

Immunotherapy with partially purified and standardized tree pollen extracts

I. Clinical results from a three-year double-blind study of patients treated with pollen extracts either of birch or combinations of alder, birch and hazel

B. NÜCHEL PETERSEN,¹ H. JANNICHE,¹ E. P. MUNCH,² J. Å. WIHL,³ H. BÖWADT,⁴ H. IPSEN⁴ & H. LØWENSTEIN⁴

¹Dept. of Lung Diseases, Copenhagen County Hospital, Gentofte, ²The Allergy Clinic, Frederiksberg, Copenhagen, Denmark. ³Dept. of Otorhinolaryngology, University of Lund, Malmö General Hospital, Sweden, and ⁴ALK Research, Hørsholm, Denmark

Fifty-four adult patients with tree pollen-induced rhinitis (28), asthma (1), or rhinitis and asthma (25) were selected for immunotherapy with standardized and partly purified tree pollen extracts using a double blind protocol. The selection was based on clinical history, results of nasal or bronchial challenge, skin prick tests and RAST. Further, based on crossed radio-immunoelectrophoresis, sex, age and severity of symptoms, the patients were allocated in matched pairs and the treatment alternatives were randomly distributed within the pairs. Twenty-three patients treated with extracts composed of any combination of alder, birch and hazel pollen which matched their IgE response in CRIE (Group 1 (ABC)) and 22 patients receiving birch pollen extracts (Group 2 (B)) completed all 3 years of treatment. The *in vivo* results comprising symptom and medicine consumption scores are given here. Changes in specific skin and nasal reactivity as well as in immunological parameters are presented separately. No significant differences were demonstrated between the treatment groups in the two parameters. Both extracts were effective and reduced in general the symptom scores to one tenth of the starting level. Expressed another way, at the end of the study, the patients tolerated 30 times more pollen until symptoms of the same severity were elicited, compared to before. In the Nordic countries, spring-time asthma and rhino-conjunctivitis caused by pollen from deciduous trees can be effectively treated with an extract of birch pollen alone.

Key words: asthma; immunotherapy; rhinitis; tree pollen allergy.

Accepted for publication 11 February 1988

Tree pollens are important airborne spring allergens in many parts of the world including Scandinavia, northwest Europe and parts of North America. Allergy against tree pollen, defined as a positive skin prick test, has been

found among 12% of a selected allergic population in Denmark (2). In the other Scandinavian countries with areas containing a greater number of birch trees, an even more pronounced allergy, especially towards birch pollen, has

Abbreviations:

Group 1 (ABC): Patients treated with mixtures of one or more of alder, birch and hazel pollen extracts; Group 2 (B): Patients treated with birch pollen extracts; RAST: Radio allergosorbent test; CRIE: Crossed radio immuno electrophoresis; A: Alder (*Alnus glutinosa*); B: Birch (*Betula verrucosa*); C: Hazel (*Corylus avellana*); HEP: Histamine equivalent prick; pg: 10⁻¹² g; MDI: Metered dose inhaler; SPT: Skin prick testing; NPT: Nasal provocation test; BPT: Bronchial provocation test; Symp.sc.: Symptom score.

been revealed (4). The most common tree pollen used for specific immunotherapy in Scandinavia is either birch or a mixture of alder, birch and hazel. Eriksson et al. (3) examined the effect of extracts composed of alder, birch and hazel compared to extracts consisting of these three kinds of pollen and other tree pollen to which the patients also reacted. A significant difference was found and it was shown that the mixture of alder, birch and hazel was the most effective. Recently, Möller & Dreborg (10) published *in vivo* results of an immunotherapy study in children treated with extracts containing either a mixture of pollen from alder, birch and hazel, or birch alone. Although birch is the dominating deciduous tree in northern Sweden where the study was performed, they could demonstrate a slight but significantly stronger decrease in symptom scores among patients treated with the mixture, compared to those treated with equipotent amounts of birch pollen alone.

Most of the molecules extracted from pollen of alder, birch and hazel are immunologically partially identical, having molecular epitopes common to the three species. Partially identical epitopes have been studied, particularly in the major allergens of these phylogenetically related pollens (6). However, it is not known whether the patients' common reactions to pollen from these three sources are due to specific sensitization (i.e. the patients' reaction towards molecular epitopes they have actually been sensitized

with) or due to cross-sensitization (i.e. reactions caused by an epitope common to the related molecules from the related pollen).

