

Immunotherapy with Grass Pollen Major Allergens

Clinical Results from a Prospective 3-Year Double Blind Study

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Perennial hyposensitization with a partially purified timothy extract resulted in a statistically significantly higher degree of clinical protection than treatment with the two timothy major allergens (Nos. 19 and 25) and protected better from the second – than during the first grass pollen season. The extracts were standardized biologically and adsorbed to aluminium hydroxide for administration. The therapy had a more beneficial influence on sneezing than on rhinorrhoea and blockage of nasal airways, and an excellent effect on grass pollen asthma was obtained with the partially purified timothy extract. Associated birch pollen allergy was not influenced by hyposensitization with grass pollen.

Key words: allergens; asthma; biological units; grass pollen allergy; hay fever; immunotherapy; major allergens.

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Recent publications suggest that specific immunotherapy (hyposensitization) with partially purified grass pollen extracts may protect better than crude extracts (2, 7, 12).

Norman et al. (15) have previously compared crude extract of ragweed with the major allergen Ag E. They found no significant difference between crude extract and Ag E, but both extracts were better than placebo.

This study compared the clinical effect of two major allergens (13) of timothy grass pollen with that of partially purified timothy extract. Results of preseasonal therapy (season 1) have been published (17, 18) and showed that the clinical effect of partially purified extract was significantly better than treatment with the two major allergens, although the dosages and side effects of the two timothy extracts were equal.

Clinical results during subsequent perennial therapy (seasons 2 and 3) compared to season 1 and to non-hyposensitized controls during season 2 are presented here.

In vivo (nasal provocation tests, skin prick tests) and *in vitro* analyses and side effects are to be published elsewhere (19, 20, 21, 22).

MATERIAL

Patients

Forty typical grass pollen allergic patients were randomly divided into two equal groups and treated with WPA (group WPA), and PPA (group PPA). Despite a primary allocation according to sex and age, no statistical difference was demonstrated between the groups in other pretreatment *in vivo* (17) or *in vitro* parameters

Abbreviations: WPA = whole pollen allergens: a partially purified timothy extract consisting of approx. 20 out of the extractable 30-45 timothy antigens, but all the potential allergens; PPA = purified pollen allergens: two isolated timothy major allergens 19, 25; Group WPA = patients treated with extract WPA; Group PPA = patients treated with extract PPA; MTD = maximal tolerated dose.

Table 1

Number of patients in group WPA (W), PPA (P), and non-hyposensitized controls (C) with grass pollen asthma in four seasons

	W	P	C
1977	7*	4*	4*
1978	5	5	4*
1979	0	5	4*
1980	0	2	—

* Number of patients using seasonal asthma medicine before immunotherapy.

Table 2

Number of patients in group WPA (W) and PPA (P) with associated birch pollen asthma and hay fever in three seasons

	Asthma		Hay fever	
	W	P	W	P
1978	2	3	2	3
1979	0	2	2	3
1980	2	4	3	5

(18). The groups were also comparable as regards associated asthma and birch pollen allergy (Tables 1 and 2), but otherwise they were pure grass pollen allergic patients. Mean age for both groups was 24 years (range 15–43 years). In group WPA, the median for the duration of symptoms was 7.5 years (range 3–21 years), in group PPA the median was 10 years (range 2–19 years).

Fourteen non-hyposensitized grass pollen allergic patients, 11 men and three women, mean age 26 years (range 19–39 years), median for duration of symptoms 11.5 years (range 2–22 years), were admitted just before season 2 with the same criteria as for groups WPA and PPA. These controls were admitted too late for hyposensitization, but during season 2 they kept the same diary cards and used the same spectrum of medicine as did groups WPA and PPA, and were later allocated for hyposensitization in another investigation.

