

Original article

Allergen-specific immunotherapy in birch- and grass-pollen-allergic rhinitis. II. Side-effects

Background: Allergen-specific immunotherapy (IT) involves the risk of side-effects. Different side-effect profiles have been reported for different allergens, and it would be of great benefit to be able more precisely to predict patient- and allergen-related risk factors.

Methods: Fifty-two patients with rhinoconjunctivitis and allergy to birch as well as grass pollen participated in a 3-year IT study, with a baseline year followed by 2 years of treatment. During the first treatment year, the patients received double-blinded IT with either birch (*Betula verrucosa*) or grass (*Phleum pratense*) pollen extracts adsorbed to aluminum hydroxide. The following year, the other allergen extract was added. Assessment of systemic reactions (SRs) was performed, and related to patient pretreatment parameters such as seasonal symptoms and medication requirement, skin prick test (SPT), conjunctival provocation test (CPT), nasal provocation test (NPT), total and specific IgE, basophil histamine release (HR), eosinophil count (EOS), eosinophil cationic protein (ECP), and eosinophil protein X (EPX).

Results: In total, 44 and 47 patients started IT with birch- and grass-pollen extracts, respectively. All SRs occurred during the dose-increase phase. No life-threatening SRs were observed. There were a higher number of patients with SRs during IT with grass pollen than IT with birch pollen, 21 vs five patients ($P < 0.001$), with SRs to 3.3% of grass-pollen injections compared to 0.7% of birch-pollen injections ($P < 0.0001$). The SRs of birch-pollen IT were mild, consisting of rhinoconjunctivitis and oral-pharyngeal itching, whereas asthma and urticaria episodes were observed in the grass-pollen IT. No difference was found in sensitivity to birch and grass, when measured by SPT, CPT, NPT, specific IgE, or HR, and no difference was found in age, duration of allergic symptoms, prevalence of asthma, mean seasonal birch/grass symptom score, eye-drop use, or antihistamine or prednisolone intake between the group with and without subsequent SRs to IT. No difference was found in EOS, serum ECP, or EPX, between the group with and without subsequent SRs to IT.

Conclusions: IT with grass-pollen extract seems to be associated with a higher number and more severe SRs than birch-pollen IT. Neither demographic data nor diagnostic tests of allergy such as specific IgE, HR, SPT, CPT, and NPT could identify the patients with subsequent SRs.

L. Winther, H.-J. Malling

Allergy Unit, National University Hospital, Copenhagen

H. Mosbech

Allergy Unit, Clinic for Internal Medicine B,
Copenhagen University Hospital, Frederiksberg,
Denmark

Key words: allergic rhinitis; aluminum hydroxide; birch pollen; immunotherapy; grass pollen; side-effects.

Lone Winther, MD
Allergy Unit 7551
National University Hospital
20, Tagensvej
DK-2200 Copenhagen N
Denmark

Accepted for publication 23 March 2000

Allergen-specific immunotherapy (IT) involves the risk of side-effects. The frequency and severity of systemic

reactions (SRs) vary between studies, with SRs in 0–100% of patients (1–3). However, the risk of side-effects in different studies is difficult to compare because of manufactural differences in allergen standardization and potency, different dose schedules, and differences in the recording and grading of SRs. The frequency and severity of SRs seems to depend on induction regimens (4–6) and disease (7, 8). The frequency and severity of SRs might also depend on the allergen in question. A retrospective study found that mite and grass pollen gave equal but higher

Abbreviations. CPT: conjunctival provocation test; ECP: eosinophil cationic protein; ELR: early local reaction; EOS: eosinophil counts; EPX: eosinophil protein X; HR: histamine release; HR15: the lowest concentration of extract giving 15 ng/ml of histamine or more in the HR test; IT: allergen-specific immunotherapy; LLR: late local reaction; MAX: maximum histamine released (ng/ml) at any concentration of extract in HR test; NPT: nasal provocation test; PEF: peak expiratory flow; SPT: skin prick test; SQ-U: standardized quality units; SR: systemic reaction.

proportions of SRs than *Parietaria* and pollen-mixture allergens (9). However, in both studies, the allergen extracts were from different manufacturers, and the IT schedules were not described. The frequency of patients with SRs varied from 9.5% to 55% in grass-pollen IT, and 28% was reported in birch-pollen IT, but studies were not comparable with respect to standardization of extracts and IT schedules (7, 8, 10–13).

IT requires dedicated patients because of the time-consuming treatment schedule. Side-effects are another reason for dropouts. It would be of great benefit if it was possible to determine parameters that could predict the SRs. It seems relevant to include not only IgE-related tests, but also markers of immunoinflammation, since in studies of pollen allergy, including rhinitis and asthma, the local allergic inflammatory reactions have been reflected in the blood with regard to concentrations of eosinophils (EOS), eosinophil cationic protein (ECP), and eosinophil protein X (EPX) (14–19).

As a part of a 3-year IT study in allergic rhinitis patients all having birch- and grass-pollen allergy, the frequency and severity of SRs were recorded. The aim was to compare side-effects to birch- and grass-pollen extracts. Furthermore, we aimed to identify parameters predictive of SRs, with focus on the diagnosis and severity of the allergic disease, described by seasonal symptoms and medication requirements; the outcome of the skin prick test (SPT), conjunctival provocation test (CPT), and nasal provocation test (NPT); and results from blood tests such as total and specific IgE, basophil histamine release (HR), EOS, serum ECP, and EPX.