The aim of this study is to reveal the efficacy of hyposensitization with either birch pollen extract alone or with that mixture of alder, birch and hazel which corresponds to the patient's diagnostic response. This paper will compare daily symptom scores of patients, including medicine consumption and subjective evaluation of the treatment, and current pollen counts. In a number of successive papers the results of diagnostic tests including skin prick tests, nasal provocation tests (14), RAST, and a detailed study of patients' IgE response to the various individual major allergens from alder, birch and hazel by means of CRIE (7) as a function of the 3 years' treatment period, will be presented.

MATERIAL AND METHODS

Subjects

Fifty-four patients from two allergy clinics in Copenhagen, Denmark, and one in Malmö, Sweden, were selected on the basis of the criteria given in Table 1. All were out-patients suffering from tree pollen allergy. The criteria for inclusion in the study were the number of accumulated scores obtained from case history, nasal or bronchial provocation tests, skin prick

Table 1
Selection of patients. Parameters used for scoring

Score	History	Pos. NPT/BPT at concentration (HEP)	Skin index (SI) using 1 HEP extract	RAST class
0	negative	> 1	< 0.5	0-1
1	doubtful	0.1	> 0.5, < 1	2
2	positive	< 0.01	> 1	3-4

The nasal provocation test/bronchial provocation test (NPT/BPT) was performed with a mixture of equal amounts (in SQ units) of pollen extracts from alder, birch and hazel (ref. 14).

Skin prick test (SPT) and radioallergosorbent test (RAST) were performed separately using pollen extracts from alder, birch and hazel (refs. 14 and 7).

$$SI = \frac{D_{EX} \times d_{EX}}{D_{Hist} \times d_{Hist}}$$

where the D's are the largest diameter of the weal and the d's the largest perpendicular to that one using extract (EX) or histaminedihydrochloride 1 mg/ml (Hist) for the skin prick tests.

tests and RAST. Patients who obtained a total score of at least 5 were included. The provocation tests were performed with a mixture of SQ standardized alder, birch and hazel pollen extracts (see below), whereas skin prick tests and RAST were performed with the individual SQ standardized pollen extracts. Patients with allergy in a season other than spring could be included. Patients with perennial allergy were excluded. Other exclusion criteria were former hyposensitization with tree pollen extracts, pregnancy and chronic diseases of the upper and lower airways.

The 24 men and 30 women selected had a mean age of 33.2, ranging from 15 to 72 years, with a mean duration of symptoms of 7.5 years (range 2-26 years). Twenty-eight of the patients had rhinoconjunctivitis, 25 had rhinoconjunctivitis and asthma, and one patient had asthma only (Table 2). All patients were tested for other allergies. Two had grass pollen allergy, but symptoms outside the tree pollen season were generally very weak.

After the allocation (see below) but before the start of the immunotherapy, three patients declined to participate, leaving a total of 51 patients in the study. Informed consent for participation was given by all patients, and the study was approved by the Committee of Ethics in Copenhagen County.

Extracts

Partly purified, standardized allergenic extracts of alder, birch or hazel were used. According to the manufacturer (Allergologisk Laboratorium ALK, Copenhagen, Denmark), the extracts were standardized relative to an internal standard (8) by a combination of three independent methods: quantitative immunoelectrophoresis for quantitation of the major allergens as well as a check on the content of other potential allergens; RAST inhibition as a measure of the total allergenic activity; and skin prick tests on a number (> 10) of selected patients clinically allergic towards the allergen in question as a control of the biological activities. Since the skin prick test biological activity (HEP value) (1) of the internal stan-

dards is known from testing on a large number of clinically allergic individuals (> 30), the HEP value of the extracts could be calculated (1, 8). Identically composed extracts were used for both treatments (aluminium hydroxide absorbed extract, Alutard SQ®), for skin prick testing (dissolved in 50% glycerol, Soluprick SQ®) for nasal and bronchial provocation test (water soluble extract, Aquagen SQ®) and for *in vitro* investigations, e.g. RAST, histamine release and CRIE (water soluble extract, aquagen SQ®). The activity of the extracts was given in SQ units. The content of birch pollen major allergen Bet v I (Ag 23) (5, 9) in the birch pollen extract was 230 pg/SQ unit. The biological activity was 10 HEP/SQ units for all three pollen extracts: alder, birch and hazel. When mixtures of the various tree pollen extracts were used for treatment, equal amounts of SQ units of the various tree pollen extracts were used for the mixtures.

