

Hyposensitization in Childhood Hay Fever

A Comparison of Refined and Whole Timothy Extracts

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Thirty-six children with well-defined criteria for hay fever (mean age 8 and range 4-15 years) were allocated at random for hyposensitization (HS) with a refined (R) or whole (W) timothy pollen extract during 3-4 years. HS was performed as rush HS with the patients hospitalized for about 1 week and thereafter with monthly injections. Scores for symptoms and antihistamine use were recorded during the season before HS and all seasons during HS. Skin and conjunctival tests were made at the start of HS and postseasonally. Blood samples for IgE and IgG measurements were drawn before and during rush HS and pre- and postseasonally each year.

The R-group patients tolerated a higher allergen dose at the end of the rush HS than the W-group ones. They also demonstrated a higher, significant increase in total and specific IgE levels within 7 days and specific IgG levels within 60 days after the start of HS. In both groups postseasonal increases in total and specific IgE levels were seen. The IgG levels increased successively during the treatment. The most remarkable difference between the groups was in scores for symptoms and antihistamine use, which in group R decreased significantly while they increased significantly in group W.

Based on these data we recommended that HS, if indicated, should be performed with purified allergen extracts.

Key words: allergen extracts; children; hayfever; hyposensitization; IgE; IgE antibodies; IgG antibodies

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Hyposensitization (=HS), i.e. injection of increasing doses of allergens, has been used widely in allergic diseases especially pollinosis, since it was introduced by Freeman and Noon in 1911 (7, 17). Controlled studies have shown that HS with pollen extracts is of benefit to the patient (1, 6, 8, 9, 14, 16, 19, 20). Different types of allergens have been used clinically, but for most of them, the available extracts have been crude ones. These contain unspecific irritants, such as proteins and carbohydrates, without allergenic activity. Allergen extracts containing such impurities may cause non-specific reactions in the skin and/or shock organs when used for testing. Thus, there is an overt risk of a falsely positive

diagnosis when using crude extracts. Furthermore, there is a risk of non-specific skin reactions when administering such extracts during HS. The treating physician may misinterpret these non-specific reactions, and thus an inadequate treatment with too low doses may result.

The first course of HS, where a refined and a crude extract were compared clinically with placebo injections, was given by Frankland & Augustin in 1954 (6). Both extracts gave the same clinical results, but better than placebo.

The purpose of the present study was to compare, in a group of children with hay fever, the clinical results and the effects on IgE and IgG antibodies of HS with two aqueous

extracts of timothy pollen: a refined extract (Pharmacia AB, Uppsala, Sweden) and a crude extract already on the market.

MATERIAL AND METHODS

Patients

Patients had to fulfil all the following criteria:

- a definite history of grass pollen allergy during two seasons or more,
- at least a 3 plus reaction (+ + +) on skin prick test (SPT) with a timothy pollen allergen extract (2),
- a positive reaction on conjunctival test (CT) with a timothy pollen allergen extract,
- a Phadebas RAST level of class 2 or more,
- no previous HS.

Thirty-six children selected from out-patients at the Paediatric Department, University Hospital, Linköping, Sweden, fulfilled these criteria and were studied after the children's parents had given their informed consent.

The diagnosis was established at the end of summer 1973. During the preceding grass pollen season, the children were observed carefully by their families. Score cards were used for the registration of symptoms as well as the use of antihistamines.

The children were allocated at random to one of two treatment groups. One group ($n=19$) was treated with a refined timothy extract (group R) and the other group ($n=17$) with a whole timothy extract (group W). The two groups were comparable as regards age and sex. The median ages were 9 and 8 years and the mean ages 8.8 ± 2.7 and 8.8 ± 2.3 years for group R and W, respectively. Fourteen and 12 children of group R and W, respectively, had histories of allergy to allergens other than grass pollen, as well.

The treatment with the two extracts (see below) was conducted as a double-blind trial, the data were collected and computerized before breaking the code.

