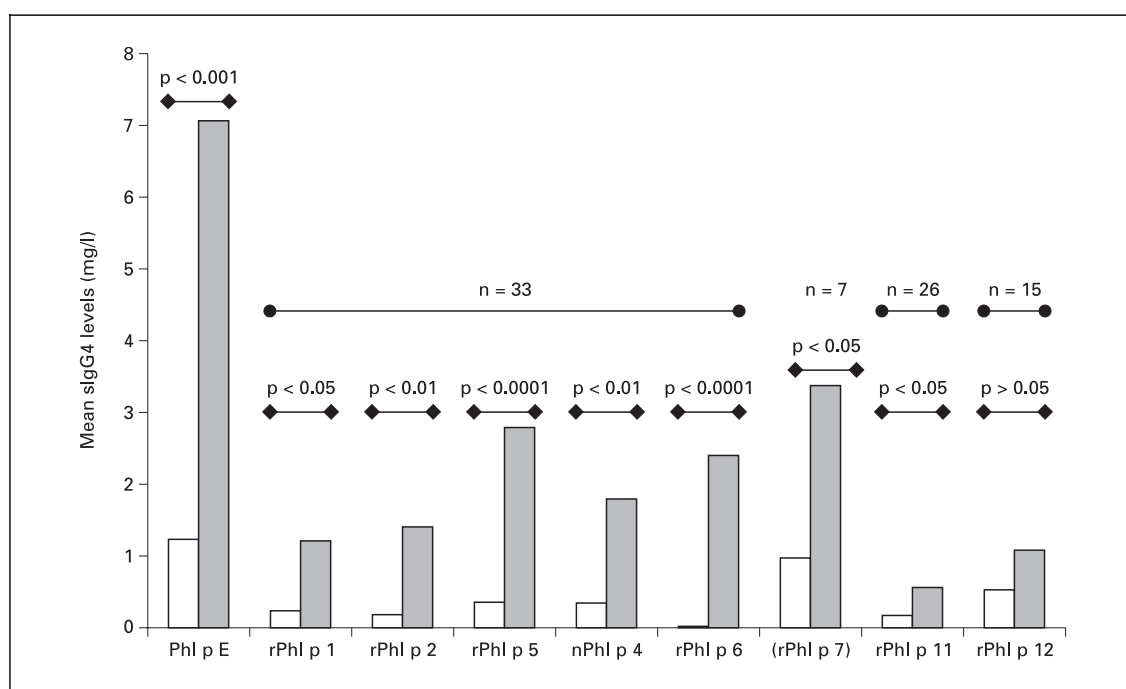


Fig. 5. Serum IgG4 to timothy grass pollen extract (Phl p E) and recombinant and native allergens) before (white bars) and after SIT (gray bars). Mean patients's age: 24 years; duration of SIT: 15 weeks.

Discussion

In our study, we used recombinant and native timothy grass pollen allergens (rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, rPhl p 12) to evaluate allergen-specific IgE and IgG4 responses in patients allergic to grass pollen who had received 13 injections of aluminium-adsorbed *P. pratense* extract (cumulative dose administered: 65 µg of Phl p 5) during 15 weeks. In our opinion, the subdivision of patients on the basis of their IgE reactivity profiles is essential to evaluate the immunogenic property of an allergen vaccine as patients allergic to pollen (and other allergen sources) may have heterogeneous IgE sensitization profiles [14] that may influence specific IgG4 response.

We have observed that only patients exhibiting significant levels of serum IgE to Phl p 7 before treatment had high serum Phl p 7 IgG4 levels after treatment. The same was observed for rPhl p 11 and other relevant allergens in some individuals. This suggests that specific allergen vaccine appears to activate a common mechanism which regulates both antigen-specific IgE and IgG4 antibody responses.

The fact that very low or undetectable IgG4 responses were induced against certain major allergens (e.g. Phl p 1, Phl p 2, Phl p 11) and minor allergens (e.g. Phl p 12, profilin) (fig. 5) may be ascribed to their low representation in the *P. pratense* extract administered. In fact, natural exposure to Phl p 1 caused an early serum rPhl p 1 IgE conversion in non-EU immigrants that became allergic to grass pollen in this geographic area [pers. observation]. Regarding profilins, despite extensive cross-reactivity among plant profilin and to the human homologue as well, it seems that a large proportion of IgE reactivities to profilins is clinically irrelevant. However, it may be assumed that a genetic background and other environmental factors may influence specific IgE response, and consequently IgG4 antibody production to an individual allergen.

These findings and previous observations [15] suggest that specific allergen vaccines should ideally contain immunogenic allergens in equivalent and defined molarities to act as an effective immunotherapeutic product. Allergen concentrations in allergen vaccine may have a pivotal role in virtue of the fact that SIT may act to change the character of allergen-specific IgG rather than simply increasing allergen concentrations. In fact, administration

of high concentrations of allergen has been shown to alter antibody affinity and specificity, as well as the quantity of antibodies produced [16, 17]. As recently reported, epitope specificity of IgG antibodies is decisive for their protective activity, in terms of blocking activity [18, 19], or negligible blocking activity, and even enhanced IgE recognition [20], respectively.

In the present study, we did not evaluate a possible correlation between the success of SIT and the change in IgG antibody levels, but simply the property of an allergen extract to induce selective antibody production. It has previously been reported that the induction of blocking antibodies represents a major mechanism underlying allergen-specific immunotherapy [21, 22]. Recently, Wacholz et al. [23] found that allergen-specific IgG antibodies induced by immunotherapy can inhibit IgE-facilitated allergen binding by B cells and presentation to T cells.

Moreover, recent data indicating that the efficacy of blocking antibodies is primarily a function of their serum concentration [24, 25], and given that the epitope complexity of allergens is less than anticipated [21], it is apparent that the measurement of allergen-specific IgG against purified allergens will serve to evaluate antibody other than IgE that could compete for a relevant allergen to which a patient is sensitized. Since most allergen extracts used for grass-pollen-specific vaccine are mainly standardized with respect to their major allergen (i.e. Phl p 5), we suggest the use of diagnostic tests based on purified major and cross-reactive allergens for selecting patients for grass pollen SIT and for the immunological monitoring of this treatment.

In conclusion, from a practical point of view, simply measuring a patient's serum IgG4 antibodies a few weeks after the onset of SIT may allow to assess the presence of relevant allergens in a given specific allergen vaccine.

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