Dosage

The immunotherapy was carried out with Alutard SQ extracts with 1-3 weekly injections starting at a dose of 20 SQ units. The dose was then increased in stages until an expected maintenance dose (10,000 SQ units) was reached, or until a maximum tolerated dose (i.e. general reactions or maximum local reactions > 8 cm in largest diameter using the same dose), whereafter this dose was given every 4th to 6th week. After 1 year of treatment the maintenance dose was further increased to the individual maximum tolerated dose, or 100,000 SQ units. This dosage was used for the remainder of the treatment period. Maintenance doses were reached before the pollen season, during which the dosage was reduced where necessary to 1/3-1/4. In the majority of cases the treatment was continued by the general practitioner after maintenance doses had been reached.

Skin prick test (SPT)

Skin prick tests were performed as a titration using the Soluprick SQ pollen extracts from

alder, birch and hazel in the following concentrations: 0.01, 0.03, 0.1, 0.3, 1.0 HEP. The procedure and results are presented in detail elsewhere (14).

Provocation tests (NPT/BPT)

The bronchial provocation tests were performed using 10-fold allergen dilutions starting from 0.01 HEP 1 ml inhaled as a mist from a Pari Inhalior Boy with recording of FEV₁. The provocation test was positive when FEV₁ decreased 20% or more compared with the highest measured value after inhalation of physiologic salt solution 1 ml.

The nasal and/or bronchial provocation tests were performed with either 0.2 or 1 ml of an allergen extract which was a mixture of equal amounts (in SQ units) of pollen extracts from alder, birch and hazel. The nasal provocations were performed using 10-fold dilutions starting from 0.01 HEP. Into each nostril 0.1 ml of the test extract was sprayed and the reaction given as a total nasal provocation score comprising sneezes, secretion and nasal blockage (measured as expiratory nasal peak flow). The procedure and results are presented in detail elsewhere (14).

In vitro tests

Allergen-specific IgE antibodies were measured by means of radioallergosorbent test (RAST), histamine release (HR) from leukocytes and crossed-radioimmuno-electrophoresis (CRIE). The analyses were performed as described in detail elsewhere (7).

Symptom and medicine consumption scores

Daily recording of symptoms and medicine consumption was performed by all the participating patients and entered on special diary cards. Eye, nose and lung symptoms were recorded separately using the following system: 0 = no symptoms; 1 = mild symptoms; 2 = moderate symptoms; 3 = severe symptoms. Daily medicine consumed was registered according to the following score system: 2 mg Polaramin = 1; 2 drops Antistina Privine eye drops = 1; 1 spray Ventoline MDI/Bricanyl MDI = 1; 5 mg prednisone = 5. The weekly symptom and medicine consumption scores for all patients were calculated and all data computerized.

After 3 years of treatment and before the code was broken, a global assessment was made by both the patient and the investigator using a

Table 2
Demographic data, concomitant allergies and medication

	Group 1 (ABC) Various mixtures of pollen extracts from alder, birch and hazel	Group 2 (B) Birch pollen extract
Male/Female	13/14	11/16
Age (years):		
mean	30.4	32.4
range	15-72	15-56
Rhinitis	13	15
Asthma	1	0
Rhinitis and asthma	13	12
Duration of symptoms (years)		
mean	8.3	6.8
range	2-26	2-20
Clinical allergy to:		
- birch pollen	3	3
- alder & birch pollen	3	3
- alder, birch & hazel pollen	21	21
- animal danders, mites, moulds	0	0
- pollens other than spring trees	1	1