Extracts

Two aqueous extracts, one whole and one refined, of timothy pollen (*Phleum pratense*) were used. Both were assayed for their allergen potency using a direct RAST titration method (21) before the start of the HS, employing a standard reference pool of sera from more than 20 patients allergic to grass pollen allergens. The whole extract (Vitrum AB, Stockholm, Lot No. 83 183), an ordinary 1/10 (w/v) extract had a potency of 172 arbitrary Allergen RAST Units/ml (ARU) and the refined extract (Pharmacia AB, Uppsala, Lot. No. 32 705) had a potency of 526 ARU/ml. The refined extract was produced from a freshly prepared 1/10 (w/v) whole extract by means of column chromatography (Sephadex G-75). Through purification approximately 95% of the pigments, 90% of the carbohydrates and 70% of the nitrogen-containing impurities could be removed. The same two batches of extracts were used for skin and provocation tests as well as for HS during the whole study. The extracts, when not used for testing or HS, were kept at 4°C.

The time schedule for the study is shown in Fig. 1.

In vivo tests

The *in vivo* tests (Fig. 1) were performed as skin prick test (SPT) and conjunctival provocation test (CT) after the grass pollen seasons of 1973–75. Throughout the period 1973–75 the SPT was performed with both extracts in all children. The CT was performed in all children with both extracts in the 1973 and 1974 tests, while in 1975 only the refined extract was used in group R and the whole in Group W. When both extracts were used in a child, the statistical calculations were made on the mean result of both extracts used.

SPT was performed with a 1/10 (w/v) concentration of the extracts, and the results were compared with those of histamine 1:1000. The mean of the prick test results

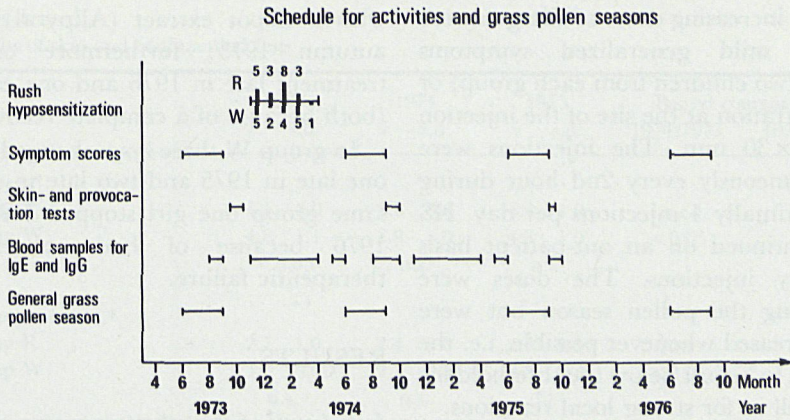


Fig. 1. Time schedule for activities and grass pollen seasons.

(number of plus signs) was used in calculations (2).

The CT was always performed as a titrated test started by application of one drop of the diluent, followed by one drop of the extract 1:100 000 (w/v) in the other eye. If no reaction occurred within 5 min, an allergen dilution 10 times stronger was applied in the first eye tested. This procedure was continued using alternatingly the right and the left eye until a 1/10 (w/v) concentration was reached or until a positive conjunctival reaction (increased vascularity and local itch) was noticed. For the calculations the log of the lowest concentration giving a positive provocation test was used; a higher figure thus indicates a higher sensitivity.

In vitro tests

Blood samples from each individual were drawn on one occasion during the different time intervals shown in Fig. 1. However, initially, blood samples were taken before the start of HS (day 0) and 1, 3, 7, 14, 30 and 60 days after the start. The results of these initial measurements are presented elsewhere (10). Sera were distributed in duplicate tubes and kept frozen at -20°C until all determinations were made. All sera of each single patient were assayed for total IgE in one run, for specific IgE in one run and for specific IgG in one run. However, the autumn sample of 1975 was run separately for specific IgG.

Total IgE analyses were performed with Phadebas IgE PRIST® and timothy-specific IgE antibodies were determined with Phadebas RAST® (Pharmacia Diagnostic AB, Uppsala, Sweden). The result of the RAST tests was expressed in Phadebas RAST Units (PRU) (15) in order to detect small differences which could have gone undetected if the conventional RAST classes 0–4 had been used. Employing this method, four reference sera of the concentration of 0.35, 0.7, 3.5 and 17.5 PRU/ml are used. Patients' specimen counts are plotted on a logarithmic graph against reference sera PRU values and then interpreted. Sera with a high IgE titre are diluted with human serum without specific IgE to fall into the range 0.7–3.5 PRU/ml.