Material and methods

Patients

Fifty-two patients (28 men and 24 women, aged 18–52 years) with a history of severe birch- and grass-pollen allergy participated in a 3-year IT study. Seventeen of the patients also suffered from mild to moderate asthma, with occasional symptoms. Demographic data have been reported (20). All patients fulfilled the criteria for IT according to the position paper of the European Academy of Allergology and Clinical Immunology (EAACI) (21).

The study was approved by the local ethics committee of Copenhagen, and written, informed consent was obtained from all patients before admission to the study.

Extracts

The *Betula verrucosa* and *Phleum pratense* extracts for SPT (Soluprick SQ); for CPT, NPT, and titrated SPTs (Aquagen SQ); and for IT (Alutard SQ) were supplied by ALK-Abelló, Hørsholm, Denmark. The activity of the extracts was given in standardized quality units (SQ-U). The content of the birch-pollen major allergen

Bet v 1 was 12 mg per 100 000 SQ-U, and the content of the grass-pollen major allergen Phl p 5 was 20 mg per 100 000 SQ-U.

Allergy and blood testing

The CPT, NPT, and titrated SPT were performed with extracts of *B. verrucosa* or *P. pratense* (Aquagen SQ), diluted in isotonic saline on the day of testing. Allergen dilutions were multiples of 10 (100, 1000, 10 000, and 100 000 SQ-U/ml). The CPT was performed by applying one drop of the diluent in the conjunctival sac and then increasing allergen concentrations every 10 min until a positive reaction (conjunctival redness and itching) occurred (22). The NPT was performed by instillation of 0.1 ml of diluent in each nostril by a mechanical pump and then increasing allergen concentrations every 10 min until two of three criteria were fulfilled: more than five sneezes, more than 0.5 ml nasal secretion, or more than 50% reduction in nasal peak flow (23). A titrated SPT was performed in quadruplicate on the volar site of the forearm with the same dilutions of aquagen, according to the recommendations of the EAACI (24). The area of wheal was outlined and transferred by adhesive tape to a sheet of paper, and determined by a SPT area scanner (25). The skin index, defined as the SPT area of 100 000 SQ-U/ml divided by the SPT area of histamine, was used for calculations.

Specific IgE to birch (t3) and timothy grass (g6) was measured by the enzyme fluorescence assay (Pharmacia CAP System, Pharmacia Diagnostics, Uppsala, Sweden), according to the instructions of the manufacturers.

Total serum IgE was measured by the microparticle enzyme immunoassay on a IMx reader, according to the manufacturer (Abbot Laboratories, IL, USA).

HR from basophil leukocytes was performed by the glass microfibre method (Reference Laboratory, Copenhagen, Denmark) (26). The HR was calculated as the maximum histamine released (ng/ml) at any allergen concentration (MAX).

The counting of EOS in blood was performed automatically by the Technicon H1 peroxidase method with a Technicon counter (Technicon Instruments Corp., New York, USA).

Serum levels of ECP and EPX were determined by ELISA procedures, as described elsewhere (27).

Symptom score and medication

Once a day, from April to August, the patients recorded symptom score and medicine intake. The severity of each of five symptoms (sneezing, rhinorrhea, nasal congestion, itchy nose and/or throat, and itchy eyes) was scored on an arbitrary scale of 0–3 (0 = no symptoms, 1 = mild, 2 = moderate, and 3 = severe symptoms). The daily use of the antihistamine acrivastine (8 mg), antazoline-naphazoline eye-drops, and prednisolone

was registered. The birch- and grass-pollen seasons were defined as the period including the mid-90% of total collected pollen of birch and grass, respectively.

Patient assignment for IT

After 1 year of observation, the patients were assigned to active IT with either birch-pollen (26 patients) or grass-pollen extracts (26 patients) by the method of minimization (28). Patient groups were comparable with respect to sex, age, duration of allergy symptoms, specific IgE, SPT, and CPT sensitivity (20).

Allergen-specific IT

During the first year of IT, the patients received double-blinded IT with either *B. verrucosa* or *P. pratense* pollen extracts (Alutard SQ). During the second year of IT, the patients continued IT with their original extract and in addition received the other extract. They were subsequently treated with both *B. verrucosa* and *P. pratense* pollen extracts. The dose-increase phase of any allergen was placed during autumn and winter outside both pollen seasons. The clustered induction schedule is shown in Table 1. The interval of maintenance dose injections was gradually increased to 2 months. The dose-increase phase of the second allergen was placed in the interval between maintenance dose injections of the first allergen. Only when the maintenance dose of both allergens was reached could the two different allergens be given on the same day. The practical administration of IT followed European guidelines (21). Before each injection, the physician evaluated the patient's condition, including peak expiratory flow (PEF), with a Mini-Wright peak flow meter (Clement Clarke International Ltd, Harlow, UK), and assessed the suitability for IT (21). Injections were postponed in patients with recent infections or exacerbations of allergic symptoms (increased need for drugs), and in patients with a

reduction of 20% or more in PEF. No additional injection was given the same day if a reduction in pretreatment PEF of 10% or more was observed. The patients received the injections at 30-min intervals and stayed in the clinic for at least 30 min after the last injection. Dose modifications were made if SRs occurred, with either no increment, or reduction of the dose up to four steps. Patients experiencing severe SRs, repeated allergic rhinitis, or inconvenient, large local reactions during cluster IT were transferred to a schedule of one injection a week.

