

Immunotherapy in Spring-Time Hay Fever

A Clinical and Immunological Study Comparing Two Different Treatment Extract Compositions

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In a study of the efficacy of two different treatment schedules for perennial immunotherapy, 47 adult patients with spring-time hay fever due to allergy against birch and other deciduous trees were randomly assigned to three treatment groups: one group received birch, alder and hazel allergen in Allpyral®, another group received the same Allpyral mixture and in addition all relevant tree pollens in aqueous extract and a control group received no injections. For determination of antibody titres the radioallergosorbent test (RAST) and the ammonium sulphate precipitation (ASP) technique were used. Cellular responsiveness was studied by measuring birch pollen (BP) induced leucocyte histamine release in peripheral blood.

The clinical and immunological response was similar in the two treated groups. Treated patients had less symptoms and a lower consumption of antihistamine tablets during the pollen season than the control group.

Non-IgE BP antibodies and IgE antibodies recorded with the ASP technique increased after immunotherapy while RAST values did not change significantly. A decrease of RAST values from postseasonal values during the first year to pre-seasonal values in the following year was seen in all patient groups but was less pronounced in treated than in untreated patients. The decrease was more pronounced in patients with high RAST values of postseasonal sera than in patients with low RAST values. Cellular reactivity increased slightly during the first phase of therapy but returned to the pre-treatment level later.

Clinical improvement was positively correlated to the percentage increase of non-IgE antibody titre and to the pre-treatment non-IgE/IgE antibody ratio. Patients with high preseasonal RAST titres or high cellular sensitivity tended to have more severe symptoms during the pollen season.

It is concluded that a mixture of birch, alder and hazel is sufficient for immunotherapy in spring-term hay fever. It is obvious that changes of a single immunological variable do not account for the therapeutic results in immunotherapy.

Key words: allergens; birch pollen allergy; blocking antibodies; hay fever; immediate type hypersensitivity; immunotherapy; pollen allergy; reaginic antibodies.

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Immunotherapy is well established in immediate type allergy although the mechanisms causing symptom amelioration are not clear (28). To achieve therapeutic results, relevant and immunologically active allergens must be present in the treatment

extract. Since many grass pollen allergens are immunologically cross-reactive (19) immunotherapy of grass pollen hay fever is commonly given as a mixture of such allergens. Similarly, cross-reactions, although less pronounced, seem to exist

between some tree pollen allergens (4, 36). In Sweden many clinicians use a mixture of birch, alder and hazel (the Allpyral Northern tree mix) for immunotherapy of spring-time hay fever, although many patients with this disorder are sensitized to tree pollens such as beech, oak and bog-myrtle as well (11). Thus, some patients with, e.g. beech allergy will be undertreated, whereas other patients, e.g. patients without a hazel allergy, may be overtreated when this type of standard allergen mixture is used without taking into account the allergies in the individual patient.

The aim of this investigation was to evaluate whether the treatment results in spring-time hay fever can be improved by including other relevant tree pollen allergens, in addition to birch, alder and hazel, in the hyposensitization extracts. In addition, the immunological events during immunotherapy were studied in relation to the clinical findings. For this purpose, clinical evaluations were supplemented with serum reagin determination, using the radioallergosorbent test (RAST) and the ammonium sulphate precipitation technique (ASP) and determination of allergen-induced histamine release from leucocytes.

PATIENTS AND METHODS

Patients. The study comprised 47 adult hay fever patients with birch pollen (BP) allergy according to nasal provocation tests (PT). All except one were also sensitive to pollen from one or several other deciduous trees (Table 1). In addition to the tree pollen allergy, 28 individuals had a grass pollen allergy and 11 a compositae pollen allergy.

Treatment schedules

All patients were given a prescription for a combined antihistamine tablet, (Lunerin®, containing 40 mg of brompheniramine and 167 mg phenylpropanolamine) and prednisolone 5 mg, and were told to take the

antihistamine tablet for mild symptoms and add prednisolone if the symptoms were intolerable in spite of this medication. The patients were randomly assigned to one of three treatment schedules. Besides the tablet medication, the patients of the three treatment groups were given the following therapy:

Group BAH (Birch, Alder, Hazel): Hypo-sensitization with Allpyral Northern tree mix (birch, alder, hazel).

Group AT (All Trees): Allpyral Northern tree mix supplemented with other aqueous tree pollen extracts, depending on the results of PTs with other tree pollens.

Controls: No immunotherapy. Placebo injections were not given.

Details of the patients in the different groups are given in Table 1.

Patients who besides tree pollen allergy also had a grass and/or compositae pollen allergy and were assigned to the treatment groups (BAH or AT) were given immunotherapy with grass and/or compositae allergens.

