

Original Article

Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System[™]

Background: New IgE sensitizations to proteins in allergen extracts have been shown to occur during allergen-specific immunotherapy (IT). However little is known about the kinetics of the changes in antibody reactivities.

Methods: Twenty-four allergic children and adults were treated with birch pollen rush IT (RIT). Fifteen matched patients served as allergic controls. Sera were obtained at regular intervals for up to three years and analyzed with immunoblotting and Pharmacia CAP System[™] with recombinant (r) birch pollen allergens (rBet v 1, rBet v 2, and rBet v 4).

Results: All birch-allergic patients had specific IgE to the major birch pollen allergen Bet v 1, but only three had IgE to rBet v 2 and/or rBet v 4 at the beginning of the study. New IgE sensitizations developed in 65% of the birch RIT-treated patients when studied by immunoblotting. Twenty-nine percent of the patients developed new sensitizations to rBet v 2 and/or rBet v 4 during RIT as measured by Pharmacia CAP System. Generally, new specific IgE reactivities occurred after at least one year of RIT, and only at low levels (<1 kU_A/l).

Conclusions: Sensitization to additional allergenic pollen components frequently occurs during prolonged birch RIT. However, the IgE levels are low and the clinical relevance is not known.

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Allergen-specific immunotherapy (IT) is a clinically well-documented treatment of allergic diseases (1, 2). However IT trials without clinical success have also been reported (3) and the immunological mechanism behind the effect of IT is not fully understood. A shift from Th2-like to Th1-like cytokine profile of allergen-specific T cells (4–6), as well as reduced T cell proliferative response (4, 7, 8) have been reported after successful IT. This is accompanied by a decreased production of IgE antibodies specific for the allergen extract, while the production of specific IgG increases (9–12). However, often the specific IgE temporarily increases during the early phase of IT (9–11). The immunological mechanism behind this increase is not known, but elevated IL-4 production by T cells during the early phase of IT has been reported (13–15).

Earlier studies have shown that *de novo* induction of new specific IgE reactivities can occur to allergenic components present in the extracts used for IT (10, 16–18). The kinetics of this sensitization process are, however, not known. We hypothesize that a transient increase in the Th2-like response to allergens during the early phase of IT might lead to the development of new allergen-specificities of IgE.

The techniques commonly used to study IgE reactiv-

ities to individual allergenic components are immunoblotting and crossed radio-immunoelectrophoresis (CRIE). These techniques provide only semiquantitative estimations of IgE concentrations. The availability of recombinant allergens on Pharmacia CAP System[™] (Pharmacia Diagnostics, Uppsala, Sweden) allows the determination of serum concentrations of specific IgE against several individual allergenic components. This new diagnostic tool has been designated component-resolved diagnostics (19). We have used both immunoblotting technique and Pharmacia CAP System[™] including three important recombinant birch pollen allergens: the 17 kDa major allergen Bet v 1 (20), and the minor allergens Bet v 2 at 13–14.4 kDa (20, 21) and Bet v 4 at 9.3 kDa (22), to study changes in IgE reactivities to individual allergenic components during birch pollen rush IT (RIT).

Material and methods

Study design

We studied sera collected from 24 allergic patients treated with RIT (RIT group), and from 15 allergic control patients (control group). The study was composed of children and adults suffering from seasonal birch pollen allergy. It was part of an open longitudinal RIT

study at the Outpatient Clinic at the Division of Allergy of the Helsinki University Central Hospital, Finland (23). The patients were polysensitized to common aeroallergens. Many of them were also skin prick positive to various fruits and vegetables (data not shown). Patient characteristics are given in Table 1.

The three-year long IT treatment was performed with an initial rush phase consisting of three daily, subcutaneous, injections of aqueous standardized birch pollen extract (Aquagen, ALK-Abello, Hørsholm, Denmark). Doses were gradually increased until the end of the first week to the highest individually tolerated dose (normally 10 000 SQ). After this initial rush phase, IT was continued every three weeks with subcutaneous injections of increasing doses of a birch pollen extract depot preparation (Alutard, ALK-Abello). The maintenance dose was individually adjusted within the range of 20 000–100 000 SQ (mean 73 000 SQ) and administered every three weeks. The concentration of the major allergen Bet v 1 in the standardized birch pollen extracts is approximately 12 µg/100 000 SQ, according to the manufacturer.