Allergen extracts

Extracts were produced and supplied by ALK, Copenhagen, Denmark, January 1978. Group WPA was treated with WPA (Alutard® SQ), a partially purified extract of timothy grass pollen. Group PPA was treated with PPA consisting of two isolated timothy major allergens, Ag 19 and Ag 25 (13). The extracts were standardized biologically before being adsorbed to aluminium hydroxide for administration. Extract WPA contained 4.0 mg aluminium hydroxide/ml diluent in 10 HEP versus 1.2 mg aluminium hydroxide/ml diluent in the 10 HEP extract PPA. The stability of the extracts has been described elsewhere (19). The amount of Ag 19 and Ag 25 was equal in extracts WPA and PPA, determined by quantitative immunoelectrophoresis. The amounts of protein, and of Ag 19 and Ag 25 in extract WPA, 1 HEP, were approximately 40, 3, and 6 µg/ml. The amounts of protein, and of Ag 19 and Ag 25 in extract PPA, 1 HEP, were approximately 9, 3, and 6 µg/ml. The extracts were used in ten-fold dilutions from 0.01 to 10 HEP (17). The Nordic Committee for Allergen Standardization has defined 1 HEP extract as 1,000 biological units (BU)/ml (1). Extracts from the same batch were used for treatment and for *in vivo* and *in vitro* tests throughout the investigation.

METHOD

Until the code for treatment and test extracts was broken in the autumn of 1978 (17), the author effectuated injections and tests assisted by staff nurses. By then all data in relation to season 1 had been collected and registered, and elaborated on in collaboration with a statistician. The data were computerized. The staff who later evaluated the analyses and treated the patients remained ignorant of the code, and the author being away from the department was not in a position to exert any influence on the patients' symptom recordings or concomitant medication during seasons 2 and 3. After season 1 approximately one-third of the patients were hyposensitized by their general practitioners.

Immunotherapy (hyposensitization)

Dosage has been described in detail, and maximal tolerated dose (MTD) was used as maintenance dosage (17). All patients received perennial treatment with one injection per week before season 1, and up to MTD just after seasons 1 and 2; otherwise one injection per month. Seasonal dosage was reduced to 1/3–1/4. The final injections were given just before season 3 after approximately 116 weeks of hyposensitization.

Diary cards

Symptom scores and concomitant medication were recorded daily throughout the pollen season. The score system has been published previously (17). One spectrum of medicine for all patients was strived for. Where treatment with antihistamines was insufficient prednisone was given at minimum dose. Patients with asthma received salbutamol aerosol. None received disodium cromoglycate or beclomethasone. Symptom scores for each shock organ (eyes, nose, lungs) and scores for concomitant medication were accumulated weekly.

Pollen counts

Pollen grains were collected in a Burkard Recording Volumetric Spore Trap placed in the centre of Copenhagen, 15 m above the ground. Determinations and counting were carried out by the Aerobiological Group in Copenhagen and published in the Danish Pollen Bulletin (25). The weekly accumulated pollen counts were used. All symptoms were recorded within a radius of 25 km of the spore trap during season 1 and within 40 km during seasons 2 and 3.

Statistics

Paired and unpaired Rank Sum Tests, Spearman's correlation analysis and Fourfold Table Test were employed, using two-tailed tests with 5% significance level.

RESULTS

Patients

In group PPA, one patient with the highest symptom scores in season 1 dropped out of the study just after season 1 due to side effects (17). One patient in group WPA without symptoms in season 1 was abroad during seasons 2 and 3 but proceeded with hyposensitization until season 3. All non-hyposensitized controls completed the trial.

Dosage

Tables 3 and 4 illustrate accumulated dosage and MTD respectively, for both groups just before the three seasons. Accumulated dosage showed no intergroup difference, but was reduced after season 2 in both groups ($P < 0.001$). Just before the end of hyposensitization MTD was lower ($P < 0.05$) for group PPA than for group WPA. Clinical effect did not correlate with dosage.

Table 3

Medians of accumulated doses (25–75% percentiles) in biological units (BU) $\times 10^{-2}$ in group WPA (W) and PPA (P) just before the three seasons. Dosage was equal in the two groups

	W	P
1978	39,4 (19,8–50,7)	41,9 (16,7–53,9)
1979	81,5 (54,9–107,7)	91,5 (48,4–107,9)
1980	112,2 (76,6–148,0)	117,2 (78,5–135,9)

Table 4

Medians of maximum tolerated doses (MTD) (25–75% percentiles) in biological units (BU) $\times 10^{-2}$ in group WPA (W) and PPA (P) just before the three seasons. Equal MTD was seen just before seasons 1 and 2, but MTD just before season 3 was significantly higher in group W than in group P at 5%* level

	W	P
1978	6,5 (4,0–7,0)	5,5 (3,0–8,0)
1979	6,0 (4,3–6,8)	5,0 (4,0–6,0)
1980*	5,0 (4,0–6,0)	3,0 (3,0–5,0)

