

Immunotherapy in Hay Fever with Two Major Allergens 19, 25 and Partially Purified Extract of Timothy Grass Pollen

A Controlled Double Blind Study. *In Vitro* Variables, Season I

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Grass pollen hay fever patients were hyposensitized in a prospective 3-year double blind study with two timothy extracts. Group WPA (20 patients) was treated with partially purified extract = whole pollen allergens (WPA) (Alutard®SQ) and group PPA (20 patients) was treated with a purified pollen allergen (PPA) = two isolated major allergens, Ag19 and Ag25. Both aluminiumhydroxide adsorbed extracts were biologically standardized. Clinical results from the first season have been recently published and WPA showed significantly fewer symptoms ($P = 0.001$) than PPA. Corresponding preseasonal and seasonal *in vitro* results are presented here.

Serum total IgE, specific IgE versus total and individual allergens of timothy pollen and allergen-specific IgG showed a rapid increase in both groups until the season but showed no further increase or decrease during the season. Specific IgE was shown to correlate to specific IgG during hyposensitization.

Group WPA, with fewer symptoms in grass pollen season than group PPA showed a significantly higher increase of specific IgG and IgE than group PPA, but individual symptom scores were not correlated to specific IgG or IgE, or their ratio.

Specific IgE and IgG increases were not correlated to dosage. Surprisingly, almost all females were low-responders to specific IgG and IgE though they had equal symptom scores, dosage, and side effects as males, while the characteristics of high-responders to antibody were: youngest individuals and shortest duration of symptoms prior to treatment.

Crossed radioimmuno-electrophoresis (CRIE) showed specific reaction to stimuli but no development of allergy against new timothy antigens.

High response of IgE to Ag 19 in CRIE during initial hyposensitization seems suitable as marker for prospective evaluation of clinical effect in grass pollen hyposensitization.

Nasal secretion was collected after methacholine provocation, and total IgE and specific IgE detected. There was no response to treatment, only a slight increase during the season. No decrease in nasal reactivity to methacholine was noted during one preseasonal hyposensitization.

Key words: allergen extracts; biological units; blocking antibodies; crossed radioimmuno-electrophoresis; grass pollen; hay fever; immunotherapy; major allergens; methacholine provocation; reaginic antibodies.

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Pollen allergy is a suitable model for studies of *in vivo* and *in vitro* variables in specific immunotherapy (hyposensitization).

Patients with a *non-hyposensitized pollen allergy* demonstrate an increase during season in specific IgE in *serum* (SSiGE) which gradually decreases until the following season (1, 8, 12, 16, 17, 18, 31, 38). This also applies for total IgE (1, 16, 31). Seasonal IgE antibodies may correlate to non-seasonal values (12), and symptom scores show positive correlation to the SSiGE level (18). Cellbound specific IgE measured in histamine release from basophils (HR) seems to correlate to SSiGE (18, 34). Antigen-specific "blocking" IgG in serum (SSiGG) has no variation in season at very low (8, 12, 20, 24, 32, 35, 36) or non-detectable levels (10). In *nasal secretion* total IgE may show seasonal increase (38) or no specific IgE (NSiGE) (8). No change was detected in low specific IgG (NSiGG) (8, 29, 36).

During *hyposensitization* SSiGE increases (8, 11, 12, 15, 16, 17, 18, 31) in most patients within a few weeks, often with no further increase during the season, and, perhaps, a decrease. During the first months of treatment SSiGG correlates to SSiGE (12) or increases to a relatively higher level (3, 8, 11, 14, 15, 16, 18, 20, 24, 25, 32, 35). When persisting with treatment the SSiGE decreases gradually (3, 7, 8, 11, 12, 15, 16, 18, 25, 31). SSiGG stays at a high level (12, 18, 32) but may decrease in concentrations after years of treatment (11). SSiGG may reduce side effects

(6, 25) and has been positively correlated to dosage (21). In *nasal secretion* results range from no change (36) in NSiGE and NSiGG to minimal increase (8) in NSiGG, NSiGE, and NSiGA (nasal allergen-specific IgA), or a significant increase in NSiGA and NSiGG (26), though with no correlation to the clinical effect.

Studies of *lymphocyte activity* show a post-treatment reduction in lymphoblast transformation (3, 10, 23), mitogenic factor (10), and migration inhibitory factor (10), and an increase in T-suppressor cells (4).

Investigators mainly agree on the variability of SSiGE and SSiGG in hyposensitization, but they disagree on the relation to *the clinical effect*, as is the case in *in vivo* variables. Some correlate the clinical effect of hyposensitization to an increase in SSiGG (10, 20, 35), or to the relative SSiGG increase and pretreatment SSiGG/SSiGE ratio (9), or to the postseasonal SSiGG/SSiGE ratio (11). A correlation between HR and symptom scores in hyposensitization has been demonstrated (16, 20, 21, 32). Reduced HR gave less symptoms, but some patients recorded very few symptoms in high HR and others showed many symptoms despite a weak HR. Some investigators have demonstrated a reduced HR of unspecific character (19, 33), but no clinical correlation (25, 26).

To the authors' knowledge no publications exist on prospective controlled studies of the immunoparameters *after the end of hyposensitization*.

In a 3-year prospective double blind controlled study from January 1978 *in vivo* results during the first preseasonal and seasonal hyposensitization (27) present evidence that treatment with WPA (Alutard®SQ), a partially purified extract from timothy grass pollen, showed significantly less symptoms than treatment with PPA, consisting of the major allergens 19, 25. The clinical effect was significantly correlated to increased thresholds in skin prick tests, but not to nasal provocation tests or dosage. Corresponding *in vitro* results are published here.

MATERIAL AND METHOD

Forty patients, aged 15–43 years, with typical

Abbreviations:

HR = histamine release from basophils; SSiGE = serum-specific IgE; SSiGG = serum-specific IgG; NSiGE = nasal-specific IgE; NSiGG = nasal-specific IgG; NSiGA = nasal-specific IgA; WPA = whole pollen allergens: a partially purified timothy extract consisting of approx. 20 out of the approx. 30 timothy antigens, but all the potential allergens; PPA = partially purified allergens: two isolated timothy major allergens, Ag19 and Ag25; Group WPA = patients treated with WPA extract; Group PPA = patients treated with PPA extract; WPA₁/PPA₁ = analysis values before treatment for group WPA or PPA; WPA₂/PPA₂ = analysis values after 8 weeks' treatment for group WPA or PPA; WPA₃/PPA₃ = analysis values just before season for group WPA or PPA; WPA₄/PPA₄ = analysis values just after season for group WPA or PPA; Ag19⁺ = Ag19-responder: patient with high response of IgE to antigen No. 19 in crossed radioimmuno-electrophoresis (CRIE); Ag 19^o = Ag 19-non-responder.

hay fever with or without asthma for more than two seasons were randomly divided into two equal groups. Twenty patients (group WPA) were hyposensitized with WPA (Alutard®SQ), a partially purified extract from timothy grass pollen, and 20 patients (group PPA) with PPA, consisting of two isolated timothy major allergens, Ag's 19, 25. Both aluminiumhydroxide adsorbed extracts were biologically standardized before being coupled to aluminiumhydroxide. No release of allergenic material is detected within a period of 2 years according to the manufacturer. The design for the investigation, and the allergen extracts have recently been presented in detail (27). During season I no non-hyposensitized pollen allergic patients were examined, but only healthy controls with no sign of timothy antibodies in either serum or nasal secretion.

In vitro samples were collected just before treatment (January 1978), after 8 weeks' treatment, after 12 weeks' treatment (just before the season), and finally, after 30 weeks' treatment (just after the season). Serum and nasal secretion were stored at -20°C and examined parallelly: In *serum*: total IgE (PRIST), total specific IgE = SSiGE (A1-RAST) to WPA and PPA, specific IgE versus the individual allergens of timothy grass pollen (CRIE), and allergen-specific "blocking" IgG = SSiGG to WPA and PPA. In *nasal secretion*: total IgE (PRIST) and total specific IgE = NSiGE (A1-RAST).