The Allpyral injections were given once a week until the highest tolerated dose or a maximum dose of 4000 PNU was reached, in accordance with the routines at our clinic at that time. This dose was then given as a maintenance dose every 6th or 8th week. The aqueous allergen extracts were given twice a week in increasing doses until the highest tolerated dose was reached. This dose was then administered every 4 weeks. The mean maintenance dose given for *beech* allergen was 1267 (200–5000) Noon units. Treatment started in January/February 1974 and continued, as a rule, for 3 years. Only a few patients were followed with *in vitro* determinations until completion of hyposensitization.

In vivo tests

Nasal PTs were performed employing 0.1 ml of allergen extracts of concentrations

Table 1

Patients

	Patient group BAH ("Birch-Alder- Hazel")	Patient group AT ("All Trees")	Controls
Allergens in immunotherapy	birch, alder, hazel	all relevant trees	
Maximum dose of treatment extract Northern tree mix ^a	1500 (40-4000) PNU	2500 (200-4000) PNU	
No. of patients	17	16	14
Males/females	8/9	8/8	7/7
Ages, years ^a	25 (15-46)	24 (15-33)	22 (14-45)
No. of patients with allergy to various tree-pollens			
Birch	17	16	14
Alder	17	15	14
Hazel	15	12	14
Beech	16	13	11
Bog-myrtle	15	14	11
Oak	7	6	8
Aspen	7	7	2
Sallow	3	2	4
Poplar	1	2	0
Linden	6	3	1
Elm	2	2	1
Maple	2	3	1
Other pollen allergies			
Grasses	11	10	7
Compositae	6	4	1

^aMedians and ranges.

between 10^{-3} and 10^{-1} w/v, as earlier described (12) and the results were graded from 0 to 9, according to the allergen concentration used and severity of symptoms elicited. In the skin tests the scratch and intracutaneous test methods were used (12). The pollens listed in Table 1 were used in the *in vivo* tests.

Clinical evaluations

The clinical effect of therapy was judged in three ways:

(a) PTs with *birch* and with *beech* pollen were

performed before the start of therapy (autumn-winter 1973), in the autumn of 1974 (year 1) and in autumn 1975 (year 2). The differences between PT results at the various points of time were used for statistical calculations.

(b) Clinical symptoms during the tree-pollen season in year 1 were evaluated on the basis of a daily record kept by the patients. Sneezing, rhinorrhea and eye symptoms were scored on a 0 to 3 scale (0=no symptoms, 1=slight symptoms, 2=moderate symptoms and 3=severe symptoms) and the number of Lunerin® and prednisolone tablets taken was

also scored from 0 to 3. The diary cards were collected every 2 weeks. A mean symptom and medication score for April (before the BP Season) and May (during the BP season) was calculated for each patient.

(c) In order to make comparisons possible between the severity of symptoms during the pollen season in year 1 and that of the preceding year, the patients were asked to rate their symptoms in a questionnaire sent to them after the pollen season. The answers were scored as 0=no symptoms, 1=much better, 2=better, 3=unchanged and 4=worse.

Antigen. The aqueous allergen extracts used for therapy, *in vivo* tests, and histamine release were made in our own laboratory, as earlier described (12), with the exception of the maple and beech extracts, which were obtained from Vitrum AB, Stockholm, Sweden. The birch, alder and hazel extracts used in a standard mixture were pyridine-extracted and alum-precipitated Allpyral extracts from Dome Laboratories, Slough, England. In the antibody determinations employing the ammonium sulphate precipitation (ASP) technique (1) a BP allergen purified according to the method of Belin (5) was used. In the radioallergosorbent test (RAST) the allergens coupled to the discs by the manufacturer of the commercial kit (Pharmacia Diagnostics, Uppsala, Sweden) were employed. These allergen extracts showed very similar activity to the ones used in the *in vivo* as well as in the other *in vitro* tests.

In vitro analyses

Blood samples were collected before the start of therapy (winter 1973–1974), after the treated patients had reached their maximum desensitization doses (spring 1974) and in the autumn of 1974. Sera for RAST analyses were also collected before and after the pollen season in 1975 and for a few patients samples were collected in January 1977, after 3 years' therapy. The sera were stored at

–70°C until analysed. The leucocytes in whole blood samples were analysed for histamine release on the day of sampling.

Antibody determinations. The ammonium sulphate precipitation (ASP) technique (1) was used to analyse the level of IgE antibodies as well as non-IgE antibodies against the BP allergen. The assay was performed on untreated serum, recording antibodies of both IgE and non-IgE classes. In addition, serum was analysed after elimination of the IgE antibodies by absorption with anti-IgE antibodies conjugated to Sepharose (Pharmacia Diagnostics, Uppsala, Sweden). This procedure left only non-IgE antibodies, probably mostly IgG antibodies (1). The amounts of IgE antibodies were calculated by subtracting the non-IgE antibody titre from the titre obtained for unabsorbed serum.

Utilizing the ASP technique and employing four different antigen concentrations, the relative *antibody avidities* were determined as previously described (1). These analyses were performed on untreated serum, recording the relative average antibody avidity for BP antibodies of all immunoglobulin classes simultaneously, and on the serum after absorption of the antibodies of IgE class as described above, recording the relative average antibody avidity for non-IgE antibodies.