Assessment of side-effects

Thirty minutes after each allergen injection, the early local reaction (ELR), i.e., the swelling at the injection site, was measured (mean of the longest diameter and the midpoint orthogonal diameter). Measurement of PEF was repeated, and any signs or symptoms of SR were recorded by the investigators. After each injection series, the patients registered the time of onset of rhinitis, asthma, urticaria, angioedema, or any other symptoms. Drug consumption to alleviate symptoms (antihistamine and/or β_2 -agonist) was recorded. The late local reaction (LLR) was measured 6 h after the last injection. If the swelling increased, the largest swelling was registered. SRs were classified as early, i.e., those occurring within 30 min, and late, i.e., those occurring 31 min to 48 h after the last injection. SRs were graded from 2 to 4, with 2 indicating mild SRs (rhinitis, asthma symptoms, or <25% reduction in PEF, responding adequately to antihistamines or β_2 -agonist spray), 3 indicating non-life-threatening SRs (urticaria, angioedema, or severe asthma with a reduction of 25% or more in PEF, responding well to treatment), and 4 indicating anaphylactic shock (rapidly evoked reaction of itching, flushing, erythema, bronchial obstruction, etc. requiring intensive treatment) (21). After 1 and 2 years of IT, subcutaneous nodules were registered, and patients were asked about the duration of the nodules, and if and how they were inconvenient. The patients were further asked about unspecific symptoms related to the treatment.

Statistics

Treatment groups were compared by the chi-square test of independence in 2 by 2 tables and by the two-tailed Mann-Whitney U-test. Statistical analyses were performed with the SPSS for Windows, Version 6.0 (SPSS, Inc., Chicago, IL, USA). Probability (*P*) values of less than 0.05 were considered statistically significant.

Results

Patients and allergen-specific IT

Three patients dropped out after assignment but before

Table 1. Schedule for dose increment

Day	Dose (SQ-U)
1	10
	100
	1000
7	2000
	4000
14	6000
	10 000
21	10 000
	20 000
28	30 000
	30 000
35	50 000
	50 000
42	100 000

IT, leaving 49 patients for IT (26 birch IT, 23 grass IT). The patient flow is visualized in Fig. 1. Forty-seven patients (26 birch IT and 21 grass IT) completed the first season of IT. Two patients (8.7%) of the grass IT group left the study due to side-effects, compared to one patient (3.8%) of the birch IT group ($P=0.48$). Patient no. 1 of the grass IT group experienced mild asthma with a peak flow reduction from 550 to 500 after the first injection of 10 SQ-U and wished to stop treatment. Patient 2 repeatedly experienced urticaria after IT, and was excluded (at 40 000 SQ-U). The patient of the birch IT group was excluded after the first year of IT due to side-effects consisting of several subcutaneous nodules with a maximum diameter of 3 cm. Four patients (one birch IT, three grass IT) did not start the second year of IT for reasons not related to side-effects of the treatment. Forty-two patients (24 birch IT and 18 grass IT) started IT with the second allergen. During the second year of IT, one patient, with grass IT during the first year, was excluded during the dose-increase phase of birch IT (at 40 000 SQ-U), because of pregnancy. In total, 44 patients started IT with birch (birch group), and 47 patients started IT with grass (grass group), with 43 and 45 reaching the maintenance dose, respectively.

The total number of injections was 1159 in the birch group, and 1241 in the grass group. The median number of injections for the patients reaching the maintenance dose was 14 (range 12–19) in the birch group, and 15 (range 14–26) in the grass group ($P=0.001$). Five patients in the birch group reached the maintenance dose ahead of schedule with intentional change in the dose-increase schedule to reach the maintenance dose before the season. In the birch group, four patients were transferred to conventional IT during the dose-increase phase because of SRs, in contrast to 11 receiving grass IT ($P=0.059$). Three grass patients experienced SRs at the conventional IT (one patient who was excluded because of repeated urticaria; one patient with atopic dermatitis who repeatedly developed macules at pre-disposed sites, but continued IT with no SRs during maintenance dose; and one patient with one episode of mild rhinitis). Six patients in the grass group were transferred to conventional IT because of inconvenient, large local reactions, as opposed to none in the birch group ($P=0.015$).

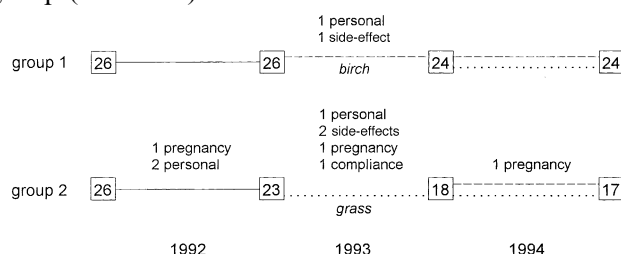


Figure 1. Study flowchart 1992–94. Number of remaining patients at beginning and end of each year for groups 1 and 2. Reason for dropouts described for each year. Straight line: baseline year; broken line: birch IT; dotted line: grass IT.