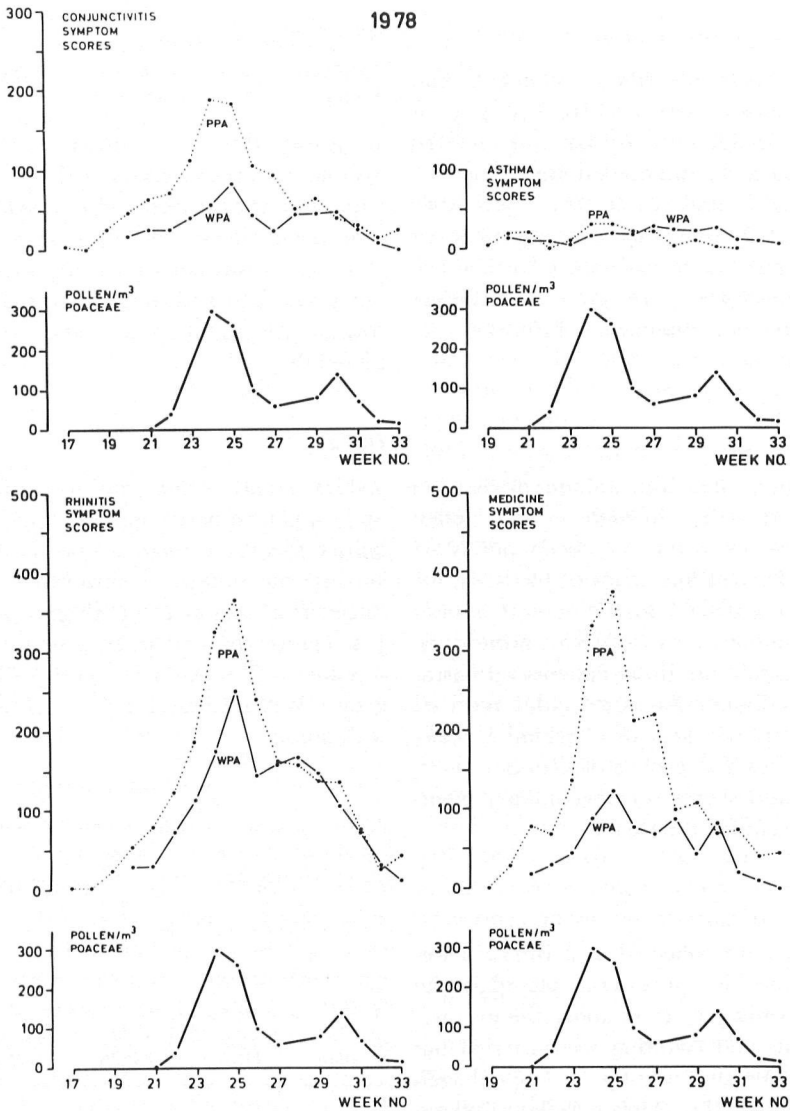


Fig. 1. Selective symptom scores in group PPA and WPA compared to grass pollen counts during season 1, 1978.

Hay fever and concomitant medication

As shown in Figs. 1, 2, and 3 and Tables 5 and 6, group PPA developed the highest symptom levels in all seasons. Response in grass pollen asthma (see below) and conjunctivitis (Table 1) was the most pronounced.

In accordance with pollen counts, symptom scores and concomitant medication during sea-

sons 1 and 2 were considerably higher than during season 3. However, as shown in Tables 5 and 6, there was a deviation in symptom scores and concomitant medication between the groups from the second pollen season, as also shown in nasal sensitivity (20).

In Fig. 4 rhinitis scores for the three pollen seasons are divided into sneezing, rhinorrhoea, and blockage of nasal airways. In all three seasons sneezing showed significant group dif-