At the end of the first study year, the allergic control subjects were offered to start an active RIT treatment as described above. Three of them who accepted RIT were followed for two more years.

Blood samples for antibody measurements were obtained before the start of RIT (which was between November and February), after two months of RIT, and during the subsequent birch pollen season of the first year (after four to five months of RIT). For some patients samples were also collected outside the pollen season (November–February) in the following three years. The sera were stored at -20°C until analysis was performed.

Both the RIT group and the control group were allowed symptomatic medication. The clinical improvements were evaluated once each year, just after the peak of the birch pollen season. When visiting the clinic, the patients reported single visual analog scores on a scale ranging from 0 to 100 for symptoms and use of rescue medication. The patients also reported their use of medication in a diary during the first year of RIT (medication index). The clinical part of this study has been described in detail in a previous report (23).

The study was approved by the ethics committee of Helsinki University Central Hospital and all subjects gave their informed consent to participate.

Skin prick testing

The patients were subjected to skin prick testing using 10 different extracts of allergens (birch, timothy, meadow fescue and mugwort pollen, cat, dog, horse and cow dander, *Dermatophagoides pteronyssinus* and *Cladosporium herbarum*; Soluprick SQ, 10 HEP, ALK-Abello). A wheal diameter larger than 3 mm was regarded as a positive reaction.

Pharmacia CAP System

Allergen-specific serum IgE concentrations were determined with Pharmacia CAP System according to the manufacturer's instruction. ImmunoCAP[®] with extracts of birch pollen, and with recombinant (r)Bet v 1 and rBet v 2 are commercially available. Recombinant Bet v 4 (rBet v 4) was a kind gift by Dr Jonas Lidholm (Pharmacia Diagnostics), and was coupled to ImmunoCAP according to standard methods. The cut off value of the assay was 0.35 kU_A/l.

Immunoblotting

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of extracts of birch pollen (Allergon, Ängelholm, Sweden) was performed on a continuous 7.5–20% gradient gel with a 6% stacking zone, using a discontinuous two-buffer system according to Neville et al. (24). The proteins were transferred to a nitrocellulose membrane using the Trans-Blot electroblotting system (Bio-Rad,

Hercules, CA, USA) during 4 h (125 V) according to Towbin et al. (25). Any remaining free binding sites were blocked for 30 min with phosphate-buffered saline (PBS) (43 mM NaHPO₄, 7.3 mM KH₂PO₄, 154 mM NaCl, pH 7.4) with 0.3% Dextran and 0.1% Tween 20. The same buffer was used for dilution of serum samples and tracer. The membrane was cut into 3.5-mm strips and incubated with 1.5 ml patient sera for 16–18 h at room temperature. The sera were diluted to give a final concentration of 3 kU_A/l of birch pollen-specific IgE. Sera with specific IgE levels less than 6 kU_A/l were diluted at least 1:2. Normally, three serum samples from each patient were evaluated. The sera were collected before start of birch RIT, during the subsequent birch pollen season, and after one–three years of RIT. After incubation, the strips were washed with 3×2 ml washing buffer (0.9% NaCl, 0.5% Tween 20). Two milliliter ¹²⁵I-labeled antihuman IgE antibodies (Pharmacia CAP System specific IgE RIA-tracer; Pharmacia Diagnostics), diluted 1:4, was added to each strip and incubated for 4 h at room temperature. The strips were then washed with 3×2 ml washing buffer followed by 2×2 ml distilled water, then dried and finally subjected to autoradiography with Hyperfilm MP (Amersham Pharmacia Biotech, Uppsala, Sweden) in BioMax MS intensifying screen cassettes (Kodak, Rochester, NY) at -70°C for 24–72 h. All three sera from each individual were analyzed in the same run to guarantee similar conditions. The IgE binding patterns to birch pollen allergens in the immunoblot were determined by a combination of visual and computer-based evaluations (ImageMaster version 4.00, Amersham Pharmacia Biotech). The detection limit for IgE to an individual allergen was less than 0.1 kU_A/l. The calculated measuring range for the SDS-PAGE/immunoblot was 10–250 kDa.