WPA₁/PPA₁ means pretreatment analysis results for group WPA, and group PPA, respectively, whilst WPA₂/PPA₂ shows the values after 8 weeks' treatment. WPA₃/PPA₃ and WPA₄/PPA₄ represent the results just before, and just after season I, respectively.

Nasal secretion

In healthy controls 48 mg methacholine bromide was used to provoke a secretion of approximately 0.5 ml within 15 min for the evaluation of specific antibodies. In relation to the first grass pollen season each of the 40 patients were provoked four times at least 24 h before or after

nasal provocation with the allergen, using nasal provocation technique (27). Nasal secretion for 15 healthy controls was examined parallelly with the patients' nasal secretions.

For performance of PRIST and NSiGE analyses the nasal secretion was diluted 1:5 with 0.9% NaCl and the serum technique used. The results were multiplied by the dilution factor and proved reproducible. All the analyses were double controlled. Based on 20 double determinations the error of analysis was: nasal PRIST: s.d. 1.3 U/ml (9.1% of the mean), NSiGE to WPA: s.d. 11.5 SU (24.2% of the mean), and NSiGE to PPA: s.d. 9.5 SU (19.6% of the mean).

Total IgE (PRIST)

Total IgE, Phadebas PRIST® (Paper RIST), determinations (5) were performed according to the manufacturer's description. The patient serum was diluted 1:8 with IgE-free diluent in order to reduce inhibitory serum factors, and the results were later multiplied by the dilution factor. All the analyses were double controlled and the mean values were determined as results. Total IgE + 2 s.d. U/ml for adults is 263 U/ml in our laboratory.

Specific IgE (A1-RAST)

Allergen-specific IgE, SSiGE, and NSiGE were measured by A1-RAST according to Weeke (37). 200 μl allergen extract, WPA (5 mg/ml) or PPA (0.6 mg/ml), dissolved in 2 ml coupling-buffer (TRIS, HCl 0.1 M pH 7.5 with 1% (w/v) Tween 20 and 0.015 M NaN_3) were absorbed to 50 μl Alhydrogel® (Superfos, Vedbaek, Denmark) containing 2% (w/v) $\text{Al}(\text{OH})_3$ (equivalent to 1.3% (w/v) Al_2O_3).

The suspension was shaken gently for 15 min at room temperature and centrifuged at 3,000 rpm for 15 min. The supernatant was gently sucked away leaving about 0.2 ml on top of the precipitate. This washing procedure was repeated four times using 2.5 ml coupling-buffer. After washing, the precipitate was resolved in 5 ml coupling-buffer and was now ready for use. The quantity containing 100 μl of the well-mixed suspension was distributed in small plas-

tic tubes and 50 μ l serum sample or diluted (1:5) nasal secretion was applied and allowed to react for 24 h at room temperature.

A coupling-buffer (2.5 ml) was added, the tubes were centrifuged at 3,000 rpm for 3 min, followed by suction leaving 0.2 ml free supernatant. This was repeated three times.

After the fourth washing procedure all tubes were turned upside down for a few seconds in order to decant the free supernatant.

50 μ l ^{125} I-anti-IgE (Pharmacia, Phadebas®) was added to the tubes. After 24 h at room temperature 2.5 ml coupling-buffer was added. The mixture was centrifuged and washed as previously described. After the fourth washing procedure the last 0.2 ml free supernatant was left on the top of the precipitate.

Finally, the amount of specific labelled anti-IgE bound to the allergen at the Alhydrogel was counted (5 min). The results were expressed as a percentage of the total activity, or in sorbent units (SU), and in allergy classes (class 0, 1, 2, 3, and 4) corresponding to < 2 SU, < 4 SU, < 20 SU, < 100 SU, and ≥ 100 SU, respectively, using the serum from a timothy allergic patient (diluted 1 + 9 (w/v) with incubation buffer) as a 100 SU reference. This reference system gave the same counts as the reference serum from Phadebas RAST, Pharmacia, using birch pollen allergic serum and birch coupled to aluminium-hydroxide.

All analyses were double controlled and the results were set as the mean value. The error of analysis for the single allergen extracts ranged from 10 to 20% of relative deviation.

Allergen-specific IgG

Specific antibodies were measured by a double-antibody assay according to Prahl et al. (30). 100 μ l serum diluted 1:40 in test-buffer (sodium borate 55 mmol/ml, NaCl 100 mmol/l BSA 1%, NaN₃ 0.1%, pH 8.0) and 100 μ l ^{125} I-labelled WPA (5 ng/100 μ l) or PPA (2.5 ng/100 μ l) were mixed at room temperature. 100 μ l ^{125} I represented approximately 200,000 cpm. After 3 h, 100 μ l rabbit antihuman IgG diluted 1:2 was added and the samples left overnight at 4°C. The precipitates were washed four times with

2.5 ml test-buffer by centrifugation for 15 min at 3,000 g. The activity of the precipitates was measured in a gamma counter and expressed in percentage of moderately positive standard serum samples. All cpm values were corrected for background, i.e. the amount of cpm precipitated by standard serum pool.

Crossed immunoelectrophoresis (CIE)

Crossed immunoelectrophoresis was carried out for performance of CRIE (see below) as described in detail elsewhere (22). Antigens: 5 μ l WPA, 5 mg/ml. Antibodies: 12.5 μ l anti-5-grass/cm². The antibodies were obtained by immunization of three rabbits with equal amounts of pollen extract from *Phleum pratense*, *Dactylis glomerata*, *Lolium perenne*, *Festuca pratensis*, and *Holcus lanatus*. The immunization, bleeding, and subsequent purification were performed according to Harboe & Ingild (13). Precipitates corresponding to individual allergens were identified by means of isolated antigens: Ag's 3, 19, 25, and 30, using crossed-line immunoelectrophoresis and different mixtures of anti-5-grass mix and timothy antibodies (22).

Crossed radioimmunoelectrophoresis (CRIE)

Crossed radioimmunoelectrophoresis was performed as an ordinary crossed immunoelectrophoresis together with subsequent binding of allergic patients' specific IgE to the native crossed immunoprecipitates (22). For 7×5 cm² plates the amounts and the procedure (at room temperature, 18–24°C) were as follows: 1) incubation with patient serum: 7 ml of 1/15 M phosphate buffer (pH 7.5) and 0.7 ml of serum added to the plastic box was sufficient to cover the gel film on the glass plate; 2) after being left to react overnight, the non-bound serum proteins were removed from the gel by washing with 10–20 ml of 0.1 M sodium chloride at least four times for 10-min periods; 3) ^{125} I-anti-IgE in 7 ml incubation-buffer (0.05 M phosphate buffer, pH 7.5, containing 0.3% (w/v) bovine serum albumin, 0.9% (w/v) sodium chloride, 0.1% (w/v) sodium azide, and 1% (w/v) EDTA), corresponding to 0.35 μ Ci and giving rise to ap-

Table 1

Normalized decay-corrected, reciprocal exposure time necessary for visible radiostaining of various CRIE precipitates

Autoradiography	Disintegration factor	Normalized values of
days (d)	(f)	$\frac{1}{d \times f}$
1	0.995	10.8
3	0.975	3.7
12	0.890	1

Table 2

Relative intensity of the radiostaining by comparing with the intensity exerted by 5 µl drops of ¹²⁵I-anti-IgE representing 1,000, 500, 250, 125, 67.5 cpm

Counts/min	Intensity factor
1000	16
500	8
250	4
125	2
67.5	1

Table 3

CRIE units calculated from the values of Table 1 (normalized exposure time) and Table 2 (intensity factor)

Normalized values of	Intensity factor				
$\frac{1}{d \times f}$	16	8	4	2	1
~ 11	170	85	45	20	10
~ 4	60	30	15	8	4
~ 1	16	8	4	2	1

proximately 200,000 cpm in a gamma counter, was added to the gel and allowed to react for at least 1 day; 4) after four 10-min washing with 10–20 ml saline and one final washing with distilled water, the gel on the glass plate was dried in hot air and placed on X-ray film in a lightproof box. The exposure time chosen

was 1, 3, and 12 days, and finally, 5) the gel on the glass plate was stained for proteins with Coomassie Brilliant Blue.