The radioallergosorbent tests (Phadebas RAST®) were performed according to the manufacturer's instructions, with slight modifications as previously described (12). RAST analyses with birch and with beech allergens were performed on sera from various points of time, whereas analyses with alder, hazel and oak only were performed on pre-treatment sera. RAST results with birch and beech pollen were expressed in per mille of the total amount of radioactivity added per tube and an activity of <20 ‰ was considered to be negative. RAST results with alder, hazel and oak were expressed in Phadebas RAST classes 0–4 (12). The pre-

treatment sera, the "top dose" sera and the post-season sera from year 1 were analysed at the same time whereas sera from year 2 and year 4 were analysed in separate assays.

Birch pollen allergen-induced histamine release from leucocytes. The assay was performed basically according to the method of Lichtenstein & Osler (21). Thus the leucocytes taken from whole blood were washed twice by centrifugation at $110 \times g$ for 8 min and resuspended in cold Tris A buffer. The BP antigen, in dilutions of 10^{-9} – 10^{-2} , was added and the samples incubated at 37°C for 60 min with gentle shaking. For determination of total histamine similar samples of leucocytes without antigen were boiled for 10 min. The samples were then centrifuged at $2000 \times g$ and the supernatant sucked off and frozen at -20°C until assayed for histamine content. The assay was performed by adding 1.0 ml of supernatant to tubes containing 1.25 ml of *N*-butanol and 300 mg of NaCl. In addition, 100 μl of NaOH 3 mol/l was added, after which the tubes were shaken and centrifuged for 2–3 min in the cold. 1.0 ml of the supernatant was taken and added to 0.6 ml of 0.12 mol/l HCl and 1.9 ml of *N*-heptane. The samples were shaken and incubated for a few minutes. 0.5 ml of the lower phase was carefully sucked off and incubated in the cold, after which 100 μl of NaOH 1 mol/l and 50 μl of 0.2% OPT in methanol was added and mixed. The samples were incubated for 40 min at $+4^{\circ}\text{C}$, after which 50 μl of H_3PO_4 2 mol/l was added and the samples mixed. The tubes were incubated at room temperature for 15 min before being read under fluorescent light at the wavelengths 350 and 450 nm. The content of histamine in the samples was estimated from a standard curve obtained by assaying 0.5 to 150 ng of pure histamine per ml.

The histamine release response in the leucocytes was characterized in two ways: *Cell reactivity* was defined as the maximum amount of histamine (expressed in per cent

of total histamine) released by any amount of antigen. *Cell sensitivity* was defined as the allergen concentration required to release the maximum amount of histamine and was expressed as the inverted logarithm of the allergen dilution.

Statistical methods

Changes in clinical and *in vitro* variables between two points of time were analysed with a linear permutation test for paired observations (30). The correlations between various parameters were studied with Pitman's test and Spearman's rank correlation coefficient and the differences between the treatment groups with Fisher's permutation test (8). In order to make it possible to calculate percentage changes of antibody titres, negative RAST values (i.e. titres $< 20\%$) with birch and beech pollen were transformed to 1 (one) in these calculations.

RESULTS

Pre-treatment values

Comparisons between the patient groups. There were no significant differences between the three treatment groups with respect to pre-treatment *in vivo* or *in vitro*-variables, with the exception of lower non-IgE titres in group BAH than in group AT ($P < 0.05$) (Table 2).

Relationship between various variables. Significant correlations were found between the PT and skin test results with BP allergen ($P < 0.05$) and between BP IgE antibodies in the serum according to both the RAST and the ASP ($P < 0.01$). Furthermore, the RAST results with BP allergen showed a significant correlation with RAST results employing alder and hazel allergen. Alder and hazel RAST values also were significantly correlated to each other as well as to the RAST titre with oak pollen ($P < 0.01$). In contrast, no correlation was found between the *cell sensitivity* or

Table 2

Pre-treatment clinical and immunological values with respect to birch pollen

	Group BAH	Group AT	Controls
Skin test (score)	5.7 (2-8)	5.5 (0-6)	6 (0-8)
Provocation test (score)	3.9 (2-8)	3.3 (2-5)	5 (3-7)
RAST, (% ₀₀)	99 (0-760)	152 (0-1000)	265 (0-1000)
ASP IgE, titre	14 (1-100)	11 (3-122)	26 (2-99)
ASP non-IgE, titre	17 (1-42)	28 (6-90)	31 (12-90)
Leucocyte histamine reactivity (%)	30 (5-92)	47 (16-86)	63 (6-95)
Leucocyte histamine sensitivity (allergen concentration $\times 10^{-1}$)	5.3 (4-7)	6.1 (4-8)	5.5 (3-8)

Figures indicate medians and ranges

the cell reactivity, on the one hand, and antibody titres or *in vivo* tests with BP allergen on the other. There was no significant correlation between the degree of BP allergy (according to *in vivo* or *in vitro* tests) and the number of tree pollen allergies.