IgG antibodies against timothy were determined with an ammonium sulphate precipitation method (3) and the antibody quantities were expressed as reciprocal values of the serum dilution causing 20% precipitation of the antigen added.

Due to the positive skew distribution of total and specific IgE and specific IgG antibodies, their natural logarithms were used in all statistical calculations.

Hyposensitization

All children were submitted to a rush HS (4) during the off-season period 1973–74: the children were hospitalized for about 1 week

during which increasing doses of allergen were given until mild generalized symptoms occurred (in two children from each group) or until the infiltration at the site of the injection exceeded 30×30 mm. The injections were given subcutaneously every 2nd hour during daytime, maximally 4 injections per day. HS was then continued on an out-patient basis with monthly injections. The doses were reduced during the pollen season but were otherwise increased whenever possible, i.e. the dose intended to be just below the threshold for general as well as for strong local reactions.

In seven children in group R and five in group W, HS therapy with a mixed tree pollen extract (birch, alder and hazel) run parallel with the timothy pollen HS.

Clinical evaluation and symptom scores

The children's parents recorded scores of the symptoms and the use of drugs during the general grass pollen season (June-August) during the whole study (Fig. 1). All of them obtained written instructions for scoring the symptoms. Scores were recorded daily for five symptoms separately: nasal obstruction, sneezing, nasal discharge, itchy eyes and cough. Each symptom was scored from 0–4 according to the intensity and duration of the symptoms: 0 indicating no symptom and 4 indicating severe symptoms for more than 4 hours. A total symptom score was calculated for each child and season.

The use of antihistamines was recorded and the total number of doses given during a season was calculated for each child. At the end of each season during the HS therapy, the parents made an assessment as to whether or not the child seemed to have improved during the recent pollen season compared with the preceding season.

Statistical evaluation and drop-outs

Student's *t*-test was used for most group comparisons, otherwise the Wilcoxon rank sum test and the Fisher's exact test were used.

In group R two children changed to HS

with a depot extract (Allpyral) during the autumn 1975; furthermore one stopped treatment late in 1976 and one early in 1977 (both because of a complete recovery).

In group W three boys changed to Allpyral, one late in 1975 and two late in 1976. In the same group one girl stopped HS in autumn 1976 because of bad cooperation and therapeutic failure.

RESULTS

Initial state of the patients

Only slight differences were found concerning the *in vitro* and *in vivo* test results before the start of the HS (Tables 1–2, Figs. 2, 4 and 6). The patients in group W seemed to be slightly more afflicted. At the start of HS they had higher levels of total IgE ($P < 0.05$) and showed stronger reactions on SPT in autumn 1973 ($P < 0.05$, Table 1).

Skin and conjunctival tests

During the study, the *in vivo* test findings showed only slight group differences (Table 1). The only significantly decreased reactivity seen was in group W when 1974 was compared with 1973 (SPT, $P < 0.05$ and CT, $P < 0.01$).

Total serum IgE levels

From autumn 1973 to autumn 1974 group W had a significantly higher level of total IgE ($P < 0.05$, Fig. 2). During 1975 the group difference became smaller and insignificant. Group R, in contrast to group W, showed a significant increase ($P < 0.01$) 3–7 days after the start of rush HS (Fig. 3).

The serum IgE levels showed characteristic ups and downs with significantly higher levels in the samples taken in September than in May (Fig. 2). This tendency was maintained throughout the study.

Timothy-specific serum IgE antibody levels

The timothy-specific IgE antibody levels showed similar, but even more pronounced,

Table 1.
Results of skin and conjunctival tests

	1973		1974		1975		Paired comparisons	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	1974/1973	1975/1973
<i>Skin tests*</i>								
Group R	4.1	1.0	3.6	0.6	4.1	1.0	n.s.†	n.s.
Group W	4.8	1.1	3.9	1.0	4.3	1.0	<0.05	n.s.
P	<0.05		n.s.		n.s.			
<i>Conjunctival tests**</i>								
Group R	2.7	1.0	2.4	0.8	2.7	1.1	n.s.	n.s.
Group W	3.3	0.6	2.7	0.5	2.5	0.8	<0.01	n.s.
P	n.s.		n.s.		††			

*The figures denote the number of plus signs obtained at the skin prick test in relation to histamine reaction, which is denoted 3.

