

Immunotherapy with Grass Pollen Major Allergens

Immunological Results from a Prospective 3-Year Double Blind Study

O. ØSTERBALLE, H. LØWENSTEIN, S. NORN, P. PRAHL, P. SKOV, P. S. SKOV and B. WEEKE

Medical Dept. TA, Laboratory for Medical Allergology, and Paediatric Dept. G, Rigshospitalet, Copenhagen, Pharmacological Institute and Protein Laboratory, University of Copenhagen, Denmark

Preseasonal hyposensitization stimulated an intercorrelated increase in both serum-specific IgE and allergen-specific IgG. Subsequent perennial treatment depressed the stimulated IgE response and the basophil cell sensitivity, whereas the allergen-specific IgG response showed further increase and persisted at a high level. Nasal IgE response was stimulated from the second pollen season and subsequently became depressed. One year after the end of hyposensitization the allergen-specific IgG response had fallen by 25–50%.

Key words: allergen extracts; biological units; blocking antibodies; grass pollen; hay fever; histamine release from basophils; immunotherapy; major allergens; reaginic antibodies.

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CLINICAL ASPECTS

Perennial hyposensitization depressed the stimulated IgE response and basophil cell sensitivity, corresponding to a higher degree of clinical protection from the second – than during the first grass pollen season. The group of patients treated with partially purified timothy extract showed a statistically significantly higher degree of clinical protection and greater increase in allergen-specific IgG than the group treated with two timothy major allergens 19, 25. However, within the groups the individual IgG levels did not correlate with symptom scores.

That specific immunotherapy (hyposensitization) with partially purified timothy extract gives higher clinical protection than with two timothy major allergens has been demonstrated in a 3-year double blind study of 40 patients (19, 21). Fourteen non-hyposensitized controls

participated during the second pollen season and showed more symptoms than the treated groups.

This paper deals with immunological data from perennial hyposensitization and 1 year after end of treatment compared to clinical results (19, 21), tissue sensitivity (22), and initially preseasonal data (20). CRIE (crossed radio-immuno-electrophoresis) results are published elsewhere (20, 23).

MATERIAL AND METHOD

Experimental design, patient selection and allergen extracts are described in detail else-

Abbreviations: SSiGE = serum-specific IgE; SSiGG = serum-specific IgG; NSiGE = nasal-specific IgE; NSiGG = nasal-specific IgG; WPA = whole pollen allergens: a partially purified timothy extract consisting of approx. 20 out of the extractable 30–45 timothy antigens, but all the potential allergens; PPA = purified pollen allergens: two isolated timothy major allergens 19, 25; Group WPA = patients treated with extract WPA; Group PPA = patients treated with extract PPA.

where (19, 20, 21, 22). Forty patients were allocated for hyposensitization with extract WPA (group WPA) or extract PPA (group PPA). Fourteen non-hyposensitized patients admitted just before pollen season were included as controls during the second pollen season but were later allocated for grass pollen hyposensitization in another study.

Serum and secretory samples

In parallel with bleeding, nasal secretions (excluding week 168) were collected after metacholine stimulation according to (20) in samples from week 0 (before treatment, January 1978) and after weeks 8, 12 (preseason 1, 1978), 30 (postseason 1), 64 (preseason 2, 1979), 82 (postseason 2), 116 (preseason 3, 1980), 134 (postseason 3) and 168 (1 year after end of treatment). The basophils were tested for histamine release but only from week 64. Serum and secretory samples were stored at -20°C until analysed.

Allergen extracts

Two purified extracts from timothy grass pollen were used for treatment and serological analyses. Extracts for treatment were adsorbed to aluminium hydroxide. Extract WPA consisted of 20 antigen components (Alutard[®] SQ) and extract PPA of two antigen components, the major allergens 19, 25 (16). Only extract WPA was used for provocation of histamine release from the basophils. The extracts in each test series were prepared from the solution referred to below. The same allergen batch was used for tests and treatment during the entire study.

Stability measurements

All the WPA types of extracts used (Alutard[®] SQ, Soluprick[®] SQ, and Aqueous SQ) were prepared yearly from the same stock preparation, stored as approximately 1 mg/1ml 0.5% (w/v) sodium chloride, 0.25% (w/v) sodium bicarbonate at -20°C . The corresponding PPA extracts were similarly prepared from freeze dried PPA extract stored at 5°C (since July 1981, the

manufacturer has stored stock preparations as freeze dried material).

Consequently, the WPA stock preparation was used as a reference in the stability determinations, and the stability of the WPA preparation controlled by means of quantitative immunoelectrophoresis (QIE) and RAST, using the stable reagents (rabbit antibodies and human serum pool II) described previously (16).

The various types of extracts used in this study were controlled for preserved activity/concentration by QIE and RAST inhibition relative to the above stock reference preparation. The tests were performed on those extracts stored under natural conditions at the clinic for 1 year according to the details given in legend to Table 8.