Clinical evaluations

Provocation tests. The results of the PTs with BP performed in the autumn of year 1 did not differ from those performed before the start of therapy. The PTs performed in the autumn of year 2 showed a significantly reduced nasal sensitivity for BP in the controls ($P < 0.05$) as well as in group BAH and in the two treated groups analysed together ($P < 0.01$). In addition, patients in group AT showed a reduction of nasal sensitivity between the autumn of year 1 and the autumn of year 2 ($P < 0.01$). The PTs with beech pollen indicated lower nasal sensitivity in the autumn of year 2, compared to the pre-treatment levels, in group AT ($P < 0.05$) as well as in the two treated groups analysed together ($P < 0.01$).

Symptom scores. The consumption of antihistamine tablets during the tree-pollen season in year 1 was higher in the controls than in the treated groups ($P < 0.01$). In addition, the total symptom score (the sum

of scores for sneezing, rhinorrhea, eye symptoms and tablet consumption) was higher in the non-hyposensitized group than in the other groups ($P < 0.05$) (Fig. 1). Neither the consumption of prednisolone tablets nor the scores for each individual symptom showed significant differences between the groups.

Patients' reports. According to the patients' reports given after the pollen season, eight out of 12 (64%) controls, 12 out of 14 (83%) patients in group BAH and 16 out of 17 (93%) in group AT had had less troublesome symptoms during the spring-time pollen season in year 1 than during the corresponding period in the preceding year. Two patients in group AT had no symptoms whatsoever. The differences between the groups were not statistically significant, however.

Comparisons between group BAH and group AT revealed no significant differences with respect to symptom scores, postseasonal reports from the patients or changes in nasal sensitivity to birch or beech pollen allergen.

Immunological evaluations

Birch antibody titres and avidities. Increases as well as decreases of both IgE and non-IgE

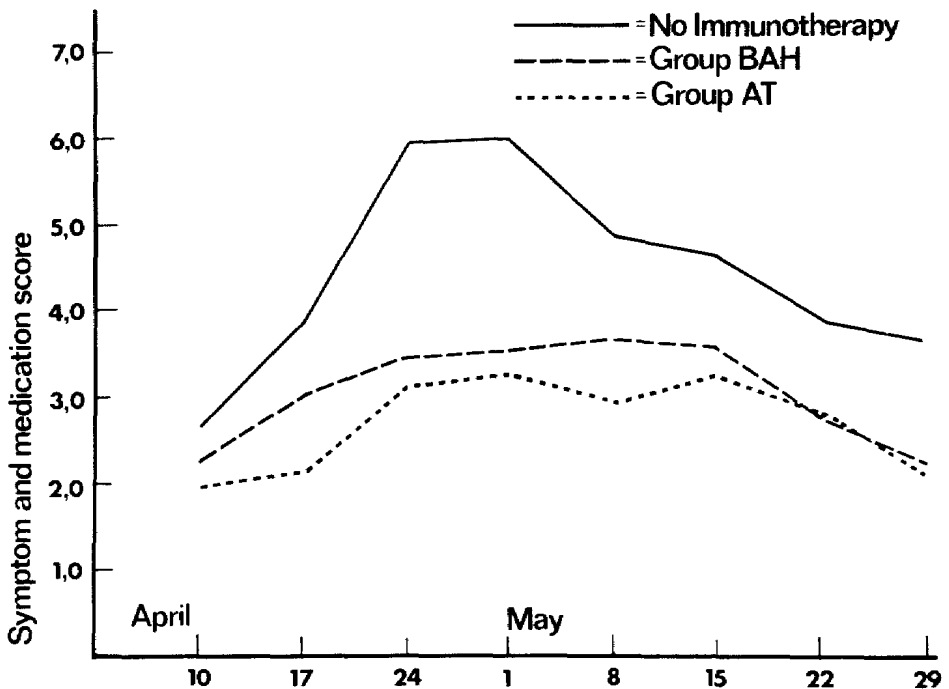


Fig. 1. Symptom and medication score during the birch pollen season in the first year of immunotherapy. The weekly mean values are indicated.

antibody titres were found after initiation of immunotherapy (Fig. 2, Table 3). The IgE antibody levels showed a particularly heterogeneous pattern, while the non-IgE antibodies were significantly increased ($P < 0.01$) at the point of time when the

maximum dose of allergen was reached in the treated groups. In the autumn of year 1, well after the pollen season, the non-IgE antibodies as well as the IgE antibodies, as determined with the ASP technique, were increased in the treated groups ($P < 0.01$) but

Table 3
Changes of birch antibody levels after initiation of immunotherapy

Patient group	When reaching maximum dose level				In the autumn of year 1			
	<i>n</i>	non-IgE (ASP)	IgE (ASP)	IgE (RAST) ^a	<i>n</i>	non-IgE (ASP)	IgE (ASP)	IgE (RAST) ^a
Treated (BAH + AT)	31	+9 ± 17**	+4 ± 33	+42 ± 151	31	+15 ± 28**	+12 ± 26**	+8 ± 170
Controls		nd	nd	nd	13	-7 ± 20	-6 ± 32	+29 ± 255

Figures indicate means ± 1 s.d. nd = not determined, ** = $P < 0.01$

^a% of radioactivity added.