**The figures denote the lowest concentration giving a reaction and are expressed as the negative logarithm of base 10, e.g. the conc. 1:1000 gives 3, 1:100 gives 2.

†n.s. = not significant.

††No group comparison was possible in 1975, since the provocation tests were performed with only that extract each group had been treated with.

seasonal changes than the total IgE levels (Fig. 4). In both groups the autumn level in 1975 was significantly higher than that in 1974. The pre-treatment levels did not show any significant group difference, while the levels of 1 year's treatment, the spring and the autumn levels in 1974 as well as those in 1975, were significantly lower in group R ($P < 0.05$ and $P < 0.01$, respectively). The early response in specific IgE on HS (3–7 days' treatment) showed a significant increase ($P < 0.05$, Fig. 5) in group R in contrast to group W where the level remained unchanged. When the 1-year change in specific IgE levels of the two groups was compared, they differed significantly ($P < 0.05$), which was based on an insignificant decrease in group R and increase in group W. This was also the case when the May level of 1975 was compared with the pre-treatment level.

Timothy-specific serum IgG antibody levels

The timothy-specific IgG levels at the start of HS, 3.3 and 3.9 (log reciprocal titre) in groups R and W (Fig. 6), respectively, were not significantly different. Within 30–60 days the

level had increased significantly to 4.2 ($P < 0.05$) in group R, but in group W it was almost unchanged (4.1) (Fig. 7) without leading to any group differences. From the start until May 1975, both groups showed increasing levels (Fig. 6) while between May and September 1975 there was an insignificant decrease for group R but a significant decrease for group W. All samples taken up to May 1975 showed a significantly increased level in group R as compared with the starting level. On the other hand, in group W only the 1-year sample and the sample of May 1975 showed significant increases.

When the pre-treatment and 1-year values of specific IgE and IgG were compared no correlations could be found in the changes of the two variables.

Symptom scores

The symptom scores in group R were lower during all seasons with HS and significantly lower scores were found in 1975 and after (Table 2). In group W a significant increase in scores was seen during the 1975 season.

Table 2.
Total symptom scores and the total seasonal use of antihistamines during the grass pollen seasons of 1973-77

	Mean $\pm$ s.d.					Paired comparisons		
	1973	1974	1975	1976	1977	1974/1973	1975/1973	1976/1973
<i>Total symptom scores</i>								
Group R	285 $\pm$ 177	202 $\pm$ 235	172 $\pm$ 150	202 $\pm$ 214	178 $\pm$ 201	n.s.	<0.05	<0.05
Group W	302 $\pm$ 153	247 $\pm$ 184	388 $\pm$ 205	302 $\pm$ 154	283 $\pm$ 194	n.s.	<0.05	n.s.
<i>P</i>	n.s.	n.s.	<0.01	n.s.	n.s.			
<i>Use of antihistamines (No. of doses)</i>								
Group R	86 $\pm$ 75	94 $\pm$ 86	60 $\pm$ 71	59 $\pm$ 62	59 $\pm$ 111	n.s.	n.s.	n.s.
Group W	91 $\pm$ 60	125 $\pm$ 87	145 $\pm$ 87	142 $\pm$ 82	129 $\pm$ 59	n.s.	<0.05	<0.05
<i>P</i>	n.s.	n.s.	<0.01	<0.01	n.s.			

Fig. 8 gives the number of children with changes in symptom scores comparing the seasons 1974–1977 with 1973. During both the 1976 and the 1977 seasons, 79% of the children in group R had less symptom scores than 1973. In group W the corresponding figures were 29 and 50%.

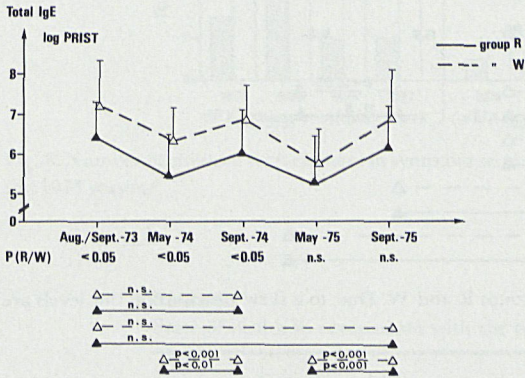


Fig. 2. Total IgE levels (mean and s.d.) of groups R and W. Due to a skew distribution the levels are given as natural logarithm levels.