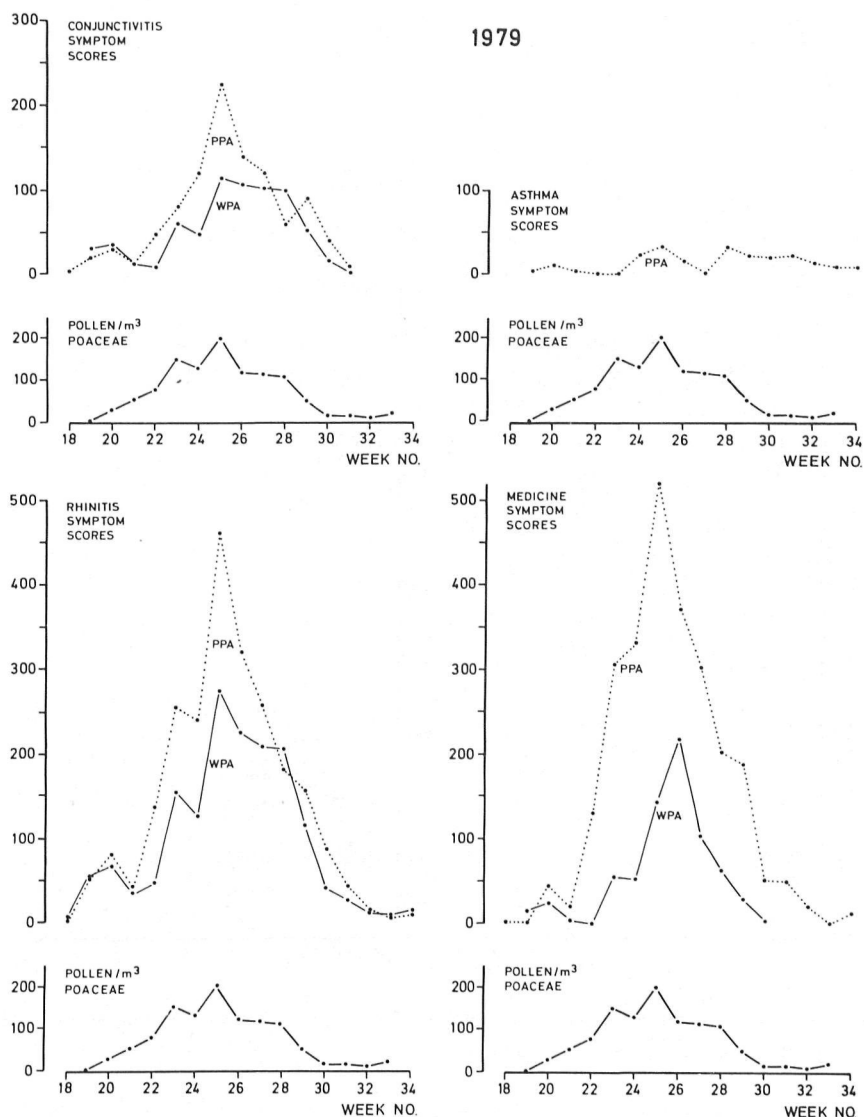


Fig. 2. Selective symptom scores in group PPA and WPA compared to grass pollen counts during season 2, 1979.

ference, whereas only blockage showed significant difference in season 1, and rhinorrhoea in season 2.

As shown in Fig. 5, non-hyposensitized controls recorded a higher total score (symptom scores + concomitant medication) than did groups WPA and PPA, but with statistical difference only for group WPA. Four patients had associated grass pollen asthma (Table 1), and two patients had associated allergy to birch and mugwort pollen.

During season 2 half of the controls received prednisone. Including the treated patients from groups WPA + PPA (six patients) 13 patients had a median (25–75% percentiles) total consumption of prednisone during season 2 of 85 mg (70–172.5 mg) and a weekly consumption of 18.5 mg (12.9–24.8 mg), or a daily maximum prednisone dose of 10 mg (5–11.25 mg). Median (25–75% percentiles) duration of prednisone therapy (in the 13 weeks of season 2) was 4 weeks (3.5–7 weeks). Most (9/13) pa-