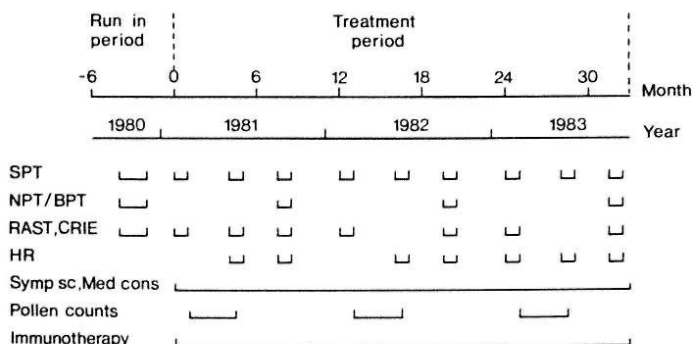


Fig. 1. Study design. SPT: skin prick test; NPT: nasal provocation test; BPT: bronchial provocation test; RAST: radioallergosorbent test; CRIE: crossed radio-immunoelectrophoresis; HR: histamine release from leukocytes; Symp sc: symptom score; Med cons: medicine consumption.

questionnaire. Evaluations of both the tolerance to the immunotherapy and recorded side effects were obtained from the individual records.

Pollen recording

The number of alder, birch and hazel pollen per cubic meter of air per 24 h was recorded in Copenhagen and Malmö. At both stations a Burchard 7 days recording spore trap was used (12). No significant difference was noted between the two observations.

Study design and allocation

The study was double-blind with parallel groups. During a 4-month pre-immunotherapy period the patients were characterized with respect to SPT, NPT, BPT, RAST, and CRIE. Based on age, sex, clinical symptoms and CRIE, the patients were matched into pairs and the two treatment alternatives, combination of alder, birch and hazel which corresponded to the patient's CRIE response (Group 1 (ABC)) or birch pollen extract (Group 2 (B)), were randomly distributed within the pairs (Table 2).

After the pre-immunotherapy period, SPT was repeated immediately before the start of the treatment (February), immediately after the season (June), and in the autumn (September, October). This was repeated for the following 2

years. RAST and CRIE were performed on blood samples taken according to the same time schedule, with the exception of June 1982 and June 1983. Provocation tests were performed before the start of treatment (February) and in the autumn (September, October) for all 3 years (Fig. 1).

Statistical methods

Student's t-test was used when measuring differences in total symptom scores or medicine consumption between the two treatment groups. All comparisons within the same group of patients at different times were made employing the Wilcoxon matched pairs rank sum test. Other comparisons between groups of patients were made at the same time by the Mann-Whitney U-test. All two-sided stochastic probabilities less than 0.05 were considered significant. The Spearman rank correlation test was used when comparing different recordings/tests.

RESULTS

Patients

Comparison between the patients in the two treatment groups revealed great similarities between

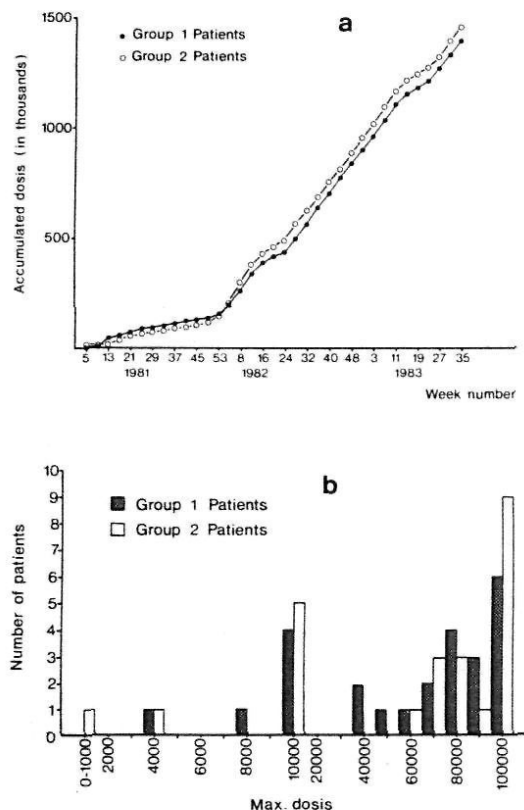


Fig. 2. Amount of allergen extracts given during 3 years of immunotherapy. a. Mean accumulated doses in SQ units for treatment Group 1 (ABC) and Group 2 (B). b. Distribution of highest tolerated doses (SQ units) for Group 1 (ABC) and Group 2 (B).

sex, age, symptoms and duration of symptoms. The patients' responses towards the various combinations of tree pollens (e.g. alder, birch and hazel) were also nearly identical (Table 2).