The evaluation was performed by measuring 1) the normalized decay-corrected, reciprocal exposure time necessary for visible radiostaining of the various precipitates (Table 1) and 2) the relative intensity of the radiostaining by comparing with the intensity exerted by 5 µl drops of ¹²⁵I-anti-IgE representing 1,000, 500, 250, 125, 67.5 cpm (Table 2). All CRIE units were run simultaneously using the same pool of ¹²⁵I-anti-IgE. CRIE units were calculated from the values of Tables 1 and 2, as shown in Table 3. According to the results of CRIE performed with healthy controls, only the CRIE units for Ag's 3 and 30 had to be reduced with value 1 (22). In the following CRIE scores > 10 will be considered as "high IgE binding". Corrections for possible co-precipitations (22) were not possible. CRIE was evaluated double blinded by one of the authors (H.L.).

Statistics

Unpaired Rank Sum Tests, Mann-Whitney or Kruskal-Wallis, Spearmann's correlation analysis and Fourfold Table Test were employed, using two-tailed tests with 5% significance level.

RESULTS

All patients fulfilled the investigation. Only 2/160 nasal secretions were lost. Patients treated with WPA (group WPA) and with PPA (group PPA) were comparable in relation to age, sex, associated asthma, accumulated dosage, local and general side effects, and in pretreatment values for skin prick tests and nasal provocation tests. The pretreatment values for *in vitro* variables were also comparable, as shown below.

Methacholine nasal provocation

The medians for nasal secretion before hypersensitization were 0.6 ml (range 0.1–1.6 ml) and just before season I, 0.5 ml (range 0.1–1.4 ml). The reactivity was equal to that of healthy controls. Some patients, especially the females,

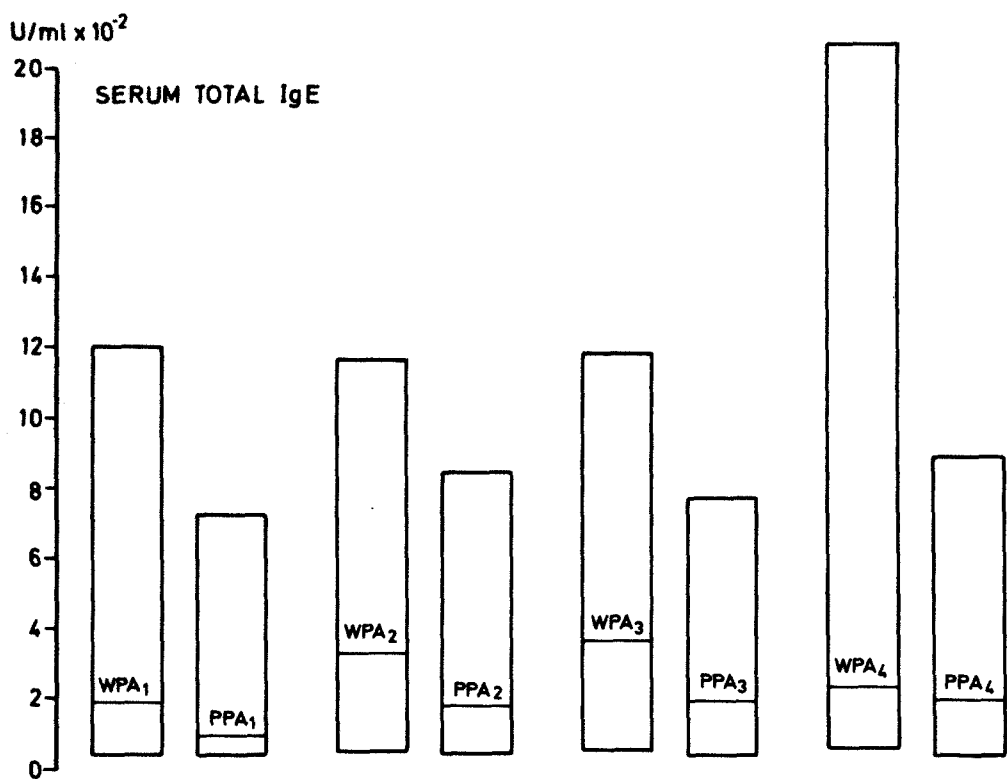


Fig. 1. Total IgE (PRIST) in serum for groups WPA and PPA. Boxes show medians and 10–90% percentiles. WPA₁/PPA₁, pretreatment analyses. WPA₂/PPA₂, values after 8 weeks' treatment. WPA₃/PPA₃ and WPA₄/PPA₄, results just before, and just after season I, respectively.

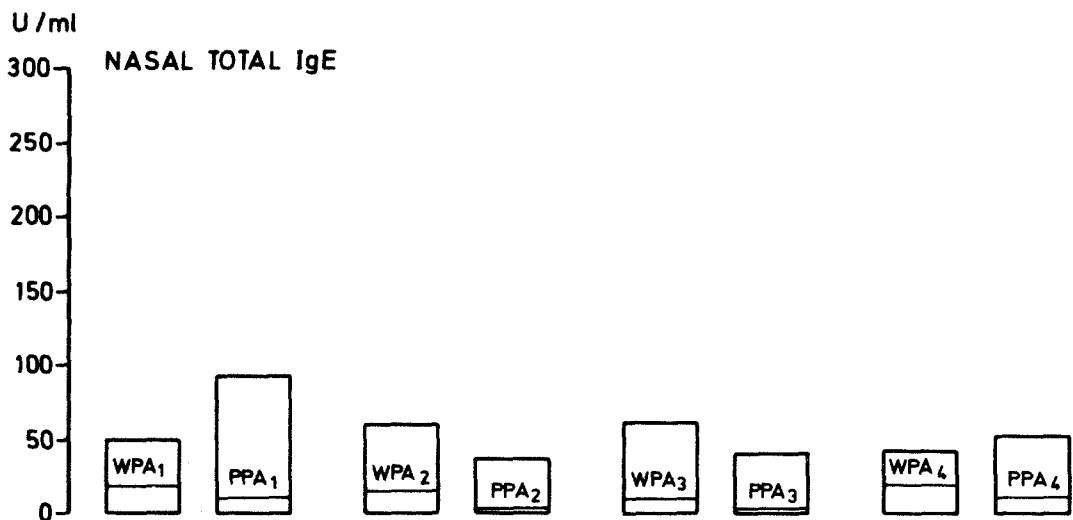


Fig. 2. Total IgE (PRIST) in nasal secretion for groups WPA and PPA. (For explanation, see Fig. 1).

showed momentary flushing, but no asthma. Only six patients were provoked twice to achieve minimum nasal secretion of 0.25 ml for the analysis. As measured by methacholine test, no decrease in nasal reactivity was detected in relation to season I.

Total IgE (PRIST)

Figs. 1 and 2 illustrate total IgE values in serum and nasal secretion.