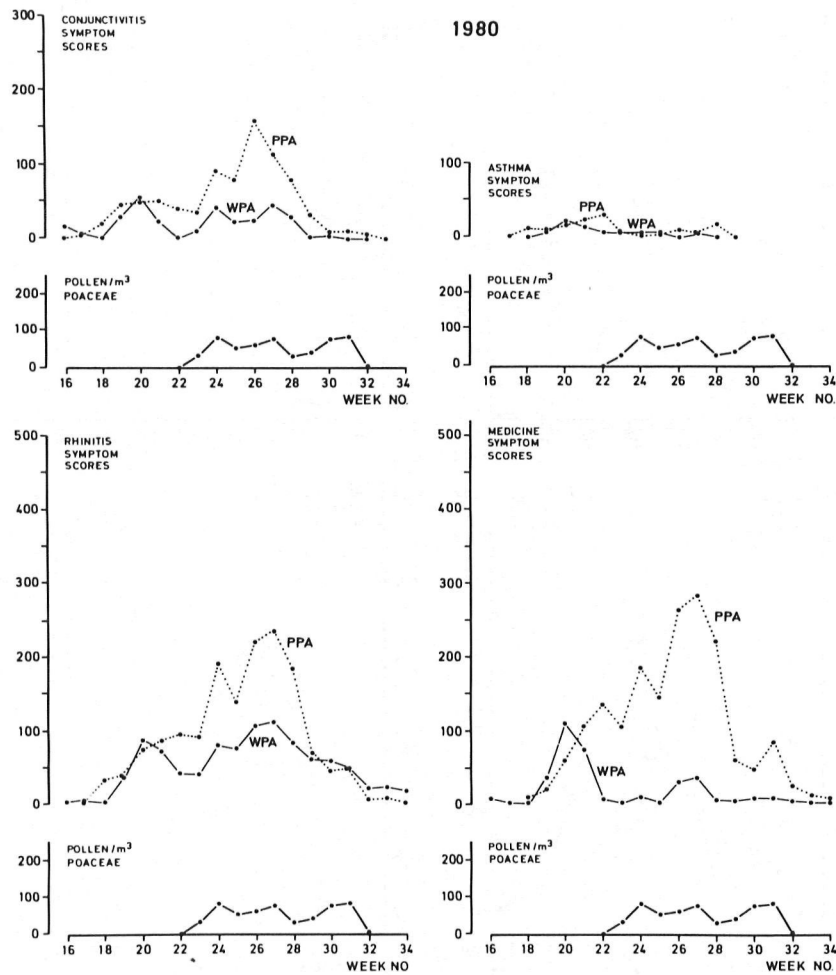


Fig. 3. Selective symptom scores in group PPA and WPA compared to grass pollen counts during season 3, 1980.

Table 5

Medians of total symptoms and medicine scores (25–75 % percentiles) in group WPA (W) and PPA (P) in the three seasons. Significant difference between the groups was shown in all seasons at 5 %* or 1 %** levels

W	P	
1978*	113 (28–202)	197 (112–335)
1979**	129 (0–315)	257 (157–472)
1980**	1 (0–76)	130 (32–229)

tients were given prednisone because of an insufficient efficacy of antihistamines, in the others (4/13) the sedative effect of antihistamines was an indication. Dosage and duration of this treatment were at equal levels in the six hyposensitized patients and in the seven controls.

Grass pollen asthma

A significant effect on asthma was recorded in group WPA although the number of asthma