Dosage

After 1 year of treatment the accumulated doses (SQ units) were approximately identical (200,000 SQ units) for both treatment groups (Fig. 2a). Because only few of the patients reached the defined threshold dosage (in SQ units), 10 times higher concentrations were made available. This resulted in a certain distribution of the highest tolerable maintenance doses, but no difference in accumulated doses after 139 weeks of treatment (approximately 1400000 SQ units) for both treatment groups (Figs. 2a and 2b). There was great individual variation in both groups concerning the highest tolerable doses with maxima around 10,000 and 100,000 SQ units, but the amounts of allergen extracts given during the 3 years of immunotherapy were almost identical (Table 3). The amount of the major birch allergenic molecule (Bet v I) received was approximately twice as much in Group 2 (B) treatment group as in Group 1 (ABC) due to the overwhelming amount of patients reacting towards alder, birch and hazel (Table 3). Theoretically, the ratio should be 69 to 36; 162 if all patients had completed the 3-year course of specific immunotherapy (Table 2).

Symptoms and medicine consumption scores

The patients' symptoms and medicine consumption showed a seasonal variation with peak values almost identical for 1981 and 1982, but with significantly reduced figures for 1983 (Fig. 3 b). The ratios between symptom plus medi-

Table 3

Amount of allergen extract given during 3 years (139 weeks) of immunotherapy. Median (25-75 percentiles)

	SQ-units ($\times 10^{-3}$)	g Bet v I
Group 1 (ABC)		
Various mixtures of pollen extracts from alder, birch and hazel	1392 (240-2160)	144
Group 2 (B)		
Birch pollen extract	1408 (312-2280)	324 (72-524)

¹See ref. 5

²Mean. Concentrations from corresponding major allergens from alder and hazel (6) have not been included

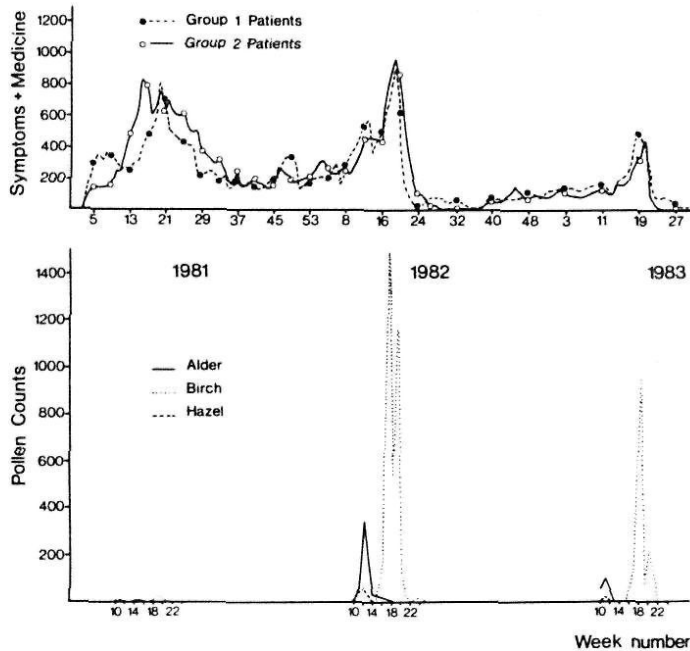


Fig. 3. a) Symptom and medicine consumption scores recorded during 3 years of immunotherapy. The curves indicate the total scores for treatment Group 1 (ABC) and Group 2 (B) according to the scoring system given in the text. b) The number of pollen grains/m³ air/week counted in Copenhagen during the 3 years of immunotherapy. Alder birch and hazel are indicated separately.

cine consumption scores relative to pollen counts changed considerably from year to year, and after the 3-year period the patients tolerated approximately 30 times more alder, birch and hazel pollen, having the same symptoms plus medicine consumption, as before the start of treatment (Table 4). The patients kept diary cards continuously, also outside the pollen season. Using the Mann-Whitney U-test it was possible to show a significant decrease in symptoms during treatment.

Table 4

Pollen counts for Copenhagen. Integrated number of pollen/m³/h for the various seasons

	1981	1982	1983
Alder	12	641	188
Birch	36	3495	1477
Hazel	6	123	24
Total	54	4259	1689

Patients' subjective assessment

After 24 months of immunotherapy the patients' subjective assessment of the efficacy of the two treatment alternatives showed that the majority of patients (68%) answering the questionnaire felt much better than before the commencement of treatment. No statistical difference between the results of the two treatment alternatives was observed (Fig. 4a). For the patients suffering asthmatic symptoms a nearly identical distribution of answers to the questionnaire for both treatment groups (75%) was obtained (Fig. 4b). Only one of the 40 patients who answered the questionnaire reported he felt worse or that there was no change in his condition.