Results

Clinical improvements

The clinical results of the RIT study from which these sera were obtained have been presented in detail in a previous report (23). The improvements in symptom scores, i.e. the decrease from baseline, for the birch RIT group (-56.7 ± 27.4 , mean $\pm$ SD) were significantly greater than the control group (-27.4 ± 20.4) already during the first pollen season after start of RIT ($p < 0.01$). During the third pollen season, the decreases in symptom scores, from baseline, for the RIT group and the control group were -60.4 ± 20.6 and -16.8 ± 31.0 , respectively ($p < 0.05$). The clinical improvements due to RIT were also reflected by decreased use of rescue medication (23).

Immunoblot analysis of birch RIT-treated patients

The IgE reactivity to Bet v 1, the major 17 kDa allergen in birch pollen extract, dominated the IgE response in these Finnish birch-allergic patients (Fig. 1). Weak IgE-reactivity at the start of RIT against an allergen of approximately 14 kDa (probably Bet v 2) was observed for patient VuJ. Sensitization to several additional allergens was also common at the start of RIT. IgE reactivities were found against allergens at approximately 15 kDa (Fig. 1, patients SnJ, PVA, VuJ and HLS), 26–30 kDa (RMJ and VuJ), 35 kDa (SaJ, VaJ, VuJ, VKA and HLS), 70 kDa (SaJ, SnJ, VaJ, PVA and HLS) and 90 kDa (SnJ, PVA and HLS). A band of about 18 kDa was also observed just above and often

Table 1. Patient characteristics

	RIT group	Control group
Number	24	15
Age, years (median with range)	15.5 (12–49)	14 (12–42)
Sex (female/male)	9/15	8/7
Clinical allergy symptoms, <i>n</i> (%)		
Rhinitis and/or conjunctivitis	24 (100)	15 (100)
Asthma	13 (54)	9 (60)
Skin prick positive, <i>n</i> (%)		
Birch pollen	24 (100)	15 (100)
Grass pollen*	16 (67)	14 (93)
Mugwort pollen	9 (38)	6 (40)
Animal dander	16 (67)	10 (67)
House dust mite	3 (13)	3 (20)
<i>Cladosporium herbarum</i>	0 (0)	0 (0)
Birch pollen specific IgE, kU _A /l (median with quartiles)	99.9 (28.3–203.9)	96.5 (8.4–159.7)
Total IgE, kU/l (median with quartiles)	308.2 (114.3–571.6)	443.0 (126.0–888.4)

*Timothy or meadow fescue.

inseparable from Bet v 1 (patients RMJ, SnJ and PVA). No bands less than 14 kDa were observed (the lower measuring limit was 10 kDa).

The IgE reactivities to various allergens, as visually observed on the immunoblot, changed during RIT. The concentration of IgE to some allergens increased, in some cases from undetectable levels. During one to three years of RIT some patients (Fig. 1, GTC, RMJ, SaJ, and PMT) developed IgE antibodies against the 14 kDa-allergen (Bet v 2), although never at as high levels as against Bet v 1. Other new IgE-reactivities were found against allergens of approximately 15 kDa (patients GTC, RMJ, SaJ and PMT), 18 kDa (SMH), 21 kDa (RMJ and PVA), 35 kDa (GTC and RMJ), 45 kDa (GTC and PVA), 53 kDa (PVA), 70 kDa (GTC, PMT

and SMH) and 90 kDa (GTC, RMJ, SaJ and SMH). Using immunoblotting, new IgE specificities were found in 17 of 26 patients analyzed (65%) after one to three years of birch RIT. In only a couple of patients new IgE specificities seemed to develop during the early phase of RIT, including the first birch pollen season (Fig. 1, patient SMH). Also in the serum from patient HGR a couple of new birch pollen IgE-reactivities were identified during the first pollen season (data not shown). Even if the IgE levels to some allergenic components seemed to decrease during RIT treatment, no bands completely disappeared from the immunoblot.