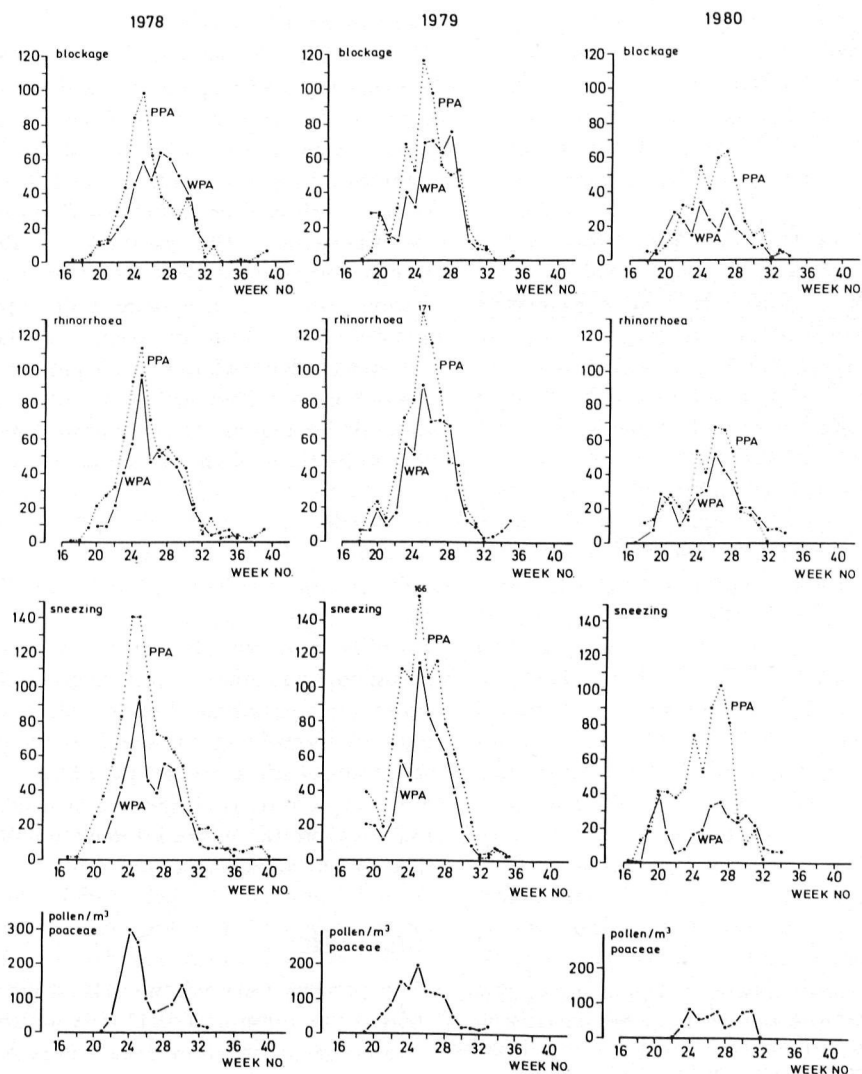


Fig. 4. Rhinitis scores divided into sneezing, rhinorrhoea, and blockage of nasal airways during three seasons.

Table 6

Number of patients in group WPA (W) and PPA (P) with grass pollen rhinitis (R), conjunctivitis (C), total concomitant medication (M), selective consumption of prednisone (P), and patients without symptoms or concomitant medication (N) in the three seasons

	R		C		M		P		N	
	W	P	W	P	W	P	W	P	W	P
1978	14	18	11	18	11	16	0	2	6	2
1979	14	19	8	17	12	14	1	5	5	0
1980	8	15	4	14	3	12	1	4	11	4

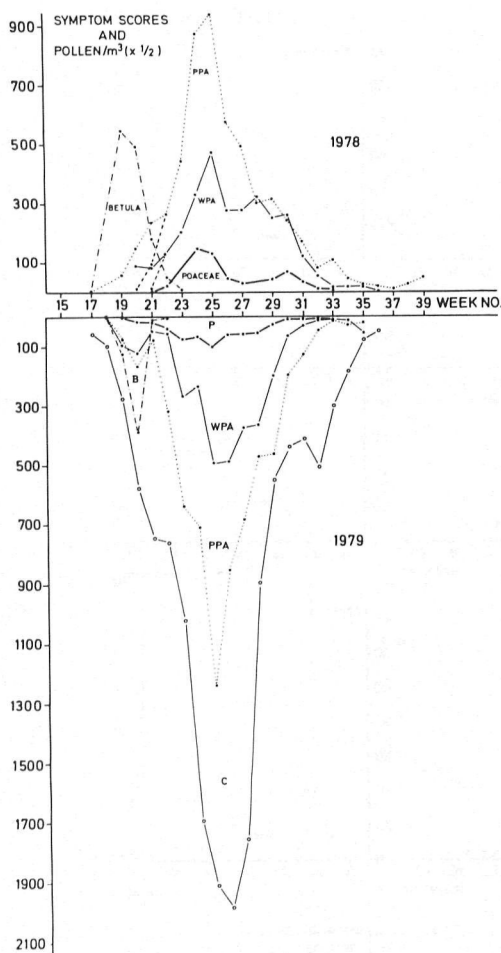


Fig. 5. Total scores (symptoms + concomitant medication) for group PPA, WPA, and non-hyposensitized controls (C) in season 2, 1979, compared with grass (P) and birch (B) pollen counts and clinical results from the first season, 1978.

patients in group WPA was higher than in group PPA just before hyposensitization (Table 1). After 12 weeks of treatment an equal number of patients in the two groups had asthma during season 1, but during season 2 no patients had grass pollen asthma in group WPA while the number with asthma was unchanged in group PPA (and non-hyposensitized controls). During low grass pollen counts in season 3, only two patients in group PPA had asthma and one patient in group WPA had minimum coughing, but no asthma.