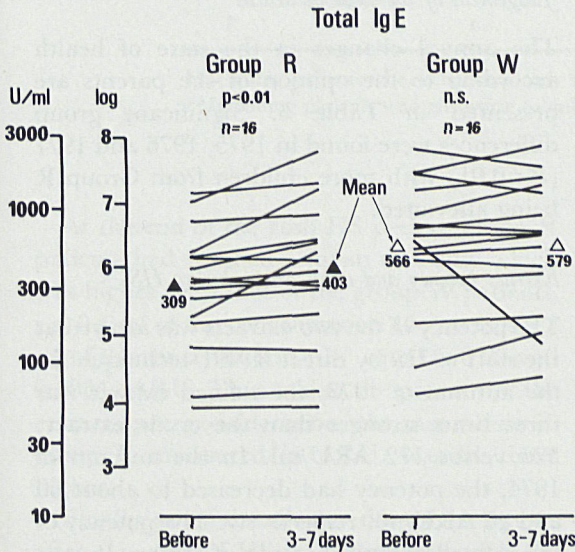


Fig. 3. Early changes (within 3–7 days) in total IgE serum levels (U/ml). Due to the skew distribution of the immunoglobulin levels the mean value is calculated on the individual natural logarithm levels and then transferred back to a real value (geometric value).

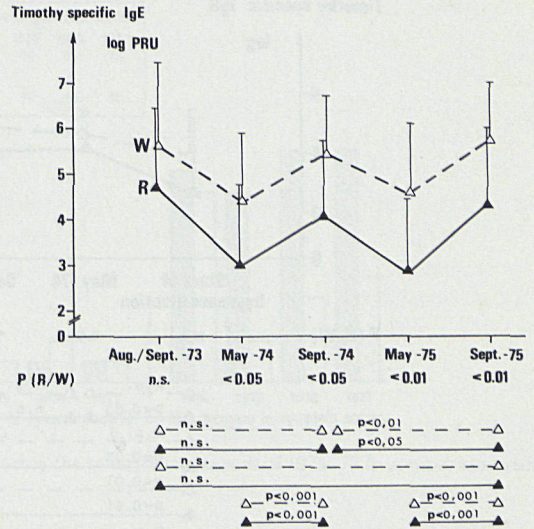


Fig. 4. Timothy-specific IgE levels (mean and s.d.) of groups R and W expressed in Phadebas RAST units (PRU). Due to a skew distribution the levels are given as natural logarithm levels.

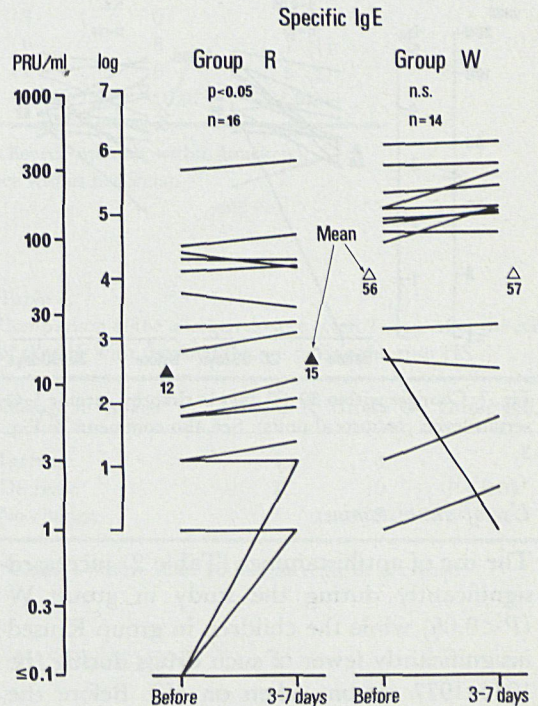


Fig. 5. Early changes (within 3–7 days) in timothy-specific IgE serum levels (PRU/ml). See also comments to Fig. 3.