Side effects

The local reactions half an hour and 8 h after injection were recorded for all patients. No sig-

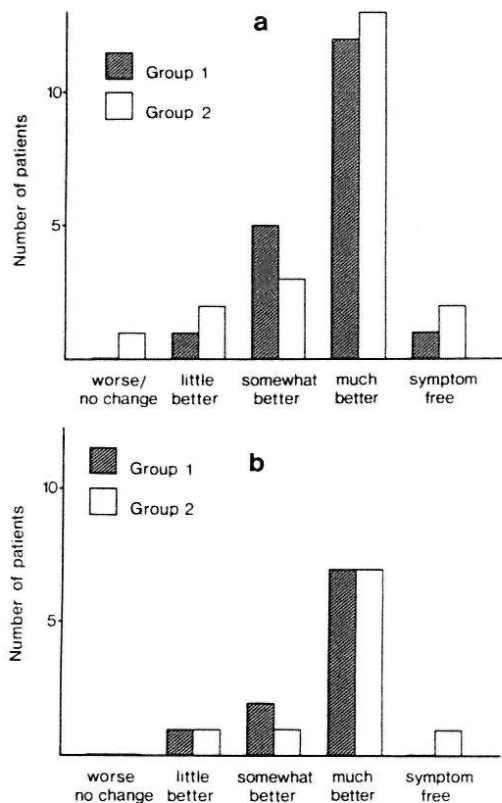


Fig. 4. a) Patients' subjective evaluation of their symptoms provoked by spring tree pollens. Changes after 2 years' immunotherapy in the two treatment Groups 1 (ABC) and 2 (B). b) Results of the same questionnaire for patients with asthmatic symptoms.

nificant differences were observed between the treatment groups. However, there was a tendency for a somewhat larger swelling at injection site for the birch-treated group. The results for the first 2 years of treatment are shown in Figs. 5a and 5b. Side effects necessitated only a few changes in the dose schedule during the treatment period. The number of systemic side effects was low and comprised mainly urticaria and slight rhino-conjunctivitis. No serious reaction of an anaphylactic type was seen.

DISCUSSION

Specific immunotherapy is generally accepted as an effective method in the treatment of allergy towards various pollens (11). In the Scan-

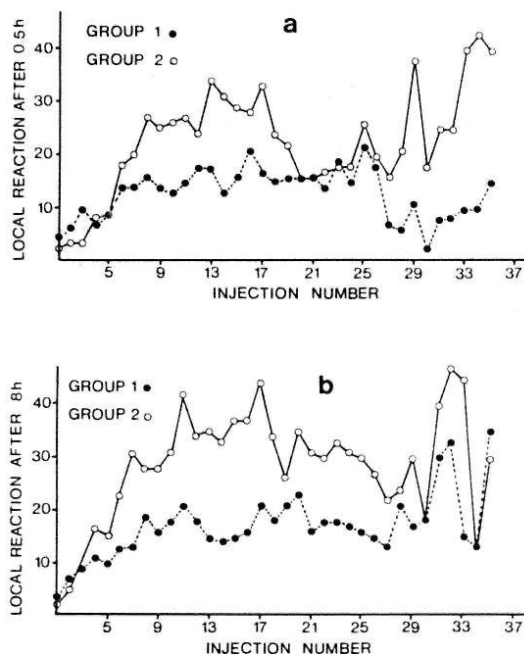


Fig. 5. Patients' mean local reaction (D + d)/2 in mm versus injection number for the first 2 years of treatment in the two Groups 1 (ABC) and 2 (B), a) after 0.5 h. b) after 8 h.

dinavian countries there is a tradition for using a mixture of extracts from the related pollens of alder, birch and hazel, although most of the exposition comprises birch pollen. In this study we have used birch or any combination of alder, birch and hazel extracts standardized by means of quantitative immunoelectrophoretic methods, RAST inhibition and biological activities assessed by skin tests on known allergic patients to investigate these two principles of treatment.