In *serum* pretreatment total IgE > 263 U/ml was detected in 11/40 patients. In WPA₂ 17/20

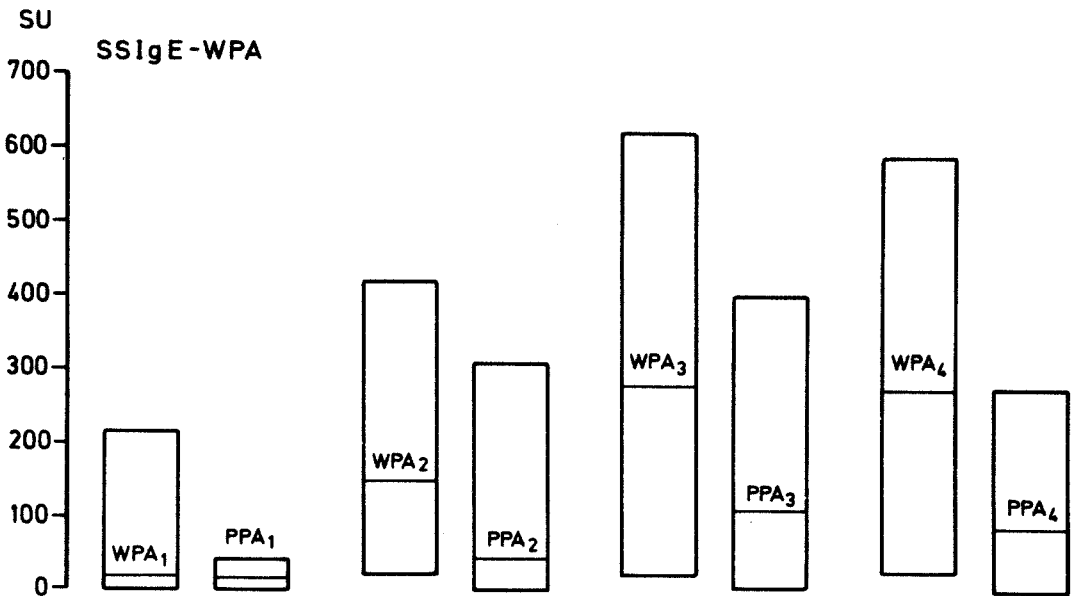


Fig. 3. Total specific IgE in serum with WPA antigen (SS IgE-WPA) in sorbent units (SU). (For explanation, see Fig. 1).

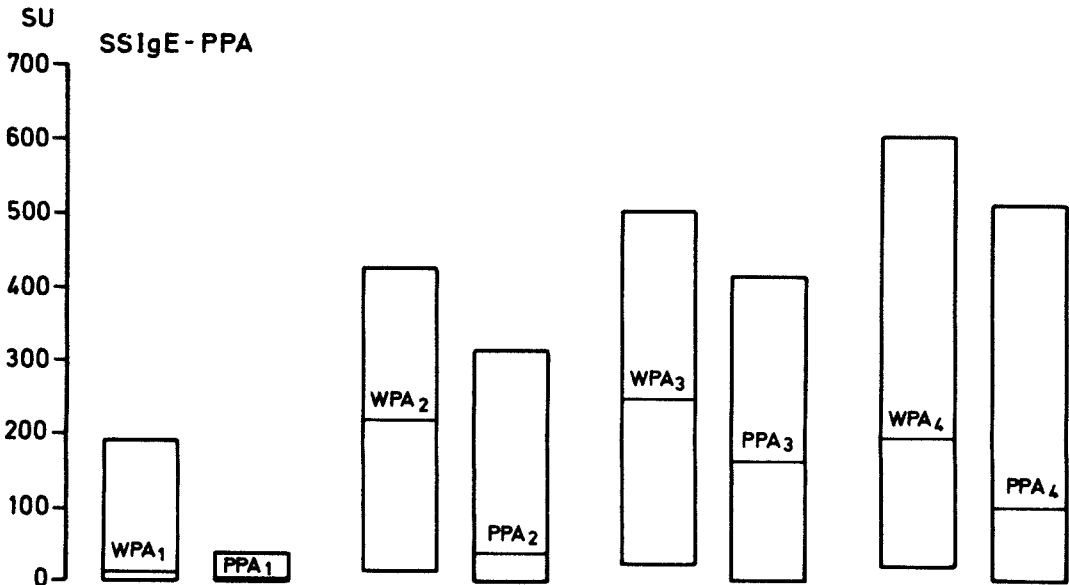


Fig. 4. Total specific IgE in serum with PPA antigen (SS IgE-PPA) in sorbent units (SU). (For explanation, see Fig. 1).

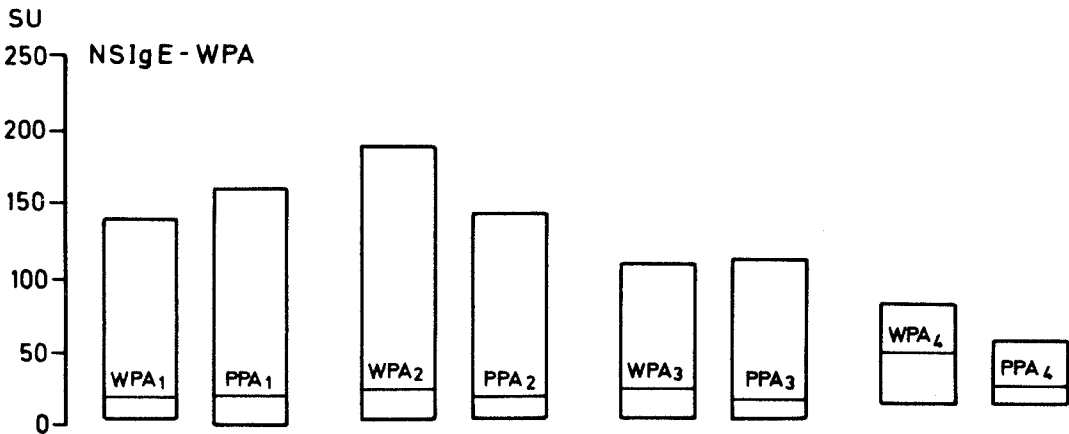


Fig. 5. Total specific IgE in nasal secretion with WPA antigen (NS IgE-WPA) in sorbent units (SU). (For explanation, see Fig. 1).

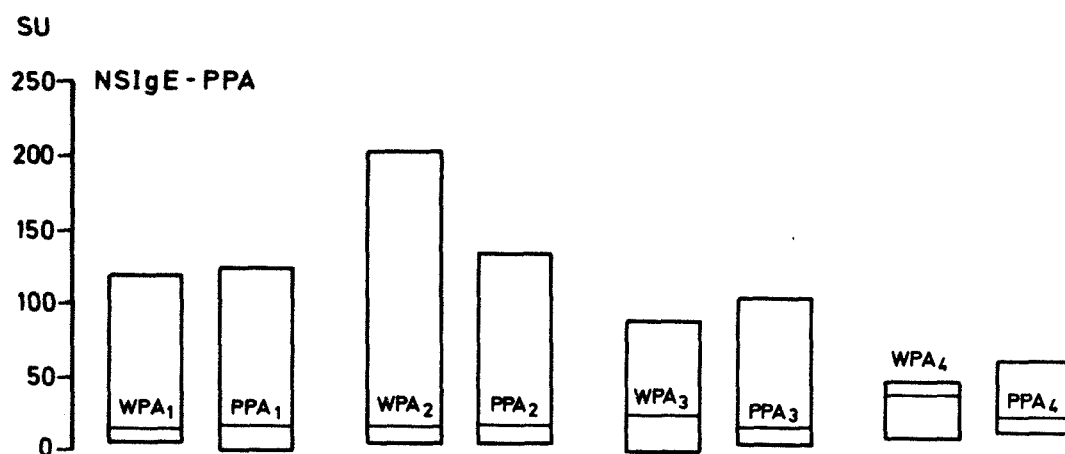


Fig. 6. Total specific IgE in nasal secretion with PPA antigen (NSIgE-PPA) in sorbent units (SU). (For explanation, see Fig. 1).

patients showed significant increase compared with 14/20 in PPA₂, with no significant difference between the groups. Total IgE increased until the season, and there was no further increase or decrease during the season.

Symptom scores showed no correlation to total IgE increase, either relative or absolute, or to change in total IgE during season.