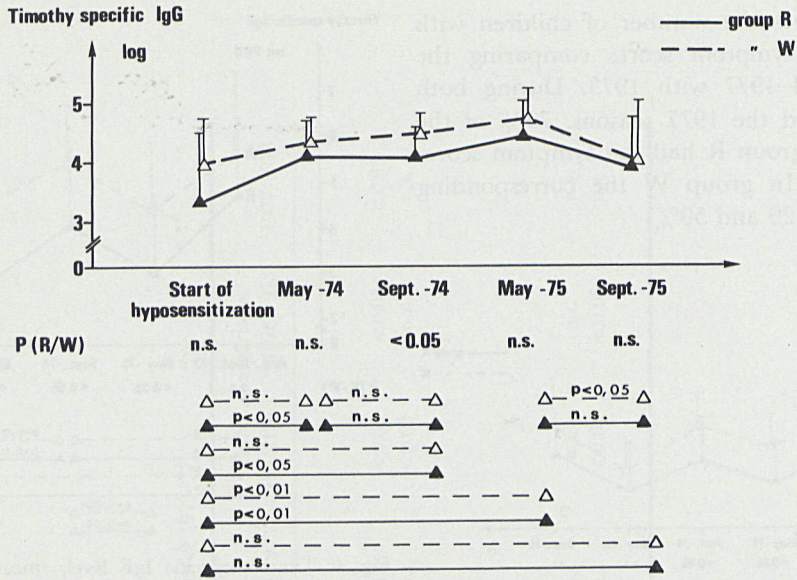


Fig. 6. Timothy-specific IgG levels (mean and s.d.) of groups R and W. Due to a skew distribution the levels are given as natural logarithm levels.

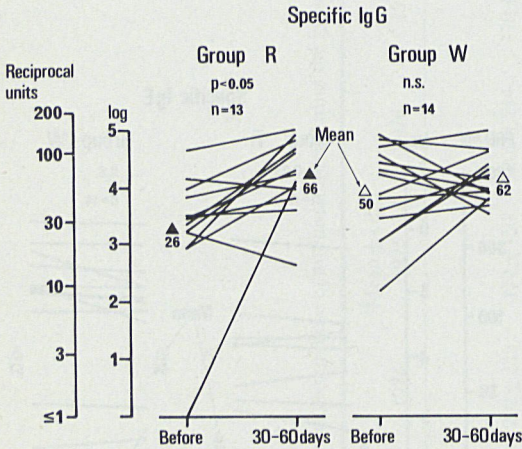


Fig. 7. Changes within 30-60 days in timothy-specific IgG serum levels (reciprocal units). See also comments to Fig. 3.

Use of antihistamines

The use of antihistamines (Table 2) increased significantly during the study in group W ($P < 0.05$) while the children in group R used insignificantly fewer of such drugs during the 1975-1977 seasons when on HS. Before the start of the study there were no differences in the two groups; but during the 1975 and 1976

seasons group R had a significantly lower intake of antihistamine ($P < 0.01$) than group W.

Judgement of the state of health

The annual changes in the state of health according to the opinion of the parents are presented in Table 3. Significant group differences were found in 1975, 1976 and 1977 ($P < 0.01$) with more children from Group R being alleviated.

Extract potency and doses given during HS

The potency of the two extracts was assayed at the start of HS by direct RAST-technique. In the autumn of 1973, the refined extract was three times stronger than the crude extract: 526 versus 172 ARU/ml. In the autumn of 1974, the potency had decreased to about 60 and 20 ARU/ml, respectively. The potency of the refined extract, but not of the crude one, was measured successively until autumn 1975: the potency had been gradually decreasing until it became stabilized at the level of approximately 40 ARU/ml.