Table 2. Number and distribution of systemic reactions (SRs)

	Birch IT	Grass IT	<i>P</i> value*
U + Q	0	1	0.005
U + A	0	2	
U + RC	0	1	
U (only)	0	4	
U (total)	0	8	
I + Q	0	1	0.005
I + RC	0	1	
I + OAS	0	2	
I (only)	0	4	
I (total)	0	8	
A + RC	0	1	<0.005
A (only)	0	3	
A (total)	0	6	
RC + OAS	1	0	<0.01
RC (only)	3	8	
RC (total)	4	11	
OAS (only)	4	1	NS
OAS (total)	5	3	
Rash [§]	0	10	
Facial erythema	0	2	
SR (total)	8	41	<0.0001
Early SR	1	6	
Late SR	7	35	
Grading of SR (2/3/4)	8/0/0	31/10/0	

U: urticaria; A: asthma; RC: rhinoconjunctivitis;

I: itching without urticaria; Q: angioedema of lips and face;

OAS: oral allergy syndrome, i.e., oral-pharyngeal itching.

[§] Patient with atopic dermatitis who repeatedly developed 1–5-mm-long macules at predisposed sites, 3–6 h after injections.

* *P* values were derived by chi-square test.

Side-effects

The number of SRs was small (2% of total injections), and the majority (79.6%) of the SRs were mild (grade 2) reactions, with no life-threatening (grade 4) reactions observed. However, a difference in the number and profile of SRs was observed between birch and grass IT (Table 2). SRs occurred after 0.7% of birch-extract injections, and 3.3% of grass-extract injections ($P<0.0001$), with eight SRs after birch IT and 41 after grass IT. All SRs after birch IT were mild (grade 2) reactions, consisting of rhinoconjunctivitis and oral-pharyngeal itching (oral allergy syndrome [OAS]). The SRs after grass IT were mostly mild (grade 2) reactions, but 10 SRs (24%) were graded as 3. The difference in severity of SRs between birch and grass IT was significant ($P<0.0001$). There were six episodes of asthma and eight episodes of urticaria after grass IT, as opposed to none after birch IT ($P<0.05$ and $P=0.005$). The difference in severity of the SRs between birch and grass IT was reflected in the number of dose reductions caused by SRs, with eight and 25 dose reductions in the birch and grass groups, respectively ($P=0.005$).

There was a difference in the number of patients with SRs, with five (11%) and 21 (45%) patients in the birch and grass groups, respectively ($P<0.001$). Seven

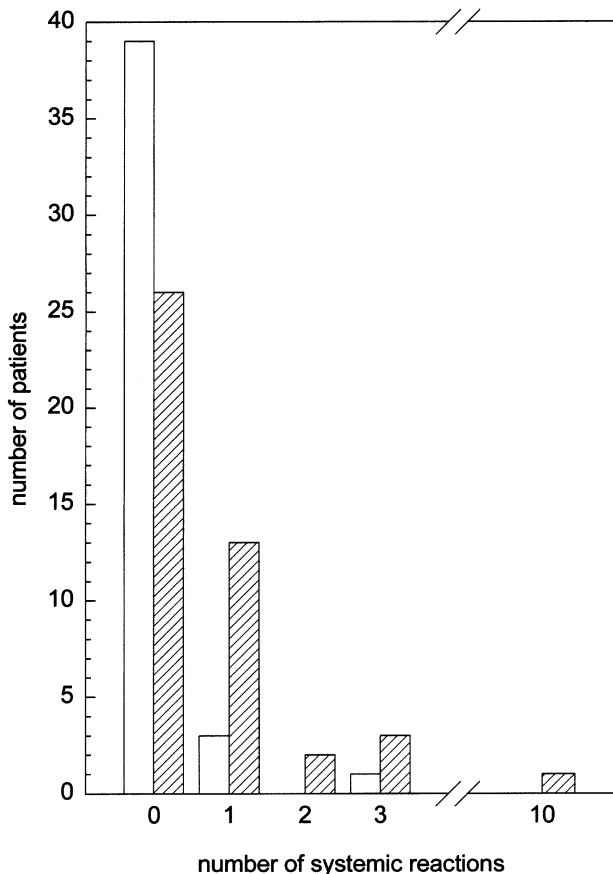


Figure 2. Number of patients with SRs during dose-increase phase (patients who reached maintenance dose 100 000 SQ-U). White bar, birch IT; filled bar, grass IT. Two grass IT patients, with one and four SRs, respectively, and one birch IT patient with two SRs, did not reach maintenance dose, and are not presented in figure.

patients were responsible for the 10 grade 3 reactions seen after grass IT. In the birch group, the maximum number of SRs per patient was three, as opposed to 10 in the grass group ($P < 0.01$) (Fig. 2). No patients in the birch group experienced asthma or urticaria, whereas, in the grass group, 29% of the patients had asthma, and 29% had urticaria ($P < 0.05$).

All SRs occurred during the dose-increase phase. Most of the SRs occurred at the lower concentrations, with more than 80% of the SRs occurring before a cumulated daily dose of 30 000 SQ-U (Fig. 3).

The time interval from the last injection to onset of mild grade 2 SRs, such as OAS and rhinoconjunctivitis, ranged from 5 min to 24 h. The 10 grade 3 SRs after grass IT, involving eight episodes of urticaria (one with concomitant angioedema of the lips and face, two with concomitant asthma, and one with concomitant rhinitis), one episode of angioedema of the face, and one episode of asthma, occurred after 1–2 h. Seventy-two percent of the grade 2 reactions were treated (64% with antihistamine alone, 8% with β_2 -agonist alone). All 10

grade 3 reactions were treated, six by antihistamine alone and four by combinations of antihistamine, β_2 -agonist, and prednisolone.