Associated birch pollen allergy

Corresponding to associated birch pollen allergy, Figs. 1, 2, and 3 illustrate a slight peak for both groups during all three birch pollen seasons. Table 2 illustrates the distribution of asthma and hay fever in the grass pollen allergic patients with coinciding birch pollen allergy for all three seasons. Groups WPA and PPA were comparable. Group WPA recorded no asthma during season 2. During season 3 one patient in group WPA, and two in group PPA, had début of birch pollen asthma. In all patients asthma symptoms coincided with hay fever. Season 3 showed the highest birch pollen counts, more than twice those of seasons 1 and 2.

Side effects

Side effects are discussed in detail elsewhere (21).

Unlike systemic side effects before season 1, no major side effects were registered during subsequent perennial therapy, where only one patient showed slight symptoms of asthma (relapse) after a single injection. A certain enhancement of local side effects after season 1 may explain the reduced dosage in both groups during perennial treatment.

DISCUSSION

The patients were admitted for hyposensitization in the autumn of 1977. Without foregoing knowledge of symptom levels before hyposensitization, the patients were divided into two groups according to age and sex and were allocated to treatment with PPA or WPA. As the groups had identical thresholds in nasal provocation tests and comparable duration of symptoms before treatment, this can be taken as evidence that symptom levels before hyposensitization also were comparable, and the absolute levels, rather than change in symptom levels, were used as parameters for clinical effect. Deviation of symptom scores and nasal sensitivity for the two groups from season 2 confirm the results. The groups were also comparable according to associated birch pollen allergy and pretreatment *in vitro* parameters.

Since others have proved the effect of hypsensitization with grass pollen (9, 10, 16, 23) no placebo group was included in this investigation, but since the results demonstrate a significant difference between the two extracts, and since group WPA showed significantly fewer symptoms than non-hypsensitized controls, this study indicates that hypsensitization against grass pollen allergy protects significantly better than placebo.

It is interesting that the only significant difference between groups WPA and PPA in rhinitis scores during all the seasons was in sneezing. That there was a significant intergroup difference in blockage of nasal airways only in season 1, and in rhinorrhoea in season 2, could be due to an equalization by concomitant medication of the rhinorrhoea symptoms in seasons 1 and 3, and of blockage in seasons 2 and 3. As antihistamines show a good effect on rhinorrhoea and sneezing but only a slight effect on blockage (5, 24) it is the author's opinion that hypsensitization with grass pollen has the greatest effect on sneezing in hay fever. This is consistent with the results of nasal provocation tests (20).

It is remarkable (Table 1) that hypsensitization with the most effective extract, WPA, completely eliminated asthma symptoms. On this account grass pollen asthma must be considered as a major indication for hypsensitization. Others have detected excellent results of hypsensitization against pollen asthma with either grass (4, 6, 14) or a high dosage of ragweed (8), but not with a low dosage of ragweed (3).

On a test level the effect manifested itself primarily in the skin with significant correlation between symptoms and skin sensitivity (17, 20). Not until season 2 was there demonstrated a significant correlation between symptoms and nasal sensitivity (20), and, simultaneously, a pronounced effect on the bronchial sensitivity was obtained.

Birch pollen allergies do not counter-influence the results for symptoms in the grass pollen season. One of the birch pollen allergic patients had especially many symptoms. This explains the double amount of symptom scores

in group PPA just before grass pollen season 1. If symptom scores in grass pollen season 1 are eliminated for all five birch pollen allergic patients (two patients in group WPA and three in group PPA), a significant difference still remains between symptom scores for groups WPA and PPA.

The patients were not hypsensitized against birch pollen. This investigation, therefore, proves a specific clinical effect of hypsensitization against grass pollen which is most marked in season 3 with high birch pollen counts, and with the début of birch pollen allergy in one patient in group WPA, and two patients in PPA.

Since extracts WPA and PPA were comparable in skin prick test and RAST inhibition (11), fewer side effects, higher dosage, and probably better clinical effect of the two major allergens could be expected. This did not prove possible. WPA, however, did prove very effective in most patients.

Partially purified pollen extracts may protect better than crude extracts (2, 7, 12) but conflicting data exist (6). Major pollen allergens may equalize crude extracts and protect better than placebo (15). These observations agree with the results of this study.

Very sensitive patients were clinically high responders to extract PPA (18, 22), but in general this extract with two timothy major allergens was too highly purified to be used for treatment in grass pollen allergy. Groups of individual allergens matching the pretreatment allergoprints may be the optimal choice for treatment.

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