Selection of patients for this study was based on a positive history of tree pollen allergy, as well as positive reaction towards tree pollen extracts in SPT, NPT/BPT and RAST. The majority of the patients (42/54) reacted to all three types of pollen, and two minor groups (6/54 and 6/54) reacted to alder and birch or only to birch pollen. The patients were matched not only by sex, age and severity of clinical symptoms, but also by the specificity of their CRIE response. Thus, there were no dif-

ferences in the molecular specificities between the two treatment groups, but a difference in epitope specificity of the patients' IgE may well exist.

No significant differences in doses (expressed both as SQ and HEP units) were observed in the two groups of patients. As only the major allergen from birch pollen has been isolated, a comparison of absolute amounts of major allergens received by the two treatment groups could only be calculated for Bet v I.

The double-peaked distribution graph in Fig. 2b is probably due to two factors. Firstly, not all patients were offered treatment with 100,000 SQ extract as it became available. Secondly, no final extraction of a still higher concentration was attempted, so a large group of patients did not receive the maximum tolerated dose. If this had been available to the clinicians, the classical Gaussian distribution curve for the defined allergy total dose would have been achieved. This also accords with the very low number of hypo-sensibility side effects noted.

In both treatment groups a significant reduction in symptom and medicine consumption scores was seen. However, no difference in efficacy could be demonstrated between the groups. This can have several explanations. The most probable is the dominating role of birch shown in both the high number of pollen counts as well as in the patients' sensibilization pattern. All the 54 initial patients were sensitized with birch allergen and hence birch was included in all the treatment extracts, and none of the patients in Group I ABC was treated with alder and hazel alone. Another explanation is that the patients were mainly sensitized against epitopes covered by birch pollen allergenic molecules, and their responses to the two other pollens were mainly due to specific IgE responses towards those molecular epitopes which are common for all the three allergens. As a consequence, a detailed molecular epitope mapping of the major allergens from alder, birch and hazel is now in progress, and the question will hopefully be answered in the near future.

Our results do not concord with those of Möller & Dreborg (10) who were able to show a

slightly stronger reduction of the symptom scores when using the mixture than with birch pollen alone. This difference was, however, first seen after more than 2 years of treatment and could not be demonstrated in the other parameter studied. It should have been expected that a difference would be easier to demonstrate in our study from the southern part of Scandinavia where alder and hazel are much more common than in the north.

The results in the objective tests performed in this study, such as changes in specific skin and nasal reaction as well as *in vitro* tests, such as RAST and CRIE, are published separately (7, 14). They concord with the subjective symptoms presented here, i.e. no difference was seen between treatment groups, but a significant improvement was observed during treatment in both groups.

In all hyposensitization therapy there is a certain incidence of systemic side effects (16). In this study we recorded only few slight reactions although the doses were increased the second year. However, access to still higher concentrations of the allergen extracts would probably have increased the number of side effects.

The pollen counts differed significantly from year to year. The greatest changes were found for birch, but they were also significant for alder and hazel. In spite of this, the integrated symptom and medicine consumption scores (Fig. 3a) decreased from 1981 to 1982 and 1983. However, the ratio between these figures and the pollen counts within the season differed from 100 (mean) for the two treatment groups to 3.0 and 3.1 for 1982 and 1983 respectively. Thus the tolerance to these particular tree pollens increased by factors of 33 and 32 during the years of observation, possibly as a result of the hyposensitization. These results correlate well with the patients' subjective assessments of the treatment, where the majority felt much better. The improvement was the same for asthma and rhino-conjunctivitis symptoms (Figs. 4a & 4b). This confirms what has been found in other studies (15) that hyposensitization is also effective in allergic asthma. The finding that symptoms outside the pollen season in some of the asthmatic patients also were significantly

reduced is important and forms an indirect proof that immunotherapy can reduce unspecific bronchial hyperactivity. This confirms with the results in a recent study on animal dander-induced asthma (13), where the hyperreactivity was demonstrated directly by histamine provocation.

The clinical message of this study, relevant for the Scandinavian countries, can thus be concluded as follows: spring-time asthma and rhino-conjunctivitis caused by allergy to deciduous trees can be treated effectively by birch pollen extracts alone.

ACKNOWLEDGEMENTS

The authors wish to thank Lone Pedersen, Hanne Steensgård, Lise Høi Hansen, Annie Lindebod and Birgit Birch from the Department of Lung Diseases, Copenhagen County Hospital, Gentofte, and Lise Jensen and Birgit Jørgensen from the Allergy Clinic, Frederiksberg Hospital for their careful handling of the patients.