When comparing the LLRs after equal doses, irrespective of the number of injections given on the same day, the LLRs were significantly smaller in the birch group at all IT stages ($P < 0.0001$ – 0.03). This was reflected by three and 34 dose modifications caused by inconvenient, large LLRs, for the birch and grass groups, respectively ($P < 0.0001$). There were 100 LLRs greater than 8 cm in the grass group, as opposed to 16 in the birch group ($P < 0.0001$). In six of these 116 LLRs (5%), injections at the next visit resulted in SRs. Ten percent of the SRs were preceded by a LLR greater than 8 cm, and in 6% of the SRs a LLR greater than 8 cm developed after the injection causing the SR (all grass) (NS).

Unspecific symptoms after IT were frequent with 48% of the patients complaining of tiredness (occasionally: 45%; frequently: 30%; always: 25%). Occasional headache was seen in 12%, and occasional malaise in 5%.

After the first year of IT, subcutaneous nodules were present in 80% of birch IT patients, and 86% of the grass IT patients (NS), with no statistical difference in the number of subcutaneous nodules between groups (birch IT: mean 2, range 0–13; grass IT: mean 1.4, range 0–5). During the second year, patients were treated with both birch and grass, and it was not possible to distinguish between nodules occurring from birch or grass during this year, as the injections were given randomly in both arms. After the first year of IT, a difference in the number of patients with inconvenient itching and swelling of nodules occurred (60% of the birch-treated patients, as opposed to 14% of the grass-treated patients) ($P < 0.001$). However, the symptoms were mainly occasional (76% of the patients had occasional, 19% frequent, and 5% constant symptoms). The itching and swelling of nodules was initiated mainly by mechanical stimulation, but some patients developed itching and swelling during the pollen season, when allergic rhinitis symptoms were present, and after

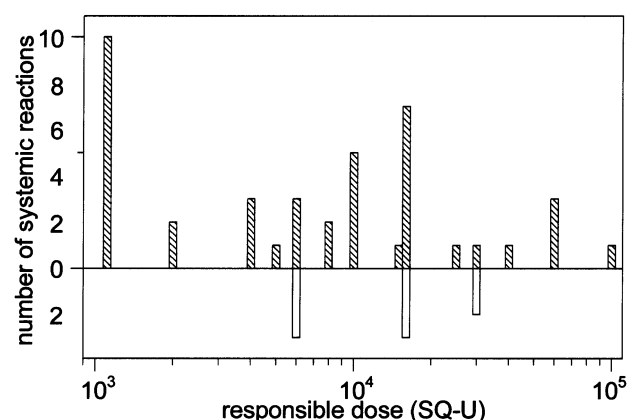


Figure 3. Number of SRs according to responsible cumulated dose on same day. White bar, birch IT; filled bar, grass IT.

Table 3. Clinical and paraclinical data of patients without and with side-effects to immunotherapy

	Patients with no side-effects (no. 27) Median (range)	Patients with side-effects (no. 22) Median (range)	<i>P</i> value
Age (years)	26 (18–52)	27 (20–41)	0.52
Duration of allergy (years)	18 (5–30)	15.5 (5–33)	0.42
Asthma (a/b/c) (No.)	18/4/5	15/3/4	0.99
Birch seasonal			
Symptom score	3.63 (0.53–10.29)	3.38 (0.67–8.90)	0.42
Antihistamine intake	1.12 (0.11–2.53)	0.25 (0–1.83)	0.66
Eye-drop use	0.48 (0–3.8)	0.39 (0–3)	0.61
Grass seasonal			
Symptom score	5.00 (1.09–10.78)	5.78 (1.75–10.08)	0.21
Antihistamine intake	1.12 (0.11–2.53)	1.41 (0.15–2.81)	0.49
Eye-drop use	0.66 (0–5.10)	0.76 (0–3.19)	1.00
Birch SPT index	1.97 (0.45–10.7)	2.06 (0.26–5.48)	0.53
Grass SPT index	2.65 (0.89–14.7)	3.13 (0.96–8.40)	0.34
Birch CPT (d/e/f/g/h) (No.)	0/2/14/11/0	2/0/14/6/0	0.29
Grass CPT (d/e/f/g/h) (No.)	0/2/22/3/0	1/2/16/3/0	0.90
Birch NPT (d/e/f/g/h) (No.)	1/2/12/11/2	0/1/8/11/2	0.77
Grass NPT (d/e/f/g/h) (No.)	0/3/19/5/1	2/1/15/4/0	0.81
Total IgE (kU/ml)	259 (20–8408)	130.5 (32–1275)	0.31
Birch-specific IgE (kU/l)	9.29 (1.27–85.9)	9.52 (0.74–57.9)	0.67
Grass-specific IgE (kU/l)	22 (2.01–143)	12.8 (1.46–120)	0.33
Birch histamine release (ng/ml)	43 (13–115)	40 (0–119)	0.65
Grass histamine release (ng/ml)	41 (15–103)	39 (9–109)	0.69
Eosinophils (10 ⁶ /ml)	0.16 (0.05–0.51)	0.23 (0.07–0.62)	0.56
ECP (ng/ml)	9.0 (0.8–36)	9.3 (1.6–42)	0.80
EPX (ng/ml)	23 (2.3–97.5)	21.5 (9.0–61.5)	0.79

(a/b/c) = (none/allergen contact only/with or without allergen contact).