In *nasal* secretion 30/40 patients showed pretreatment total IgE > 5 U/ml, median 14 U/ml (range 0–160 U/ml). Pretreatment nasal total IgE was not correlated to serum total IgE. No increase after hyposensitization was demonstrated. Fifteen healthy controls had total IgE < 5 U/ml in nasal secretion.

Specific IgE (A1-RAST)

Figs. 3–6 illustrate SSiGE and NSIgE for both groups for each A1-RAST allergen, WPA and PPA.

In *serum* 30/40 patients showed positive pretreatment values $\geq$ allergy class 2; 19/30 patients $\geq$ allergy class 3, of these 10/19 belong to group WPA. Both groups showed equal pretreatment medians, but WPA₁ had a higher range than PPA₁. A significant increase was observed in both groups until the season, but there was a significantly higher increase for group WPA than PPA. During the season there was no change in either group. Already in WPA₂ 20/20

patients showed an increase compared with 17/20 in PPA₂. At all dosage levels there was demonstrated SSiGE high- and low-responders, and, therefore, SSiGE increase was not correlated to dose. No correlation was found between individual symptom scores and relative or absolute SSiGE increase before the season, or SSiGE change during the season. SSiGE-WPA and SSiGE-PPA were significantly intercorrelated before and during hyposensitization. Though there were equal parts of major allergens 19, 25 in WPA and PPA, group WPA had a higher SSiGE-PPA response than group PPA. During hyposensitization SSiGE was correlated to SSiG (Fig. 10). (For SSiG/SSiGE ratio and SSiGE high- and low-responders, see below).

In *nasal* secretion 38/40 patients showed positive pretreatment values $\geq$ allergy class 2, 23/38 $\geq$ allergy class 3, 12/23 in group WPA. Pretreatment NSIgE was not correlated to SSiGE. No certain preseason NSIgE increase was found during hyposensitization, but during the season a slight increase was recorded in both groups, and especially in group WPA, although no positive or negative correlation to symptom scores was found. Fifteen healthy controls had no NSIgE.

Allergen-specific IgG

Figs. 7 and 8 illustrate serum “blocking” SSiG.

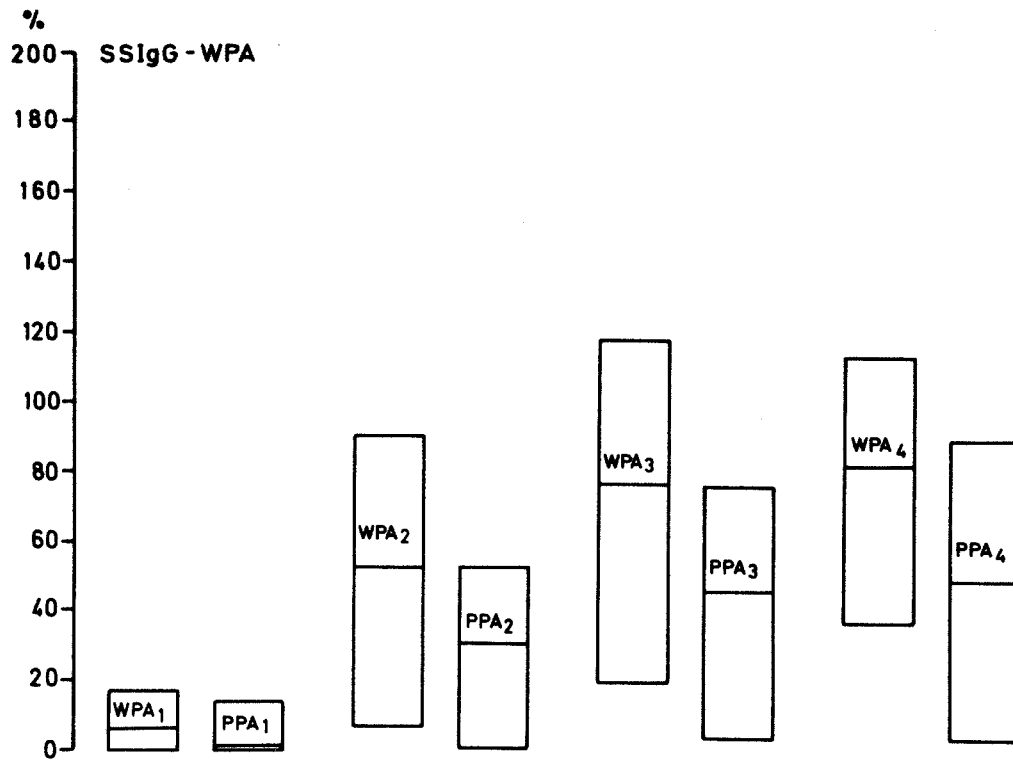


Fig. 7. Allergen-specific IgG "blocking" serum antibodies with WPA antigen (SSIgG-WPA) in per cent of reference positive standard serum. (For explanation, see Fig. 1).

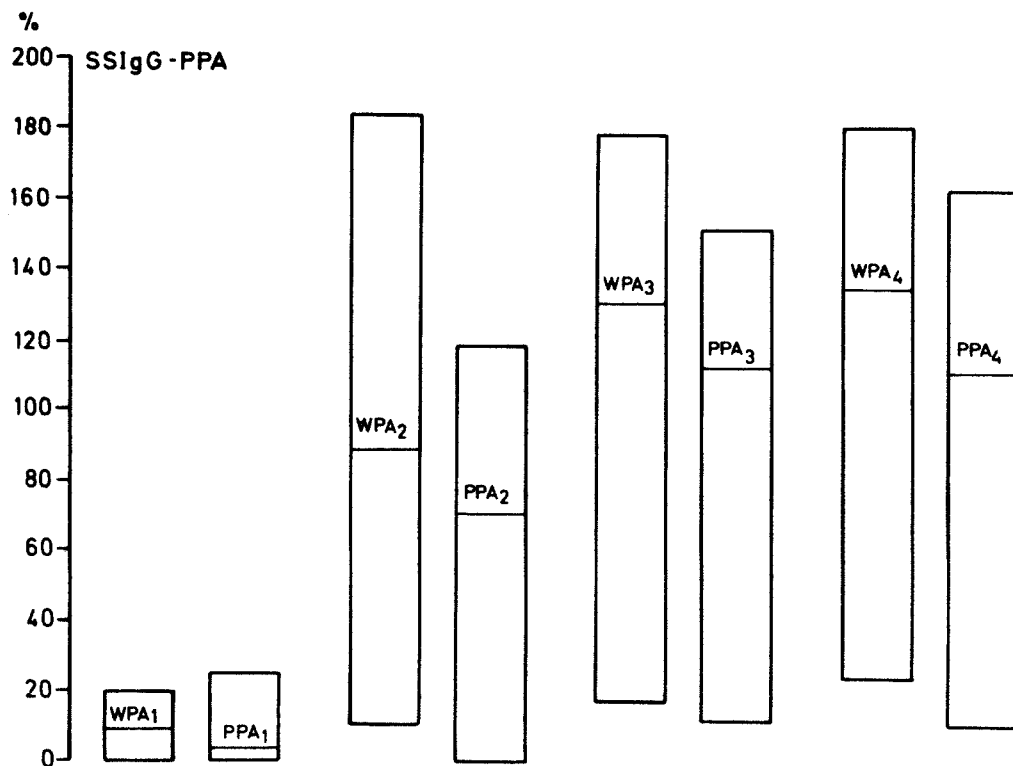


Fig. 8. Allergen-specific IgG "blocking" serum antibodies with PPA antigen (SSIgG-PPA) in per cent of reference positive standard serum. (For explanation, see Fig. 1).