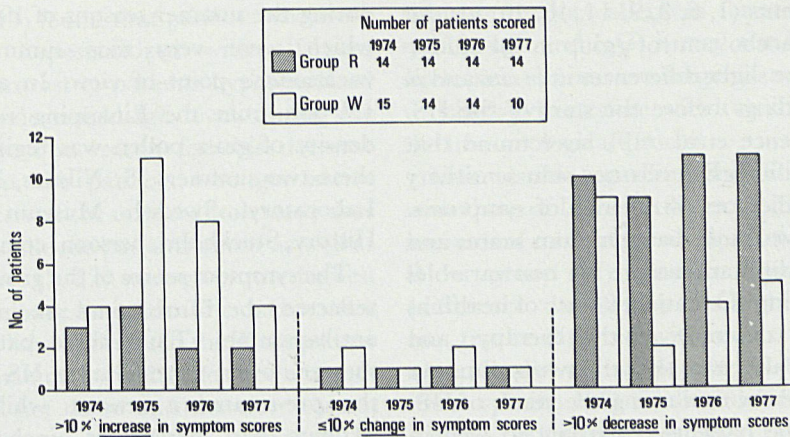


Fig. 8. Number of children with changes in symptom scores during the grass pollen seasons of 1974-77 in comparison with the 1973 season.

Table 3.

State of health in comparison with the preceding season according to the parents

Group	State of health	Seasons compared			
		1974/1973	1975/1974	1976/1975	1977/1976
R	Improved*	16	14 (2)	17 (2)	17 (8)
	Not improved	2	3	0	0
W	Improved*	14	6	8	7 (1)
	Not improved**	2	11	6	6 (2)
P		n.s.	<0.05	<0.01	<0.01

*Including patients who were completely relieved (number within brackets).

**Including patients who got worse (number within brackets).

At the end of the rush HS period, group R patients had reached a mean end dose which was higher than that of the group W patients, i.e. 0.4 ml of the concentration 10^{-4} versus 0.2 ml of the concentration 10^{-5} equal to 0.20 and 0.00344 ARU. Thus taking the potency of the extracts into consideration, the mean dose in group R, measured in ARU, was 61 times higher.

Finally, when the allergen dose given 2 years after the start of HS was compared with the end dose of the rush HS, it became obvious that more patients ($P < 0.05$) in group R had increased their dose during this interval than in group W (Table 4).

Table 4.

Comparison of the allergen dosage given 2 years after the start of the HS with the final dosage during the rush HS

Change in dosage*	Group R	Group W	Group diff.
Increase	4	0	$P < 0.05$
Decrease	7	10	
No change	7	7	

*Change = more than 10-fold increase or decrease.

DISCUSSION

Since it has been shown earlier that immunotherapy has a beneficial effect in

allergic patients (1, 6, 8, 9, 14, 16, 20) we did not use a placebo control group in the study.

There were slight differences in *in vivo* and *in vitro* test findings before the start of the HS. However, Pence et al. (19) have found that neither specific IgE levels nor skin sensitivity are good indicators of severity of symptoms. Therefore, we think that symptom scores and the use of antihistamines are the best variables for determining the patient's state of health as well as the outcome of the therapy, and therefore we also state that the two groups are well matched for evaluating the result of HS. Moreover, the patients corresponded well in sex and age.

The refined extract seemed to be better tolerated than the crude one especially as higher doses were reached during rush HS. Also, more children in group R increased their doses during the 2 year's treatment than did the children in group W. This is an important factor as clinical improvement correlates positively to the amount of allergen given (8, 9, 12, 18). Thus, treatment with the refined extract may be one reason for a better therapeutic result in group R.

Skin and provocation tests

As the potency of an extract decreases after a while and because the potency varies from batch to batch, it is extremely difficult to obtain conclusive results from *in vivo* tests. For the same reasons it is also very difficult to establish a correlation between skin or provocation tests and the clinical effect unless standardized and freeze-dried extracts become available. Therefore, our significant results of SPT and CT have to be questioned, especially as they do not correlate with any other variables.

Use of antihistamines, symptom scores and subjective state of health

In the course of the study, group R patients used less and less antihistamines while group W patients used more than before HS. Especially high amounts were consumed

during the summer seasons of 1975 and 1976, which were very nice summers from a vacationer's point of view. In areas situated 150 km from the Linköping region a high density of grass pollen was registered during these two summers (S. Nilsson, Palynological Laboratory, Swedish Museum of Natural History, Stockholm, personal communication).