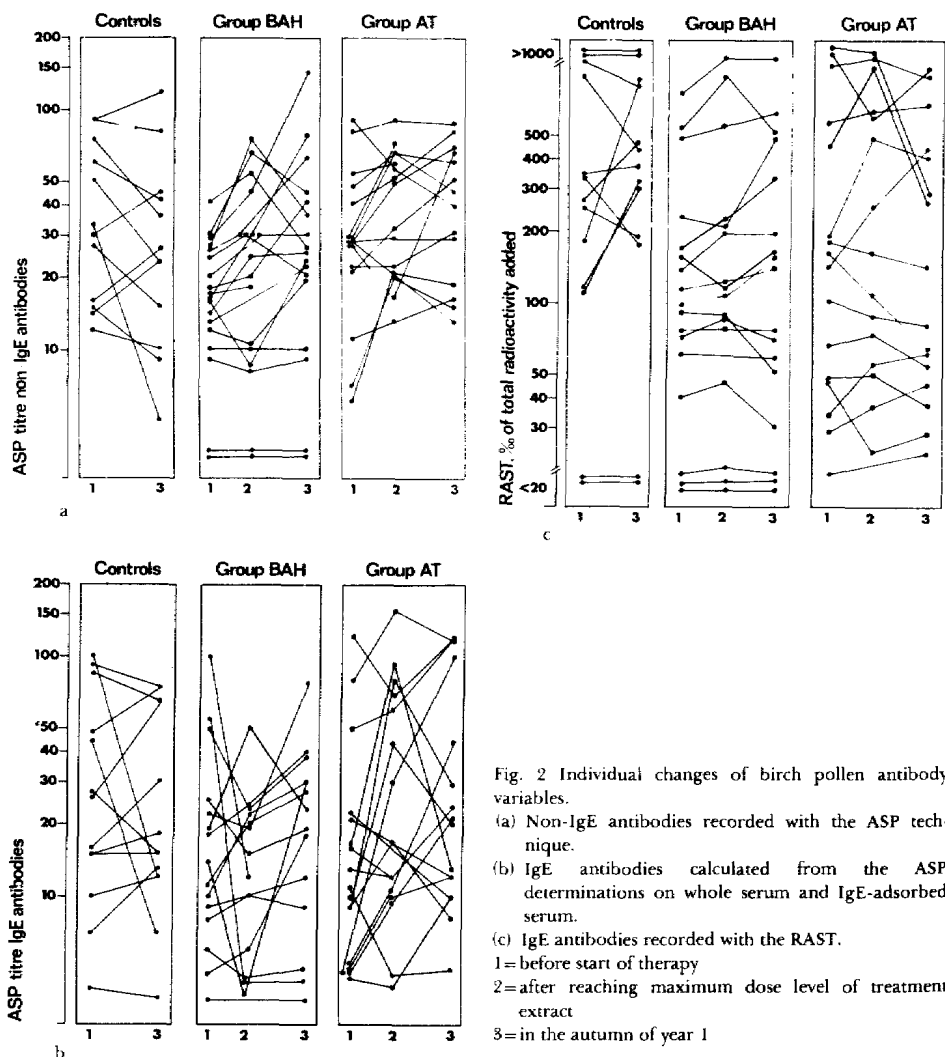


Fig. 2 Individual changes of birch pollen antibody variables.

(a) Non-IgE antibodies recorded with the ASP technique.

(b) IgE antibodies calculated from the ASP determinations on whole serum and IgE-adsorbed serum.

(c) IgE antibodies recorded with the RAST.

1=before start of therapy

2=after reaching maximum dose level of treatment extract

3=in the autumn of year 1

not in the controls. In contrast, the RAST values did not show significant changes in either the treated or the untreated groups (Table 3). Three years after the start of therapy (1977) no significant changes of the RAST values were found compared to the pretreatment values ($n=9$ for controls, $n=11$ for treated patients). ASP analyses were not performed on these sera.

Inter-group analyses revealed a significant difference between treated patients and controls with respect to the rise in non-IgE antibodies ($P<0.01$). Furthermore, the ASP titre rose more in group AT than in group BAH ($P<0.05$).

Seasonal changes. During the first year's pollen season no change of RAST values was

observed whereas a decrease of RAST values occurred in both untreated and treated patients from the autumn of year 1 to the spring of year 2 ($P < 0.05$ for treated patients, $P < 0.01$ for controls) (Table 4). This decrease was more pronounced in untreated than in treated patients ($P < 0.01$). From pre-season year 1 to the autumn of year 2 a further decrease of RAST values was seen in the controls while the treated patients did not show any significant changes during year 2. When the group-to-group analyses were applied to this seasonal change no significant difference was found between the groups, however.