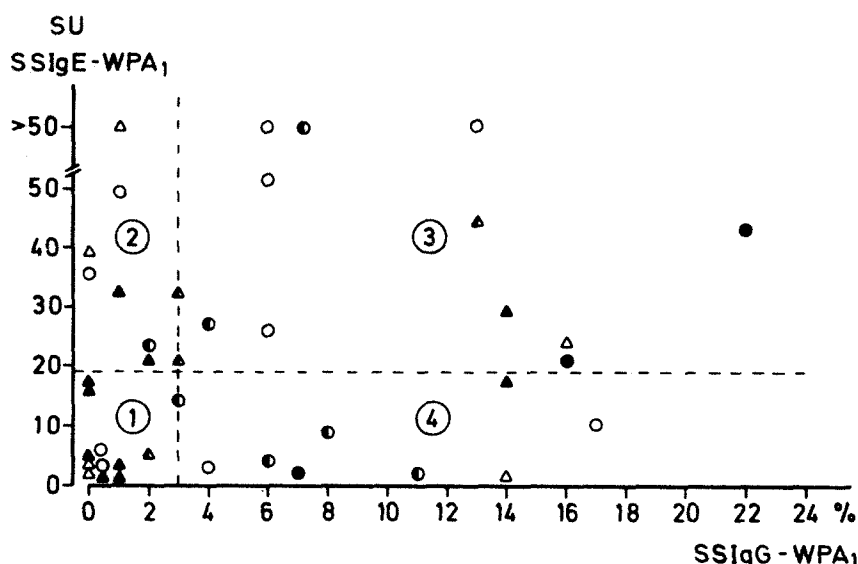


Fig. 9. Allergen-specific serum IgG against WPA (SSiG-WPA) in per cent of a moderate positive standard serum and specific serum IgE against WPA (SSiE-WPA) in sorbent units (SU) before (WPA₁) hyposensitization just before season I. Stippled lines show medians for SSiG and SSiE for groups WPA and PPA. Thus patients are divided into four subgroups: 1) SSiG and SSiE low-responders, 2) SSiE high-responders but SSiG low-responders, 3) SSiG and SSiE high-responders, and 4) SSiG high-responders and SSiE low-responders. 2) + 3) correspond to SSiE high-responders, 1) + 4) to SSiE low-responders, 3) + 4) to SSiG high-responders, and 1) + 2) to SSiG low-responders. SSiE was not correlated to SSiG, only during hyposensitization (Fig. 10). Group WPA (circles) and group PPA (triangles) symptoms: ○-△, mild or none (score ≤ 113); ◐-◑, moderate score ≤ 218; ●-▲, severe (score > 218). (For symptom scores, see ref. (27)).

In both groups the pretreatment values were low but could be demonstrated (Fig. 9) in most patients. SSiG-WPA was significantly correlated to SSiG-PPA before and during hyposensitization. During treatment in both groups SSiG showed a rapid increase significantly correlated to SSiE (Fig. 10) and a significantly higher increase for group WPA than PPA.

In WPA₂ 18/20 patients showed increase compared with 17/20 in PPA₂. Like in the SSiE analyses, the major increase was seen in group WPA and with no correlation to accumulated dosage due to SSiG high- and low-responders. Neither the relative nor absolute increase in SSiG was correlated to individual symptom scores. (For SSiG/SSiE ratio and SSiG high- and low-responders, see below). No analysis of NSiG was carried out. Fifteen healthy controls had SSiG equal to a standard serum pool.

SSiG/SSiE ratio

Figs. 9 and 10 show SSiG-WPA and SSiE-WPA for WPA₁ and WPA₃. The stippled lines indicate the medians for SSiG and SSiE for groups WPA and PPA. Thus the patients are divided into four subgroups (see text Fig. 9). Relations between the subgroups are shown in Tables 4 and 5.

Table 4 and Fig. 9 show the subgroup relations before hyposensitization. SSiE-WPA₁ was not correlated to SSiG-WPA₁. No correlation was demonstrated between SSiG/SSiE ratio and symptom scores, or between the subgroups and frequencies of asthma, major general side effects (urticaria, angioedema, asthma), or local side effects. However, it was significant that the SSiG low-responders were mostly females (group 1 + 2), whereas the SSiG +

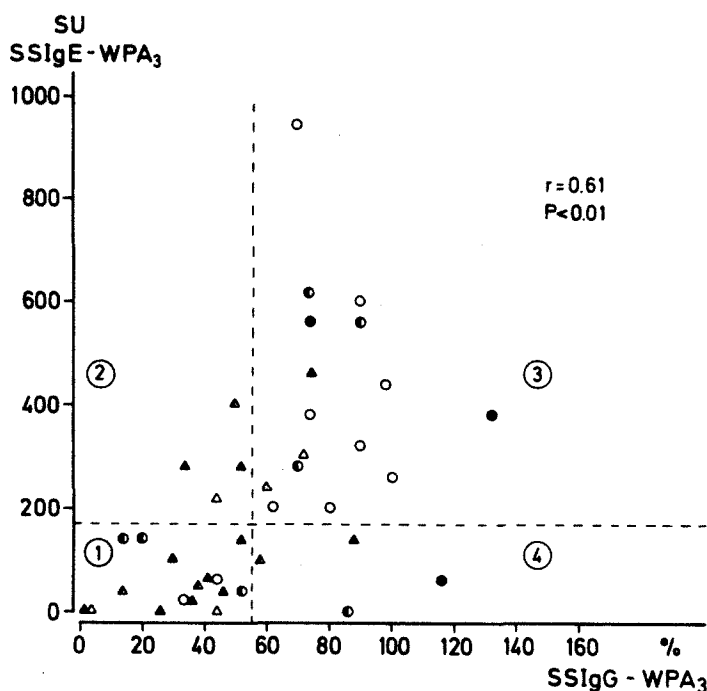


Fig. 10. Allergen-specific serum IgG against WPA (SSiG-WPA) in per cent of a moderate positive standard serum and specific serum IgE against WPA (SSiGE-WPA) in sorbent units (SU) during (WPA₃) hyposensitization just before season I. Stippled lines show medians for SSiG and SSiGE for groups WPA and PPA. Thus patients are divided into four subgroups (see Fig. 9). SSiGE was significantly correlated to SSiG, but only during hyposensitization. Group WPA (circles) and group PPA (triangles) symptoms: ○-△, mild or none (score ≤ 113); ●-▲, moderate (score ≤ 218); ●-▲, severe score > 218). (For symptom scores, see ref. (27)).

SSiGE high-responders (group 3) were usually youngest individuals with the shortest duration of symptoms before treatment.

Table 5 and Fig. 10 show the subgroup relations during hyposensitization. As SSiGE-WPA₃ was now significantly correlated to SSiG-WPA₃, only few patients were placed in groups 2 and 4, and because SSiG/SSiGE ratio was almost equal in groups 1 and 3, ratios were of no use for correlation analyses. As in prehyposensitization, nearly all females were in group 1, and group 3 was dominated by the youngest individuals and shortest pretreatment duration of symptoms. No difference was demonstrated in dosage or side effects between the subgroups. A tendency towards lower symptom scores was seen in group 3 because of highest SSiG and SSiGE response in group WPA, but for the individual groups, WPA and PPA, symptom

scores were equally distributed in groups 1 and 3.

Crossed radioimmuno-electrophoresis (CRIE)

The accumulated CRIE units for the IgE binding to individual allergens are shown in Table 6. Antigens 25 a, b, c, and v are considered as one group (Ag 25) partly because all the precipitates were absorbed *in situ* by isolated Ag 25, and partly because the IgE binding to these precipitates always correlated. As WPA₂/PPA₂ mainly corresponds to WPA₃/PPA₃, it has been excluded, whereas WPA₄/PPA₄ has been included to evaluate the influence of the season.