A few patients showed transient increases of IgE antibodies against certain allergens during the pollen season of the first study year, for instance the control

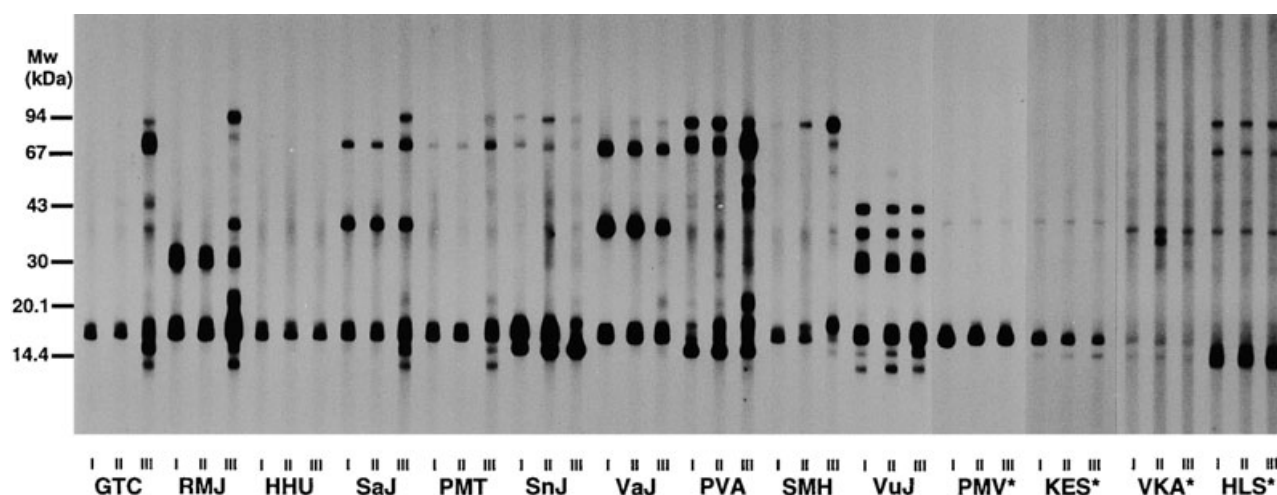


Figure 1. An example of immunoblot analysis with representative sera collected before start of birch RIT (I), during the birch pollen season after four–five months of RIT (II), and after one–three years of birch RIT (III). New IgE sensitizations were found in six of the 10 RIT-treated patients shown (GTC, RMJ, SaJ, PMT, PVA and SMH). No new sensitizations were found in the control patients (marked with *).

patient VKA (Fig. 1). However, we could not observe any induction of new IgE specificities in the birch-allergic control group by immunoblotting.

IgE levels in birch RIT-treated patients

Analysis with Pharmacia CAP System revealed that all birch-allergic patients included in this study had specific IgE to the major allergen Bet v 1. When comparing the levels, the rBet v 1-specific IgE (121.9 ± 120.0 kU_A/l, mean $\pm$ SD) seemed to represent the major part of the IgE antibodies binding to birch pollen extract (136.3 ± 138.1 kU_A/l). Accordingly, the kinetics of the IgE response to rBet v 1 during RIT was the same as the IgE response to the birch pollen extract, that is comprising an early rapid increase in IgE concentration followed by a decreased level (Fig. 2). In the control group only seasonal changes in serum levels of IgE specific for rBet v 1 were observed, similar to IgE for the birch pollen extract.

All of the birch RIT-treated patients were negative (<0.35 kU_A/l) for IgE reactivity to rBet v 2 and rBet v 4 at the beginning of the study (Table 2). In the control group one patient was initially positive for rBet v 2 and 2 subjects were positive for rBet v 4 (Table 3).