Correlations between initial values and changes in antibody titres. A negative correlation was found between the pre-treatment level of non-IgE antibodies and the percentage increase of these antibodies from pre-treatment level to the level in the autumn of year 1 ($R = -0.40$, $n = 43$, $P < 0.01$). A similar correlation was found between the initial titre of IgE antibodies, recorded with the ASP technique, and the percentage rise of IgE antibodies ($R = -0.45$, $n = 44$, $P < 0.01$), whereas no such correlation was seen between pre-treatment RAST values and the changes of RAST values. The decreases in

RAST from postseasonal values of year 1 to preseasonal values of year 2 were related to the RAST levels before the decrease ($R = -0.68$, $n = 28$, $P < 0.001$). Thus, patients with low levels of IgE antibody in postseasonal sera tended to have smaller decreases than patients with high levels (Fig. 3).

The BP antibody avidities did not show significant changes either in treated patients or in controls.

Beech antibody titres. The RAST titres with beech pollen showed an inconstant pattern, like those with BP. The mean values were increased in both treated groups when the maximum allergen doses were reached but the increases were not statistically significant. Nor were there any significant changes in any patient group in the autumn of year 1. After 1 year a significantly decreased level of beech IgE antibodies was found for the two treatment groups analysed together ($P < 0.05$). Seasonal variations were noted: controls as well as treated patients grouped together showed a decrease of RAST titres from the autumn of year 1 to the spring of year 2 ($P < 0.05$). The controls also showed a postseasonal decrease in year 2 ($P < 0.05$)

Table 4
Seasonal changes of birch and beech antibody levels according to RAST

Patient group	From autumn year 1 to spring year 2		From spring year 2 to autumn year 2	
	<i>n</i>	Change	<i>n</i>	Change
<i>Birch antibodies</i>				
Treated (BAH + AT)	20	$-33 \pm 63^*$	16	$+7 \pm 98$
Controls	8	$-289 \pm 182^{**}$	8	$-71 \pm 51^*$
<i>Beech antibodies</i>				
BAH	9	-18 ± 26^a	8	-6 ± 35
AT	12	$+2 \pm 33^a$	10	-1 ± 8
Controls	9	$-45 \pm 37^*$	9	$-16 \pm 18^*$

Figures indicate % of radioactivity added (means $\pm$ s.d.), $^* = P < 0.05$, $^{**} = P < 0.01$

^aWhen treated patients (BAH + AT) were analysed together a significant decrease ($P < 0.05$) of beech antibody levels was noted from autumn year 1 to spring year 2.

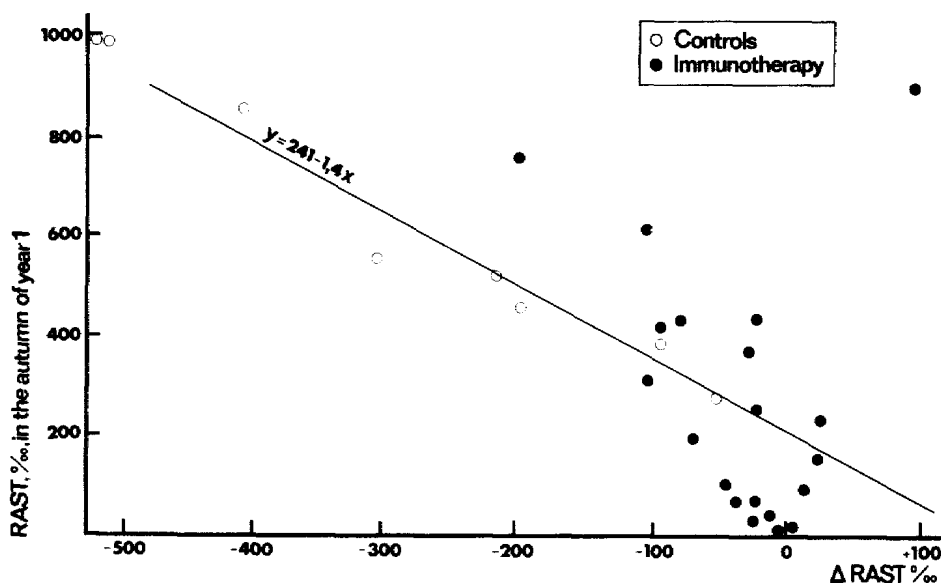


Fig. 3. Relationship between the RAST values (birch pollen) in the autumn of year 1 and the changes of RAST values from the autumn of year 1 to the spring of year 2. $R = -0.68$, $n = 28$, $P < 0.001$

(Table 4). The inter-group comparison did not reveal any differences between patients treated with all relevant tree pollens (group AT) and patients treated with birch, alder and hazel only (group BAH).