Before treatment groups WPA and PPA were comparable without significant difference in individual IgE binding. The accumulated IgE binding towards Ag's 27, 15, and 19 was lower in

Table 4

SSIgG/SSiGE relations before (Fig. 9) hyposensitization in the subgroups 1–4 and SSiGE or SSiG high- and low-responders

Groups	No. patients								Duration of hay fever median years (range)	Age median years (range)
	Total	Females	≤ 113	≤ 218	> 218	Asthma	MGS	LS		
1	11	8	4	1	6	2	3	9	8.0 (2–19)	26.0 (19–38)
2	7	2	4	1	2	2	2	4	11.0 (6–21)	23.0 (15–35)
3	11	2	5	3	3	3	5	9	6.0 (3–18)	21.0 (15–27)
4	8	1	3	3	2	2	2	5	13.5 (6–20)	24.0 (16–36)
SSiGE-high	20	5	9	6	5	5	8	13	7.5 (3–21)	21.0 (15–35)
SSiGE-low	20	9	7	5	8	5	5	14	9.0 (2–20)	26.0 (16–38)
SSiG-high	19	3	8	6	5	5	7	14	10.0 (3–20)	23.0 (15–36)
SSiG-low	18	10	8	2	8	4	5	13	9.0 (2–21)	24.5 (15–38)

≤ 113 scores = mild or no symptoms.
≤ 218 scores = moderate symptoms.
> 218 scores = severe symptoms.
(For symptom scores, see ref. (27)).
MGS = major general side effects (urticaria, angioedema, asthma).
LS = local side effects.
(For subgroups, see text Fig. 9).

group PPA than in WPA. The patients showed individual variations in their pretreatment IgE binding, but specific IgE binding to the major allergens 15, 19, 25, and 30 dominated in both groups. Patients previous to treatment showed different antigenic responses (allergoprints) to the four major allergens, Ag's 15, 19, 25, and 30. High IgE binding was exerted to only one allergen by 12 (30%) patients (Ag 15 (3), Ag 25 (9)); towards two allergens by 11 (27.5%) patients (Ag's 15 and 25 (7), Ag's 15 and 30 (1), Ag's 25 and 30 (3)); and to three allergens by five (12.5%) patients (Ag's 15, 19, and 25 (2), Ag's 15, 25, and 30 (3)).

In most cases Ag's 15 and 25 showed high pre-treatment values, whereas the values for Ag 30 were low, and Ag 19 was characterized by very low pretreatment values. A high qualitative variation in reactions to the less frequent allergens (minor allergens) was recorded.

An evaluation in relation to grass pollen sea-

son I revealed no tendency beyond error of the analysis (see Material and Methods) to develop specific IgE towards new timothy antigens.

The patients showed specific reaction to stimulation. Group WPA reacted to the major allergens 15, 19, 25, and 30 (Fig. 11). Group PPA only reacted to Ag's 19 and 25 stimuli, whereas the CRiE units for Ag's 15 and 30 decreased until the season, but otherwise showed an increase in specific IgE due to normal exposition during the season (Fig. 11).

Like total IgE and SSiGE, the accumulated CRiE units increased up to the season and decreased during the season, but there was no significant difference between the two groups.

Ag 19 compared with the other timothy antigens was characterized by very low IgE binding in CRiE before hyposensitization and unchanged or high IgE binding during hyposensitization, dividing patients into specific IgE Ag 19-responders (Ag 19⁺) and Ag 19-non-respon-

Table 5

SSIgG/SSiGE relations during (Fig. 10) hyposensitization in the subgroups 1–4 and SSiGE or SSiGG high- and low-responders

Groups	No. patients								Duration of hay fever median years (range)	Age median years (range)
	Total	Females	≤ 113	≤ 218	> 218	Asthma	MGS	LS		
1	16	10	5	4	7	2	4	10	8.3 (2–21)	25.5 (15–38)
2	4	1	1	1	2	2	2	2	11.5 (6–13)	26.0 (21–27)
3	16	2	10	3	3	4	4	14	7.5 (3–20)	23.0 (15–43)
4	4	1	0	3	1	2	3	2	14.0 (10–19)	23.0 (15–24)
SSiGE-high	20	3	11	4	5	6	6	16	8.5 (3–20)	23.5 (15–43)
SSiGE-low	20	11	5	7	8	4	7	12	10.5 (2–21)	24.0 (15–38)
SSiGG-high	20	3	10	6	4	6	7	16	9.5 (3–20)	23.0 (15–43)
SSiGG-low	20	11	6	5	9	4	6	12	9.5 (2–21)	26.0 (15–38)

≤ 113 scores = mild or no symptoms.

≤ 218 scores = moderate symptoms.

> 218 scores = severe symptoms.

(For symptom scores, see ref. (27)).

MGS = major general side effects (urticaria, angioedema, asthma).

LS = local side effects.

(For subgroups, see text Fig. 10).

ders (Ag 19°). Sixteen patients were Ag 19+, seven in group WPA and nine in group PPA.

Surprisingly, a high response to Ag 19 could predict low symptom scores in the grass pollen season. All Ag 19+ but one in each group were already recorded in WPA₂/PPA₂ values, i.e. after 8 weeks' treatment. Fig. 12 illustrates symptoms for Ag 19+ and Ag 19°. Almost all Ag 19+ showed good clinical response with symptom scores below 218 in both groups and fewer symptoms than Ag 19° (27). The difference in symptoms between Ag 19+ and Ag 19° was only significant ($P < 0.05$) for group PPA, but most patients in group WPA generally showed excellent clinical response and were treated with several antigens other than Ag's 19, 25. Ag 15+, Ag 25+, and Ag 30+ showed higher symptom scores than Ag 19+.

DISCUSSION

As in most previous investigations, this study demonstrated a high preseasonal increase in SSiGE and SSiGG, but no further increase or decrease during the season. The highest increase in both SSiGE and SSiGG against PPA was recorded in group WPA. This confirms the tendency of the *in vivo* results: PPA has a more stimulating effect when combined with the other allergens in WPA. Partial identity between Ag's 19, 25, and non-Ag's 19, 25 in WPA could be the reason for this enhanced antigen effect, but the CRIE results with specific reaction to stimulation together with previous immunochemical studies (22) did not indicate the presence of a partial identity. Though the groups demonstrated a significant difference in SSiGE and SSiGG responses none of these parameters were correlated to individual symptom scores, associated asthma, or side effects.

Table 6

CRIE values for the individual timothy allergens in both groups in relation to WPA₁/PPA₁ (before treatment), WPA₃/PPA₃ (just before season), and WPA₄/PPA₄ (just after season)

	Antigen Nos. in CRIE												
	1	3	7	13	15	16	19	25a	25b	25c	25v	27	30
WPA-1 ^a units	32	2	10	16	261	0	70	124	265	235	169	139	111
-3	49	2	12	16	405	0	233	278	439	310	270	238	208
-4	44	2	9	18	346	0	181	254	399	356	282	161	140
WPA-1 ^b No. pos.	7	2	9	2	16	0	14	16	16	16	15	9	18
-3	9	1	11	2	16	0	18	17	17	17	17	10	16
-4	10	0	9	4	16	0	16	16	17	17	18	10	18
WPA-1 ^c No. > 10	0	0	0	1	9	0	2	4	11	11	5	5	5
-3	0	0	0	1	13	0	8	11	14	13	10	7	9
-4	1	0	0	1	11	0	8	10	16	15	12	5	5
PPA-1 ^a units	21	4	6	0	183	0	45	113	252	231	145	25	91
-3	22	3	12	2	148	10	163	279	402	350	217	62	104
-4	32	8	9	1	211	8	132	199	383	324	199	52	139
PPA-1 ^b No. pos.	6	1	6	0	12	0	11	15	15	14	13	3	17
-3	8	3	7	2	14	2	14	14	16	16	17	4	18
-4	6	6	8	1	17	1	16	15	15	16	16	7	19
PPA-1 ^c No. > 10	0	0	0	0	7	0	0	3	13	9	6	1	2
-3	0	0	0	0	8	0	9	10	14	13	11	3	3
-4	1	0	0	0	8	0	7	7	14	13	4	2	3

^a Accumulated CRIE units for IgE binding to individual allergens.
^b Number of patients with positive CRIE.
^c Number of patients with high IgE binding, i.e. > 10 CRIE units.