The symptom scores of the group R-patients reflected the same trend as in the use of antihistamines. Thus, these patients always showed lower scores during HS than during the pre-treatment season, while group W patients had higher or unchanged scores during HS. As the use of antihistamines masks many of the symptoms, the actual symptom scores of group W during HS ought to have been higher in relation to the values of 1973. It is notable that during seasons where a high pollen challenge was probable, HS with crude extract did not give adequate protection whereas HS with refined extract gave satisfactory protection.

The subjective state of health reflected by symptom scores and the use of antihistamines was appraised by the patients' parents. Their evaluation showed that the results in group R were significantly better than in group W after the second season of HS.

For judging the effect of HS, these three variables must be considered the most important ones. The results obtained show that treatment with refined extracts benefits the patient more than treatment with crude extracts.

Immunoglobulin levels

In our own study as well as in other immunotherapeutic studies by Foucard & Johansson (5) and Yunginger & Gleich (22), characteristic preseasonal to postseasonal increases were seen in total and specific IgE levels. However, this was not the case in other HS studies where it was noticed that specific IgE levels decreased (19) or remained unchanged (11) during pollen seasons. The reason for these varying results is not known.

Pence et al. (19) found that patients who were preseasonally hyposensitized with allergens showed, in contrast to patients hyposensitized with placebo, decreased IgE levels during the pollen season. The reason for this decrease could be that the patients who got active treatment received the maximal tolerated dose once a week while the patients in our as well as in other studies (5, 22) were given the highest tolerated dose once a month. The once a week regimen may have stimulated the T-suppressor mechanism maximally while the once a month regimen does not do so and therefore cannot suppress the postseasonal IgE increase. In order to suppress the seasonal increase of both total and specific IgE levels it is perhaps necessary to give high doses at short intervals. As the pollen density of a season may influence the postseasonal specific IgE levels, it was not surprising to find a significantly higher level in both groups in 1975 as compared with 1974 levels since the summer in 1975 was more rich in pollen.

The better clinical effect of HS in the R-group patients may depend on the different immunological response of the two groups. Thus, there was a significant increase in both total and specific IgE levels in group R within 3–7 days whereas levels in group W remained almost unchanged. Initial increases of these variables during HS have been described by Berg & Johansson (4). After 1 year, the raised level of specific IgE of group R had decreased to somewhat below the pretreatment level (3.7 vs 3.9) whereas the 1-year level of group W was higher than before treatment (5.0 vs 4.8). Another and probably more important reason for the better results in group R may be the stronger and more rapid response of specific IgG. In this group there was a significant increase after 30–60 days, and this increase persisted on a significant level throughout to spring 1975. A less pronounced increase which did not reach the level of significance until spring 1975 was observed in group W.

The reason for the better immunological response in R group patients may be that the refined extract has a higher concentration of

relevant (major) allergens for stimulation of specific IgE and IgG; it may also be devoid of irrelevant (minor) allergens and irritants which could interfere with the stimulation of the immunological process.

Our results regarding specific IgG agree with those of Lichtenstein et al. (13) as higher amounts of cumulative allergen injected (group R vs group W) gave a higher response (difference: day 30–day 0). On the other hand, our results disagree with theirs in as far as group R always (except after 30–60 days) had lower specific IgG levels and lower symptom scores. Lichtenstein et al. (13) discovered furthermore that patients with the highest response in specific IgG also had the greatest reduction in specific IgE but we could not find such a correlation.

Serum samples collected from each patient up to and including May 1975 were assayed for IgG in the same run while the autumn samples of 1975 was estimated separately. The 1975 autumn sample was run apart from all the other IgG serum samples, which may be one reason for the unexpected drop in the IgG levels of both groups between May and September 1975. Speculatively, the pollen-rich summer of 1975 could have caused the drop.

In summary, we have found that patients treated with purified timothy pollen extracts in comparison with patients treated with crude timothy pollen extracts

- have tolerated a higher allergen dose (measured as ARU) at the end of rush HS;
- have shown lower symptom scores during the pollen seasons;
- have used less antihistamine drugs during the pollen seasons;
- have shown a higher immune response measured by increase in total and timothy specific IgE (0–7 days);
- have shown a higher immune response measured by increase in timothy specific IgG (0–30 to 60 days).

On the basis of these data, we recommend that hyposensitization, if indicated, should be performed using purified allergen extracts.

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