New IgE sensitizations to rBet v 2 and/or rBet v 4 started during the first study year in three birch RIT-treated patients (KT, HHU, JALV). For one of them (HHU) the new IgE reactivity to rBet v 4 started

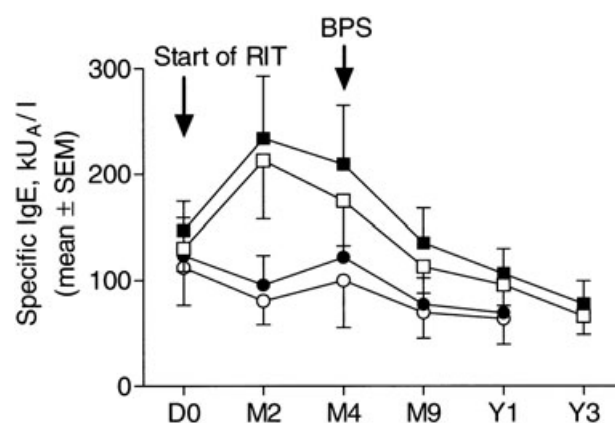


Figure 2. Comparison of the levels of specific IgE to birch pollen extract (filled symbols) and rBet v 1 (open symbols) in birch RIT-treated allergic patients (squares) and birch pollen-allergic control patients (circles). D: day; M: month; Y: year; BPS: approximate start of birch pollen season.

early, after two months of RIT. In three additional patients (OEM, KEL, and RMJ) new IgE reactivities developed after two to three years of birch RIT.

Three control patients (RKA, MSM and KAK) started treatment with birch RIT after the first study year. Patient RKA developed a minor IgE reactivity to rBet v 2 (0.4 kU_A/l) after 2 years of RIT, while in the sera of patient MSM the concentration of rBet v 2-specific IgE decreased from 6.0 kU_A/l to 2.9 kU_A/l after two years of RIT (Table 3).

During the birch pollen season of the first study

Table 2. Bet v 2- and Bet v 4-specific IgE (kU_A/l) in the RIT group

Patient ID	Bet v 2					Bet v 4				
	D0	M2	BPS	Y1	Y3	D0	M2	BPS	Y1	Y3
PSH	<	<	<	<	<	<	<	<	<	<
HGR	<	<	<	n.a.	n.a.	<	<	<	n.a.	n.a.
MAL	<	<	<	<	n.a.	<	<	<	<	n.a.
LLA	<	<	<	<	n.a.	<	<	<	<	n.a.
LAP	<	<	<	<	n.a.	<	<	<	<	n.a.
KT	<	n.a.	n.a.	1.4	0.5	<	n.a.	n.a.	<	<
OEM	<	<	<	<	0.4	<	<	<	<	<
KEL	<	<	<	<	1.6	<	<	<	<	<
KE	<	<	<	<	<	<	<	<	<	<
AKO	<	<	<	<	n.a.	<	<	<	<	n.a.
GTC	<	<	<	<	<	<	<	<	<	<
RMJ	<	<	<	<	0.4	<	<	<	<	0.6
HHU	<	<	<	<	n.a.	<	0.4	0.4	0.5	n.a.
SaJ	<	<	<	<	<	<	<	<	<	<
PMT	<	<	<	<	<	<	<	<	<	<
SnJ	<	<	<	<	<	<	<	<	<	<
VaJ	<	<	<	<	<	<	<	<	<	<
PVA	<	<	<	<	<	<	<	<	<	<
SMH	<	<	<	<	<	<	<	<	<	<
VuJ	<	<	<	n.a.	n.a.	<	<	<	n.a.	n.a.
JALV	<	<	<	<	<	<	<	<	0.4	0.5
HR	<	<	<	n.a.	n.a.	<	<	<	n.a.	n.a.
JTK	<	<	0.4	<	<	<	<	<	<	<
MRO	<	<	<	<	<	<	<	<	<	<

D0: at the start of study; M2: two months after start of study; BPS: birch pollen season (four–five months after start of study); Y1: one year after start of study; Y3: three years after start of study; <: below the cut-off value of the assay (<0.35 kU_A/l); n.a.: not available.