We also wish to thank the Aerobiological Group, the Danish Asthma and Allergy Association for the pollen data provided.

REFERENCES

1. Aas, K., Backman, A., Belin, L. & Weeke, B.: Standardization of allergen extracts by appropriate methods. The combined use of skin prick testing and radio-allergosorbent tests. *Allergy* 33, 130-137, 1978.
2. Dirksen, A. & Østerballe, O.: Common components in pollen extracts. *Allergy* 35, 611-616, 1980.
3. Eriksson, N. E., Ahlstedt, S. & Lövhagen, O.: Immunotherapy in spring-time hay fever. A clinical and immunological study comparing two different extract compositions. *Allergy* 34, 233-247, 1979.
4. Eriksson, N. E., Wihl, J. Å., Arrendal, H. & Strandhede, S.-O.: Tree pollen allergy. Sensitization to various tree pollen allergens in Sweden. A multi-center study. *Allergy* 39, 610-617, 1984.
5. Ipsen, H. & Löwenstein, H.: Isolation and immunochemical characterization of the major allergen of birch pollen (*Betula verrucosa*). *J. Allergy Clin. Immunol.* 72, 150-159, 1983.
6. Ipsen, H., Bøwadt, H., Janniche, H. et al.: Immunochemical characterization of reference alder (*Alnus glutinosa*) and hazel (*Corylus avellana*) pollen extracts and the partial immunochemical identity between the major allergens of alder, birch and hazel pollens. *Allergy* 40, 510-518, 1985.
7. Ipsen, H., Schwartz, B., Wihl, J. Å., et al.: Immunotherapy with partial purified and standardized tree pollen extracts. III. Specific IgE response towards the major allergens of alder, birch and hazel pollen during a three year double-blind study on patients treated with pollen extracts of birch or combinations of alder, birch and hazel. *Allergy* 40, 510-518, 1985.
8. Löwenstein, H.: Physico-chemical and immunochemical methods for the control of potency and quality of allergenic extracts. Proc. of the First International Paul-Ehrlich Seminar on Regulatory Control and Standardization of Allergenic Extracts, pp. 122-132. Gustav Fisher Verlag, Stuttgart, New York, 1980.
9. Marsh, D. G., Goodfriend, L., King, T. P., Löwenstein, H. & Platts-Mills, T. A. E.: Allergen nomenclature. Bulletin of the World Health Organization, 64 (5), 767-770, 1986.
10. Möller, C. & Dreborg, S.: Cross-reactivity between deciduous trees during immunotherapy. I. In vivo results. *Clin. Allergy* 16, 135-143, 1986.
11. Norman, P. S.: An overview of immunotherapy: Implications for the future. *J. Allergy Clin. Immunol.* 65, 87-96, 1980.
12. Rung Weeke, E., Jørgensen, G., Nüchel Petersen, B., Torp, U. & Buch, H.: Pollenregistreringen i København 197. *Ugeskr. Læger* 141, 1194-1199, 1979.
13. Sundin, B., Lilja, G., Graff-Lonnevig, V., et al.: Immunotherapy with partly purified and standardized animal dander extracts I. Clinical results from a double-blind study on patients with animal dander asthma. *J. Allergy Clin. Immunol.* 77, 478-487, 1986.
14. Wihl, J. Å., Ipsen, H., Bøwadt, H., Nüchel Petersen, B., Munch, E. P., Janniche, H. & Löwenstein, H.: Immunotherapy with partially purified and standardized tree pollen extracts. II. Results of skin prick tests and nasal provocation tests from a three year double-blind study of patients treated with pollen extracts of birch or combinations of alder, birch and hazel. *Allergy* 43, 363-369, 1988.
15. Østerballe, O.: Immunotherapy with grass pollen major allergens. Clinical results from a prospective 3-year double blind study. *Allergy* 37, 379-388, 1982.
16. Østerballe, O.: Side effects during immunotherapy with purified grass pollen extracts. *Allergy* 37, 553-562, 1982.

Address:

Bruno Nüchel Petersen
Lungemedicinsk afdeling
Københavns Amtssygehus i Gentofte
Niels Andersensvej 65
DK-2900 Hellerup
Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.