(d/e/f/g/h) = (not done/10³/10⁴/10⁵/ > 10⁵ SQ-U/ml).

subsequent IT injections. The duration of the nodules varied among the patients, but there was no difference between the groups. After 2 years of IT, the nodules were present for less than a month in 19%, 1–3 months in 35%, 3–6 months in 11%, and more than 6 months in 35% of the patients.

The results from pretreatment birch- and grass-specific SPT, CPT, NPT, IgE, and HR, as well as total IgE, EOS, and serum ECP and EPX, were similar in the group with, and the group without SRs to IT (Table 3). Furthermore, these groups did not differ with respect to age, duration of allergic symptoms, and prevalence of asthma, or in seasonal means of birch/grass symptom score, eye-drop use, and antihistamine intake (Table 3). No correlation was found between the number of SRs and the above-mentioned parameters for either birch or grass, except that the number of eosinophils correlated to the number of side-effects to grass IT (Table 4).

Discussion

In this study, the number of patients with SRs, and the number of, and the severity of, SRs were significantly higher in the patients treated with grass IT than birch IT, with identical dose schedules.

Different potency of the birch- and grass-pollen

extracts is the most likely explanation. The LLRs were greater in patients treated with grass extract; furthermore, the SPT index was greater for grass ($P=0.001$), and the sensitivity of the NPT to grass was higher ($P=0.01$) than to birch.

One could argue that the difference in SRs was caused by a difference in the severity of clinical birch- and grass-pollen allergic rhinitis symptoms. In fact, during the initial observation period, the patients had a higher

Table 4. Correlation coefficients and (*P* values) of relationship between number of birch IT/grass IT side-effects per patient and clinical and paraclinical data

	Number of birch IT side-effects	Number of grass IT side-effects
Age (years)	0.11 (0.50)	−0.08 (0.62)
Duration of allergy (years)	−0.20 (0.20)	−0.25 (0.09)
Birch or grass seasonal symptom score	0.08 (0.60)	0.12 (0.44)
Antihistamine intake	−0.04 (0.79)	−0.12 (0.41)
Eye-drop use	0.12 (0.44)	−0.06 (0.71)
Birch or grass SPT index	−0.04 (0.81)	0.07 (0.64)
Birch or grass CPT	−0.05 (0.74)	0.19 (0.20)
Birch or grass NPT	−0.05 (0.76)	−0.09 (0.56)
Total IgE (kU/ml)	−0.07 (0.66)	0.01 (0.97)
Birch- or grass-specific IgE (kU/l)	0.01 (0.97)	−0.11 (0.48)
Birch or grass HR (ng/ml)	0.15 (0.32)	−0.01 (0.94)
Eosinophils (10 ⁶ /ml)	−0.11 (0.47)	0.41 (0.005)
ECP (ng/ml)	−0.12 (0.43)	−0.03 (0.82)
EPX (ng/ml)	−0.06 (0.73)	−0.03 (0.83)

seasonal mean symptom score in the grass-pollen season than in the birch-pollen season (medians 5.4 and 3.4; $P < 0.001$). However, differences in duration of pollen seasons and in pollen load might explain this finding (20). Furthermore, no difference in mean birch- and grass-seasonal symptom score was found between the groups with and without side-effects.

Neither *in vivo* nor *in vitro* test parameters could predict SRs in our patients. We found a correlation between the number of grass IT-induced SRs per patient and the concentration of eosinophils in the blood. This finding was not observed for birch IT, and there was no difference in concentration of eosinophils in the groups with and without SRs, bringing into question the relevance. In a previous study, a correlation was found between side-effects and results from the NPT, but not the SPT (13). Two mite studies found the SPT to be predictive of SRs (29, 30). Differences in sample size may explain the conflicting results. The clinical status of the patients (age, duration of allergic symptoms, and asthma) gave no relevant information either. This is in accordance with one study, which found no relationship to age (31). Other studies did find asthma to be a predictive factor for SRs. In an IT study with 256 asthma patients and 179 rhinoconjunctivitis patients, 21 of 25 patients with SRs were asthmatic (7). In a study on asthma patients, the pulmonary function before IT had a predictive value for the onset of asthma symptoms during IT, since 73% of the patients with a FEV₁ of <80% of predicted values developed asthma, as compared with 13% with a FEV₁ above this threshold limit (30). The lack of predictive effect of asthma in our study could have occurred because of the low frequency, the mild asthma symptoms, and the pretreatment asthma monitoring.

Delayed skin reactions greater than 8 cm after IT with Alutard SQ extracts did not seem to predict SRs at the next visit, and SRs were not followed by great LLRs. The missing predictive value of LLRs to SRs was in accordance with other studies (13, 29, 32, 33).

All SRs occurred during the dose-increase phase, bringing into question the present cluster induction schedule more than the actual size of the maintenance dose. As IT is a time-consuming treatment, an IT schedule with few injections during the dose-increase phase would be preferable. At the same time, we need a treatment with no or few side-effects. An aggressive schedule implies a higher risk of side-effects, and consequently more dose modifications (5). In this study, 45% of grass IT patients experienced side-effects, compared to 9.5% in a study where the same grass-pollen extract and maintenance dose were used but with a conventional one injection per visit (15 injections) schedule (11). However, mild grade 2 reactions such as rhinoconjunctivitis might not have been registered, narrowing the difference in side-effects between the studies (15% vs 9.5%). In placebo-controlled studies,

side-effects such as rhinitis and urticaria have been observed at placebo injections (34, 35). The present study was not placebo controlled, and consequently the frequencies of side-effects caused by allergen injection might have been lower. Still, this did not influence the observed difference in side-effects between birch and grass IT.