Table 3. Bet v 2- and Bet v 4-specific IgE (kU_A/l) in the birch-allergic control group

Patient ID	Bet v 2					Bet v 4				
	D0	M2	BPS	Y1	Y3	D0	M2	BPS	Y1	Y3
RKA*	<	<	n.a.	<	0.4*	<	<	n.a.	<	<*
MSM*	8.2	6.2	6.5	6.0	2.9*	<	<	<	<	<*
KAK*	<	<	<	<	<*	<	<	<	<	<*
VKA	<	<	<	<	<	<	<	<	<	<
HLS	<	<	<	<	<	<	<	<	<	<
KES	<	<	<	<	n.a.	<	<	<	<	n.a.
WJ	<	<	0.7	n.a.	n.a.	<	<	<	n.a.	n.a.
HJE	<	<	<	n.a.	n.a.	0.8	0.5	0.6	n.a.	n.a.
PHP	<	<	<	n.a.	n.a.	<	<	<	n.a.	n.a.
JAM	<	<	<	n.a.	n.a.	<	<	<	n.a.	n.a.
RCH	<	<	<	<	n.a.	<	<	0.5	<	n.a.
PMV	<	<	<	<	n.a.	1.0	0.7	0.5	0.6	n.a.
TMS	<	<	n.a.	<	n.a.	<	<	n.a.	<	n.a.
LRJ	<	<	n.a.	<	n.a.	<	<	n.a.	<	n.a.
VJK	<	<	n.a.	n.a.	n.a.	<	<	n.a.	n.a.	n.a.

D0: at the start of study; M2: two months after start of study; BPS: birch pollen season (four–five months after start of study); Y1: one year after start of study; Y3: three years after start of study; <: below the cut-off value of the assay (<0.35 kU_A/l); n.a.: not available.

* RKA, MSM and KAK started birch RIT treatment after the first study year.

year, new IgE reactivities were observed in two allergic control patients (Table 3; WJ, and RCH) and in one RIT-treated patient (Table 2; JTK), probably as a consequence of the natural allergen exposure.

To summarize, after one to three years of RIT-treatment with birch pollen extract, new IgE reactivities to rBet v 2 and rBet v 4 arose at low levels in five (21%) and three (12%) patients, respectively. In one patient (RMJ) new IgE reactivities were developed to both allergens, meaning that new reactivities to rBet v 2 and/or rBet v 4 started in seven birch RIT-treated patients (29%).

Discussion

The standard *in vitro* assays for measuring allergen-specific IgE do not permit identification and measurement of specific IgE to individual allergenic components in the extract. The cloning and expression of recombinant allergens offers new possibilities to study IgE reactivities (19), for example to monitor allergen-specific IT.

Birch pollen-sensitized patients differ in their IgE binding pattern to individual allergens present in the extract. More than 95% of birch pollen-allergic patients are sensitized to the major birch pollen allergen Bet v 1 (20, 26), and about 10–20% to Bet v 2 (27, 28). The newly cloned and characterized Bet v 4 is recognized by serum IgE in approximately 5% of birch pollen-allergic patients and in 10–15% of grass- and mugwort pollen-allergic patients (22, 29). Accordingly, only a minority of the Finnish birch pollen-allergic patients participating in this study was positive for IgE specific to rBet v 2 or rBet v 4, while all of them had serum IgE specific to rBet v 1 when analyzed with Pharmacia CAP System. Sensitization patterns to individual allergenic compo-

nents in grass or birch pollen can differ between allergic populations (30), and IgE reactivity to Bet v 2 seems to be less frequent among birch pollen-allergic patients in northern Europe compared to those in central Europe (31).

Both Bet v 1 and Bet v 2 share common epitopes with proteins in other types of pollen, vegetables and fruits (32, 33). IgE to Bet v 1 and Bet v 2 are often cross-reactive with these homologous proteins, as shown by inhibition experiments (33), and are believed to be of great importance in the oral allergy syndrome resulting from consumption of fruits and vegetables (34). IgE reactivity to Bet v 1 seems to be the major cause of the common birch–apple syndrome, whereas reactivity to Bet v 2 is associated with polysensitization to grass and weed pollen (28, 35). Bet v 4 represents a cross-reactive allergen in tree, grass, and weed pollen allergies (22, 29). Therefore, it was of interest to study the development of new IgE reactivities to these allergenic birch proteins during birch pollen IT because of their reported associations with allergy to other types of pollen, vegetables and fruits.