Cellular changes. Before treatment the leucocytes of the patients in the treatment groups released on average 42% (range 5–92%) of their total histamine content on challenge with optimum concentrations of BP allergen. After desensitization therapy, at the point of time when the maximum treatment dose was reached, an increase of the *cell reactivity* to 55% (range 13–91%) was seen ($P < 0.01$) while after the pollen season a decrease to initial values was found. The BP allergen extract concentration required for maximum histamine release, the *cell sensitivity*, in pretreatment samples varied from 10^{-4} to 10^{-8} , with a mean of $10^{-5.7}$. No significant changes of this variable were seen

after immunotherapy. The controls showed no changes of *cell reactivity* or *cell sensitivity*.

Correlations between clinical and immunological variables

The symptom score for the first year's BP season showed significant positive correlation to the initial ASP non-IgE BP-antibody titre ($P < 0.01$), as well as to the *cell sensitivity* measured when the maximum allergen dose was reached ($P < 0.05$) and the pre-treatment RAST-titre of *beech* pollen allergen ($P < 0.05$). A negative correlation ($P < 0.05$) was found between the symptom scores during the pollen season and the percentage increase of the ASP non-IgE antibody titre. In other words, the more pronounced the rise of non-IgE antibodies, the less severe were the symptoms and the lower was the tablet consumption (Fig. 4). The therapeutic effect of hyposensitization, assessed from the patients'

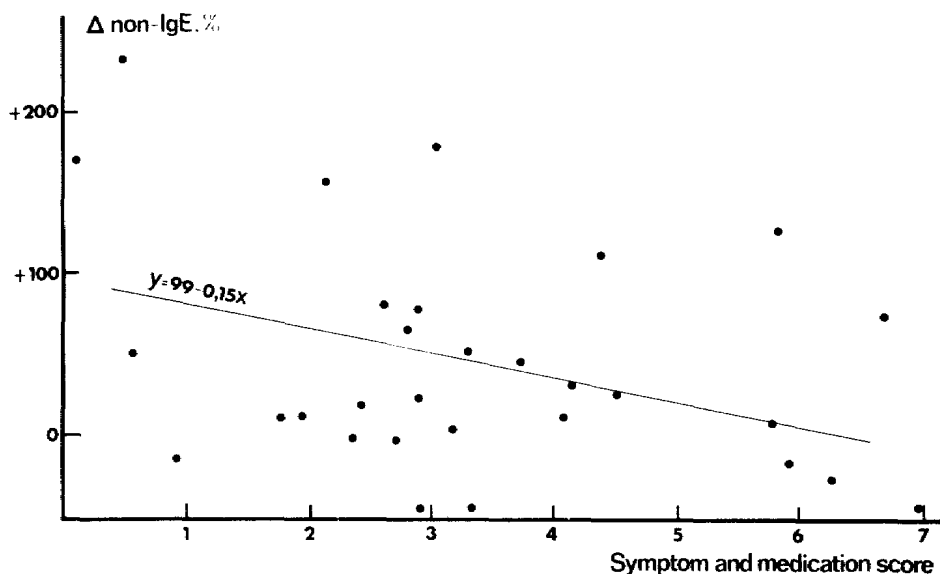


Fig. 4. Relationship between percentage change of non-IgE antibodies against birch pollen and the symptom and medication score during the birch pollen season in year 1.
 $r = -0.37$, $n = 31$, $P < 0.05$

ratings of their symptoms during the pollen season in year 1 compared to the previous year, was significantly correlated to the ASP non-IgE/IgE ratio in pretreatment sera ($P < 0.01$); the higher the ratio, the better the therapeutic effect. The difference between PTs with BP performed before treatment and in the autumn of year 1, i.e. the reduction of nasal sensitivity, was also correlated to the initial ASP non-IgE/IgE ratio ($P < 0.05$). The higher the initial nasal sensitivity, the more pronounced was the reduction of nasal sensitivity ($P < 0.01$).

When, in a separate statistical analysis, only patients with high-degree BP-allergy (patients with positive PTs at allergen concentrations of 10^{-2} or 10^{-3}) were included, significant correlations were also found between symptom scores and the RAST titres ($P < 0.05$) at the point of time when the patients received maximum doses, between symptoms score and the increase of RAST ($P < 0.05$) and between the patients' opinions

regarding the efficacy of the treatment and the percentage change in non-IgE antibodies ($P < 0.05$).

DISCUSSION

Almost all patients with spring-time hay fever in Sweden are found to have a BP allergy, although allergy against other trees is often found as well (11, 36). In the present investigation a similar therapeutic effect of immunotherapy was shown with a birch-alder-hazel allergen mixture in Allpyral and with this mixture supplemented with other relevant tree pollens in aqueous extract. Both treatment groups showed less pronounced symptoms and lower medication scores during the tree pollen season than the control group. Of the patients treated, 83% and 93%, respectively, reported improvement, which is in agreement with results previously reported after immunotherapy in

ragweed and grass pollen allergy (18). In accordance with the routines at our clinic in 1974, an allergen dose of 4000 PNU was never exceeded. Still better results might have been achieved if higher doses and more frequent injections had been given (27).