The majority of SRs were mild, with a high proportion of rhinoconjunctivitis as sole symptom. These reactions were self-treated by antihistamines if needed. We saw no anaphylactic reactions, but 24% (10) of SRs to grass-pollen IT were grade 3 reactions with asthma, angioedema, and urticaria, which all occurred 1–2 h after the patients had left the clinic. Most of these SRs were self-treated with antihistamines at home, and the patients did not contact the clinic. But on three occasions, the patients returned to the clinic, and on one occasion, the patient visited the local emergency clinic to be treated with a combination of antihistamine, β_2 -agonist, and prednisolone. The late grade 3 SRs of this study with an Alutard induction schedule emphasize the need for careful instruction of the patients.

Most of the grade 3 reactions comprised urticaria, and might have been avoided if the patients had been pretreated with antihistamines, as antihistamine premedication in one placebo-controlled study has been shown to reduce IT-induced urticaria (33). Further placebo-controlled studies of antihistamine premedication, however, are still needed before premedication with antihistamines should be recommended as a routine procedure. The optimum for grass-pollen extracts might be a conventional dose-increase schedule to reduce side-effects to an acceptable level. Further comparison of different dose-increase schedules for grass IT are needed. The cluster schedule with only seven visits was acceptable for the birch-pollen extract.

The LLRs were larger, and the dose modifications because of inconvenient LLRs were higher when patients were treated with grass IT than with birch IT. However, there was no difference in the number of patients with subcutaneous nodules, and the number of subcutaneous nodules, between the two extracts. It has previously been shown that the frequencies of patients with subcutaneous nodules is proportionate to the quantity of aluminum in the extracts (13). That study found 60% of patients with subcutaneous nodules, in contrast to approximately 90% in the present study, with the same amount of aluminum. In the present study, patients were actively asked about the nodules by questionnaires, and the arms of the patients were palpated. This could explain the difference. Only 50% of the patients with nodules felt inconvenience, mostly occasional, and the majority of nodules were transient; however, other adjuvants with no long-term accumulation might help to reduce local side-effects further.

In summary, all side-effects in this clustered Alutard IT study occurred during the dose-increase phase, and

the vast majority were mild reactions. The profile of side-effects, however, differed with the allergen extract applied. With a 7-week, clustered, up dosing regimen, grass-pollen IT seemed to be associated with a higher number, and more severe, side-effects than that of birch-pollen IT. In order to reduce side-effects during grass-pollen IT, alternative measures, such as a lower dosing, more time-consuming conventional schedule, might be applied. The number of side-effects in the present study was probably influenced by the meticulous questioning and monitoring performed. However, this could not explain the difference between the grass- and birch-pollen extracts. It would be of great benefit to be able to find patient-related factors that

could predict side-effects in IT. Diagnostic tests for allergy such as specific IgE, HR, SPT, CPT, and NPT are no help for this purpose. The age, duration of allergy symptoms, and asthma status of the patients gave no information either, nor did inflammatory markers such as ECP, EPX, and eosinophil counts.

Acknowledgments

We thank the patients for their kind and devoted participation during this immunotherapy study, and also the staff at the allergy department for their practical assistance. This study was supported by a grant from ALK-Abelló.