SSiGE was well correlated to skin prick tests and nasal provocation tests, but only during pre-treatment. SSiGE was correlated to SSiGG, but only during hyposensitization, as previously shown by Gleich et al. (12).

SSiGE or SSiGG was not correlated to dosage, possibly due to an almost maximal increase recorded already after 8 weeks (WPA₂/PPA₂). Frequent recordings between WPA₁/PPA₁ and WPA₂/PPA₂ may demonstrate a relation to dosage but at comparable dosage the patients were seen to be divided in to SSiGE and SSiGG high- and low-responders and among these almost all females were low-responders.

SSiGG/SSiGE ratio showed no correlation to symptom scores either before or during hypo-sensitization. Group 1 comprised mostly females, both before and after treatment, and as

no difference in dosage was demonstrated in groups 1 and 3, females appear to give a low immunoresponse to both SSiGG and SSiGE in grass pollen allergy. Despite a low response to SSiGG and SSiGE in group 1 and a high response in group 3, the number of major general and local side effects was equal in both groups. Thus the low-responders (group 1) may represent patients (mostly females) with relatively high IgE cellbinding or increased intracellular reactivity of the mast cells or basophils. HR analyses were not used until the following seasons.

Nasal total IgE and NSiGE showed no response to hyposensitization, but a slight increase due to normal exposition during the season. Most patients had positive nasal total IgE and NSiGE. The bronchial provocation test with methacholine is a well-accepted method for

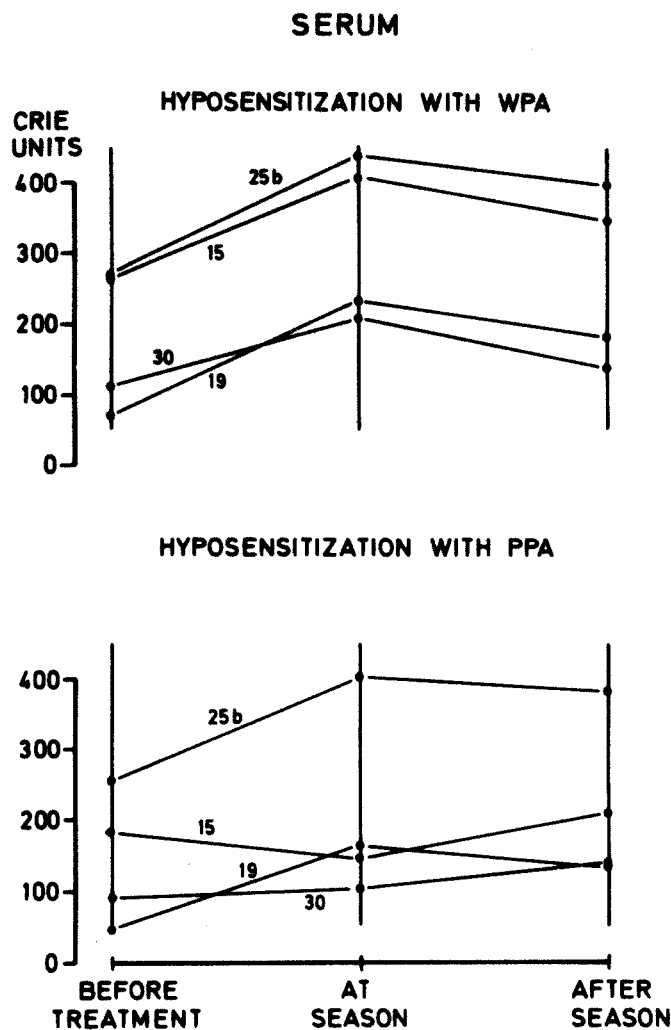


Fig. 11. Accumulated CRIE units in serum for specific IgE binding to the four major allergens 15, 19, 25, and 30 in the two groups, WPA and PPA, before treatment, just before season I, and just after season I. During hyposensitization patients showed specific reaction to stimulation and a decrease in specific IgE binding in season compared with seasonal increase in non-hyposensitized patients (Ag's 15 and 30 in group PPA).

characterizing bronchial reactivity. In a recent publication (2) on nasal reactivity applying this method a linear increase in nasal secretion was demonstrated when increasing the amount of methacholine. The methacholine nasal provocation test is well suited to provoke nasal secretion for further analysis. No decrease in nasal reactivity to methacholine was noted after hyposensitization during the first 4–5 months.

As previously shown (27), the skin prick thresholds significantly increased during hyposensitization despite increasing SSIgE. This may be due to SSIgG "blocking" antibodies, but local side effects were not correlated to SSIgG levels in this study, and increased thresholds in skin prick tests, but not SSIgG, were correlated to

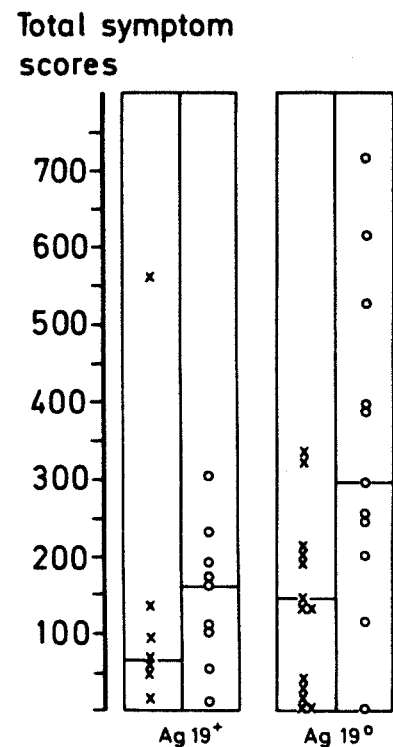


Fig. 12. IgE binding to Ag 19 in group WPA (x) and PPA (o) during initial hyposensitization as a marker for prospective evaluation of clinical effect (symptom scores). Ag 19⁺: patients with high IgE response of IgE to Ag 19 or Ag 19-responders. Ag 19⁰: Ag 19-non-responders.

symptom scores, so other cellular mechanisms could be involved.

CRIE has not previously been followed up during hyposensitization. This study is the first one to demonstrate that patient-specific IgE response only correlates with exactly those allergens which are used for treatment, and confirms (Fig. 11) a decrease in SSIgE in hyposensitization during season compared with an increase in non-hyposensitized patients (Ag's 15 and 30 in group PPA).

In relation to season I CRIE showed no development of allergy against new timothy antigens. If the following seasons reveal no new timothy allergens during hyposensitization, groups of major allergens approximately matching the pretreatment allergoprints may compromise specific treatment and antigenic competition, instead of the highly purified and expensive mono- and dicomponent specific antigens.

No patients in group PPA had high IgE binding against Ag 19 before treatment. Seven patients in group PPA had high IgE binding against Ag 25 before treatment, but their symp-

toms during the season were equal to the rest of group PPA. Six of the seven Ag 25-patients had low IgE binding against minor allergens before treatment, therefore, only one patient in group PPA received individual therapy. This patient was almost symptom free.

Hyposensitization with a partially purified allergen extract seems superior to isolated major allergens although it can not be excluded that an even higher efficacy could be obtained by using extracts matching patients' antigen-specific IgE.

High response of IgE to Ag 19, but not the pretreatment IgE level to Ag 19, was very suitable as a marker for clinical effect in this trial as both patient groups were stimulated with Ag 19, and Ag 19-response was easy to visualize by a heavy chimney-like radiostaining in this "all-or-none" reaction. All Ag 19⁺ but one were Ag 25⁺ but nine patients were Ag 25⁺/Ag 19⁰. The fact that Ag 25⁺ generally showed higher symptom scores than Ag 19⁺ seems to be the reason why SSiGE-PPA values were unreliable as markers for clinical effect. Further investigations about Ag 19 as a marker for effect of hyposensitization in grass pollen allergy are in progress.

Results from seasons II and III, including non-hyposensitized hay fever patients and stability of the allergenic extracts, will be published elsewhere (28).

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