It thus seems sufficient to use the "Northern tree mix" for immunotherapy in spring-time hay fever, in spite of the presence of sensitization to pollen from other trees. On the other hand, when this mixture is used some patients will receive injections of allergens to which they are not sensitized. Such a treatment might mean a risk of inducing sensitization (34). It might be justified to exclude hazel and possibly alder from the three-tree mixture, although this requires further investigation.

In agreement with the results of our previous studies, (1, 2) the immunological variables studied showed no consistent pattern, although a rise of non-IgE antibodies was seen after initiation of immunotherapy. The latter finding is in agreement with several other studies (9, 15, 17, 20, 26, 35). In contrast to our previous results (2), as well as to the findings of some other workers (6, 17, 24, 26), neither an initial increase of the IgE antibodies after initiation of therapy, nor a later gradual decrease of the antibodies was observed in the present study. The reason for these discrepancies is not clear. However, the fact that our patients were not regularly treated with maximum tolerated doses of allergen might be one explanation for the small immunological changes seen.

The seasonal increase of reaginic antibodies in untreated patients with pollen allergies reported by other authors (7, 15, 24) was not seen in our patients. On the contrary, a decrease of RAST titres with BP allergen was seen in our control group from the spring to the autumn of year 2. The BP season was very short and the pollen counts were very low in Sweden in 1975 (10) and the absence of a strong allergenic stimulation during the pollen season might be one explanation for the absence of increased RAST

values. Another explanation could be that an antibody peak appearing immediately after the pollen season might have been overlooked by us, since the postseasonal sera of our patients were taken 3–4 months after the BP season.

According to the findings of Gleich et al. (15) the decrease of reagin titres from post seasonal values of 1 year to the preseasonal values of the following year commonly seen is depressed by immunotherapy. Our results corroborate this finding. The decrease of RAST values which was recorded from the autumn of year 1 to the spring of year 2 in both treated patients and controls was less pronounced in the treated patients. The correlation found in our patients between the postseasonal RAST titres and the decreases from the autumn to the spring of the next year is also in agreement with the finding of Gleich et al. (15) in ragweed allergic patients.

The fact that no significant changes of BP antibody avidities were observed either in our previous study (2) or in the present one does not exclude the possibility that changes of affinities may occur during immunotherapy and may be of therapeutic importance, as proposed by Ahlstedt et al. (2). In other biological systems it has been shown that increased antigen-binding will follow rises in antibody avidity induced by immunotherapy (32).

Not only the antibody function but also the metabolic balance of the cell systems involved, affecting the release of vasoactive amines, is of importance in the allergic reaction. Several studies have shown a decreased ability of the leucocytes of desensitized patients to release histamine on challenge with the appropriate allergen (20, 26, 31). Such cellular changes seem to be immunologically unspecific (23, 25) and good treatment results can be achieved without any observable cellular changes (29). In the present study no decreased *cell sensitivity* or *cell reactivity* was seen. On the contrary, during the initial phase of the treatment an increased *cell reactivity* was observed. Similar

findings have been reported by Assem & McAllen (3), who observed increased histamine release in spite of clinical improvement during immunotherapy with mite allergen.

Conflicting data have been reported regarding the correlation between immunological variables and therapeutic effect of immunotherapy. In some studies, including our previous studies (1, 2), no correlations whatsoever have been found. In others a correlation has been found between symptom amelioration and blocking antibody titre (22, 33) or decreased cellular histamine release capacity (20). In the present study a weak correlation was found between therapeutic effect and the percentage increase of non-IgE antibodies. This finding is in agreement with those of Irons et al. (16), who reported significant correlations between the change in antigen-binding capacity and various other variables. In highly sensitive patients we also found a negative correlation between the RAST increase and the therapeutic effect. Furthermore, a correlation was found between therapeutic effect and the non-IgE/IgE antibody ratio of the pretreatment sera. Other authors have also found the IgG/IgE relationship to be of importance. Thus, D'Souza et al. (9) found that clinical improvement during immunotherapy with house-dust mite antigen occurred mainly in subjects who showed increase of IgG antibodies but little or no increase of specific IgE antibodies. Foucard & Johansson (14) found, when treating children with birch and/or grass pollen allergy, that at the end of their study the almost symptom-free children had a higher IgG/IgE antibody ratio than the children who had not improved. It seems obvious that changes of a single immunological variable do not account for the treatment results in immunotherapy but that an interplay of various known factors as well as perhaps other, as yet undiscovered, factors is in operation.

Immunotherapy is a time-consuming and

potentially hazardous form of treatment, giving improvement in most patients but seldom giving complete freedom from symptoms. According to the results of an extension of the present study (13), in which our control group was treated the following year with disodium cromoglycate, immunotherapy and disodium cromoglycate give comparable treatment results. With the availability of new effective drugs, acting allergen-unspecific, it seems probable that the number of patients demanding desensitization therapy will decrease, unless more efficient allergen extracts than those available at present can be developed.

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