We observed a few cases where new IgE reactivities developed during the first year of RIT. However, the majority of the RIT-treated patients developed IgE reactivities to new allergenic components after the first year of RIT, studied by immunoblotting and Pharmacia CAP System on sera collected outside the pollen season. No particular birch pollen allergen seemed to predominate in the sensitization process during RIT. The allergens that were recognized by patient IgE in the immunoblot cannot be identified with accuracy. However, by comparing their approximate molecular weights on the immunoblot with known purified or cloned allergens some assumptions and guesses can be undertaken. Besides the identified location of the 17-kDa allergen

Bet v 1 on the immunoblot, the band at approximately 14 kDa most probably corresponds to Bet v 2 (21). The bands at approximately 35 kDa and 18 kDa could be the recently characterized birch pollen allergens Bet v 6 and Bet v 7. Bet v 6 has a reported molecular weight of 35 kDa and belongs to a family of isoflavone reductase-related proteins (36). The pan-allergen Bet v 7 is a cyclophilin of 18 kDa (37). Bet v 3 is a Ca^{2+} -binding protein of 24 kDa, but no band in our immunoblot corresponds to that molecular weight. However, Bet v 3 needs Ca^{2+} for its native conformation, and IgE reactivity to Bet v 3 cannot be observed on a conventional immunoblot (38). Furthermore, Bet v 4 with a molecular weight of 9.3 kDa is probably not observed by immunoblotting performed by this method, since it is too small and moves out of the gel (22).

Pharmacia CAP System identified newly developed IgE reactivities to Bet v 2 and/or Bet v 4 in 29% of the birch RIT-treated patients. Immunoblotting revealed new IgE binding in 65% of the treated patients. However, immunoblotting included most allergenic components in the extract, while Pharmacia CAP System included only three recombinant birch pollen allergens. Moreover, immunoblotting is semiquantitative and some new bands were very weak. The cut-off value of the Pharmacia CAP System is 0.35 $\text{kU}_\text{A}/\text{l}$, while the sensitivity of the immunoblot method for IgE against individual allergens is less than 0.1 $\text{kU}_\text{A}/\text{l}$. This could explain why the development of IgE antibodies against Bet v 2 was more frequent when analyzed by immunoblotting than with Pharmacia CAP System. During birch pollen season, a few RIT and control patients showed new IgE specificities against rBet v 2 or rBet v 4. However, these were transient reactivities, since the IgE levels were below the cut-off value of the assay outside the pollen season.

The establishment of clones of Th2 cells with the capacity to produce high levels of IL-4 is believed to be an early crucial step in the development of allergen-specific IgE sensitization (39). Thus, although new IgE reactivities evolved late during RIT, we cannot exclude that new clones of allergen-specific Th2 cells might have been established earlier, preceding the switch of allergen-specific B cells.

The clinical improvements in the present study were significantly greater in the RIT group compared to

the control group, as previously shown in detail (23). We have no indication that the development of new IgE reactivities to Bet v 2 or Bet v 4 in birch RIT-treated patients resulted in any additional clinical symptoms to grass or weed pollen, nor when eating fruits or vegetables. The IgE levels to these new allergenic components were always low (RAST class 1–2). Furthermore, many of the patients were already polysensitized to other pollen, fruits and vegetables when starting RIT. However, in a study by van Ree et al. (40), it was indicated that IT with house dust mite extract can lead to the development of new cross-reacting IgE antibodies to snail and shrimp, and to aggravated clinical symptoms when eating shrimp. On the other hand, other studies have showed that IT reduced the risk of developing new IgE sensitizations in asthmatic children monosensitized to house dust mite (41, 42). To our knowledge, no similar study has been performed with monosensitized pollen-allergic patients undergoing IT treatment.

In conclusion, the development of recombinant allergens on Pharmacia CAP System enables studies of the development of new IgE reactivities in patients undergoing IT treatment. During birch RIT, new IgE reactivities to allergenic components in birch pollen extract developed in about 65% of the patients, usually one to three years after initiation of treatment. New IgE sensitization was observed against the two highly cross-reactive allergens Bet v 2 and Bet v 4. This might result in clinical consequences for some patients, even if the IgE concentrations to recombinant Bet v 2 and Bet v 4 are low. A mixture of recombinant allergens, tailor-made for each patient and based on *in-vitro* measurements of IgE reactivities to individual allergens, has been suggested as an alternative to the crude pollen extracts that are used today in IT (19). This might reduce the development of new IgE reactivities during IT.

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