References

1. MALLING H-J. EAACI/PTA symposium reviews. Immunotherapy as an effective tool in allergy treatment. *Allergy* 1998;**53**:461–472.
2. STEWART EG, LOCKEY RF. Systemic reactions from allergen immunotherapy. *J Allergy Clin Immunol* 1992;**90**:567–578.
3. WEKKELI M, ROSENKRANZ A, HIPPMANN G, et al. [Systemic side-effects in immunotherapy of allergic diseases – a comparative study]. *Wien Klin Wochenschr* 1989;**101**:639–652.
4. HAUGAARD L, DAHL R, JACOBSEN L. A controlled dose-response study of immunotherapy with standardized, partially purified extract of house dust mite: clinical efficacy and side-effects. *J Allergy Clin Immunol* 1993;**91**:709–722.
5. HEJJAOUI A, DHIVERT H, MICHEL FB, BOUSQUET J. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. IV. Systemic reactions according to the immunotherapy schedule. *J Allergy Clin Immunol* 1990;**85**:473–479.
6. HEJJAOUI A, FERRANDO R, DHIVERT H, et al. Systemic reactions occurring during immunotherapy with standardized pollen extracts. *J Allergy Clin Immunol* 1992;**89**:925–933.
7. TABAR AI, GARCIA BE, RODRIGUEZ A, et al. A prospective safety-monitoring study of immunotherapy with biologically standardized extracts. *Allergy* 1993;**48**:450–453.
8. ØSTERGAARD PA, KAAD PH, KRISTENSEN T. A prospective study on the safety of immunotherapy in children with severe asthma. *Allergy* 1986;**41**:588–593.
9. RAGUSA FV, PASSALACQUA G, GAMBARDILLA R, et al. Nonfatal systemic reactions to subcutaneous immunotherapy: a 10-year experience. *J Invest Allergol Clin Immunol* 1997;**7**:151–154.
10. MÖLLER C, DREBORG S. Cross-reactivity between deciduous trees during immunotherapy. I. *In vivo* results. *Clin Allergy* 1986;**16**:135–143.
11. VARNEY VA, GAGA M, FREW AJ, et al. Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by antiallergic drugs [see comments]. *BMJ* 1991;**302**:265–269.
12. WALKER SM, VARNEY VA, GAGA M, et al. Grass-pollen immunotherapy: efficacy and safety during a 4-year follow-up study. *Allergy* 1995;**50**:405–413.
13. ØSTERBALLE O. Side-effects during immunotherapy with purified grass-pollen extracts. *Allergy* 1982;**37**:553–562.
14. BENTLEY AM, JACOBSON MR, CUMBERWORTH V, et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. *J Allergy Clin Immunol* 1992;**89**:877–883.
15. CARLSON M, HÅKANSSON L, KAMPE M, et al. Degranulation of eosinophils from pollen-atopic patients with asthma is increased during pollen season. *J Allergy Clin Immunol* 1992;**89**:131–139.
16. CARLSON M, HÅKANSSON L, PETERSON C, et al. Secretion of granule proteins from eosinophils and neutrophils is increased in asthma. *J Allergy Clin Immunol* 1991;**87**:27–33.
17. MARONE G, SPADARO G, PATELLA V, GENOVESE A. The clinical relevance of basophil releasability. *J Allergy Clin Immunol* 1994;**94**:1293–1302.
18. NACLERIO RM, BAROODY FM, KAGEY-SOBOTKA A, LICHTENSTEIN LM. Basophils and eosinophils in allergic rhinitis. *J Allergy Clin Immunol* 1994;**94**:1303–1309.
19. SCHROEDER JT, KAGEY-SOBOTKA A, LICHTENSTEIN LM. The role of the basophil in allergic inflammation. *Allergy* 1995;**50**:463–472.
20. WINTER L, MOSEHOLM L, MOSBECH H, MALLING H-J. Allergen-specific immunotherapy in birch- and grass-pollen-allergic rhinitis. I. Efficacy estimated by a model reducing the bias of annual differences in pollen counts. *Allergy* 2000;**55**:818–826.
21. MALLING H-J, WEEKE B. EAACI position paper. Immunotherapy. *Allergy* 1993;**48 Suppl 14**:9–45.
22. MÖLLER C, BJÖRKSTÉN B, NILSSON G, DREBORG S. The precision of the conjunctival provocation test. *Allergy* 1984;**39**:37–41.
23. DRUCE HM, SCHUMACHER MJ. Nasal provocation challenge. The Committee on Upper Airway Allergy. *J Allergy Clin Immunol* 1990;**86**:261–264.
24. DREBORG S, FREW A. EAACI position paper. Allergen standardization and skin tests. *Allergy* 1993;**48 Suppl 14**:49–82.
25. POULSEN LK, LIISBERG C, BINDSLEV-JENSEN C, MALLING H-J. Precise area determination of skin-prick tests: validation of a scanning device and software for a personal computer. *Clin Exp Allergy* 1993;**23**:61–68.
26. NOLTE H, SCHIÖTZ O, SKOV PS. A new glass microfibre-based histamine analysis for allergy testing in children: results compared with conventional leukocyte histamine release assay, skin prick test, bronchial provocation test and RAST. *Allergy* 1987;**42**:366–373.
27. REIMERT CM, POULSEN LK, BINDSLEV-JENSEN C, et al. Measurement of eosinophil cationic protein (ECP) and eosinophil protein X/eosinophil derived neurotoxin (EPX/EDN). Time and temperature dependent spontaneous release *in vitro* demands standardized sample processing. *J Immunol Methods* 1993;**166**:183–190.
28. ALTMAN DG. Practical statistics for medical research. New York: Chapman & Hall, 1992.

29. MOSBECH H, DIRKSEN A, DREBORG S, et al. Hyposensitization in asthmatics with mPEG-modified and unmodified house dust mite extract. IV. Occurrence and prediction of side-effects. *Allergy* 1990;**45**:142–150.
30. BOUSQUET J, HEJJAOU A, DHIVERT H, et al. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. Systemic reactions during the rush protocol in patients suffering from asthma. *J Allergy Clin Immunol* 1989;**83**:797–802.
31. TINKELMAN DG, COLE WQ, TUNNO J. Immunotherapy: a one-year prospective study to evaluate risk factors of systemic reactions. *J Allergy Clin Immunol* 1995;**95**:8–14.
32. NELSON BL, DUPONT LA, REID MJ. Prospective survey of local and systemic reactions to immunotherapy with pollen extracts. *Ann Allergy* 1986;**56**:331–334.
33. NIELSEN L, JOHNSEN CR, MOSBECH H, et al. Antihistamine premedication in specific cluster immunotherapy: a double-blind, placebo-controlled study. *J Allergy Clin Immunol* 1996;**97**:1207–1213.
34. ZENNER HP, BAUMGARTEN C, RASP G, et al. Short-term immunotherapy: a prospective, randomized, double-blind, placebo-controlled multicenter study of molecular standardized grass and rye allergens in patients with grass-pollen-induced allergic rhinitis. *J Allergy Clin Immunol* 1997;**100**:23–29.
35. BOUSQUET J, HEJJAOU A, SOUSSANA M, MICHEL FB. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. IV. Comparison of the safety and efficacy of two dosages of a high-molecular-weight allergoid. *J Allergy Clin Immunol* 1990;**85**:490–497.