Dilutions of aqueous WPA and PPA extracts (10 HEP) for nasal provocation tests were prepared at the clinic and used within 1 day. The 10 HEP solutions were used within approximately 1 month of their arrival to the clinic. Dilutions of Soluprick[®] SQ (10 HEP) for titrated skin prick tests were prepared yearly by the manufacturer and used within approximately 4 months of their arrival.

Control experiments for released antigenic material from Alutard[®] SQ (WPA) and the corresponding PPA preparation were performed by rocket immunoelectrophoresis of supernatants of the 10 HEP preparations.

Total IgE

Total serum IgE was performed by means of Phadebas PRIST[®] (Paper RIST) (3) according to the manufacturer's instructions. All of the analyses were examined in duplicate and the mean values given in units (U)/ml. The serum technique was used for nasal total IgE with a coefficient of variation of 9.1% as published previously (20).

Specific IgE

Serum-specific IgE (SSIgE) was measured by Al-RAST according to Weeke (20, 32). All the analyses were examined in duplicate and the mean values determined as results in sorbent units (SU) using diluted serum from a timothy

allergic patient as 100 SU reference. The error of analysis for the single allergen extract ranged from 10–20% of relative deviation. The serum technique was used for nasal-specific IgE (NSIgE) with a coefficient of variation of 19.6% for extract PPA and 24.2% for extract WPA (20).

Specific IgG

Serum-specific IgG (SSIgG) was measured by a double-antibody assay according to Prahl et al. (24). The activity of the precipitates was measured in a gamma counter and expressed in per cent of moderately positive reference serum. All cpm values were corrected for background, i.e. the amount of cpm precipitated by a standard serum pool. All samples were examined in duplicate. The coefficient of variation was about 10%.

Histamine release from basophils

Leucocytes from peripheral blood with about 2% basophils isolated by the Ficoll-Hypaque gradient were incubated at 37°C for 40 min with WPA in final concentrations from 10^0 – 10^{-9} w/v of the stock solution in absence of serum, and release of histamine was determined by a fluorometric assay (28). The release of histamine was expressed as a percentage of the total histamine content of the sample. Correction was made for a spontaneous histamine release amounting to 10%. The sensitivity of the patients' basophils is given as the WPA concentration giving 25% release.

Statistics

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

RESULTS

All patients but one (19) from group PPA completed the trial. Initial *in vitro* data including

week 30 (just after the first pollen season) have been described in detail previously (20).

Serological immune response

The results for groups WPA and PPA are shown in Tables 1, 2, and 3, and the changes (medians) of SSiGE and SSiGG are illustrated in Fig. 1.

No statistically significant intergroup difference was observed in pretreatment values. Pre-seasonal treatment induced increase in total IgE as well as SSiGE and SSiGG before the first pollen season. SSiGE and SSiGG increased to higher levels in group WPA than in group PPA, and SSiGE intercorrelated with SSiGG ($r_s = 0.61$, $P < 0.01$). SSiGG or SSiGE did not correlate with dosage.

The IgE response reached a maximum before the first pollen season and was followed by depression through season 1 to pre-season 2. The second IgE response initiated by treatment and/or the second pollen was very much re-

Table 1

Serum total IgE (U/ml).

Medians of serum total IgE (25–75% percentiles) before (week 0), during (weeks 8–116), and up to 1 year after the end of perennial hyposensitization

Week No.	Group WPA	Group PPA
0	194 (63–364)	94 (55–259)
8	329 (104–956)	177 (61–316)
12	296 (91–763)	195 (75–345)
season -----		
30	237 (86–1011)	199 (81–413)
64	193 (46–522)	94 (51–282)
season -----		
82	209 (44–320)	130 (59–296)
116	134 (36–425)	59 (35–170)
season -----		
134	271 (39–651)	101 (49–297)
168	99 (34–489)	80 (38–160)

Statistics: * $P \leq 0.05$ or ** $P \leq 0.01$.

Intergroup differences: Not statistically significant.

Intragroup changes: Groups WPA and PPA: weeks 0–12* and 134–168**.

Table 2

Serum-specific IgE (SSIgE) (SU).

Medians of SSiGE-WPA and SSiGE-PPA (25–75 % percentiles) before (week 0), during (weeks 8–116), and up to 1 year after the end of perennial hyposensitization

Week No.	Group WPA		Group PPA	
	SSiGE-WPA	SSiGE-PPA	SSiGE-WPA	SSiGE-PPA
0	21 (5–48)	12 (2–62)	18 (3–31)	3 (1–19)
8	147 (64–336)	219 (76–338)	43 (11–119)	38 (11–226)
12	280 (96–543)	249 (142–353)	114 (37–276)	165 (18–287)
season –				
30	227 (48–368)	197 (103–355)	88 (9–148)	105 (18–236)
64	8 (4–30)	11 (7–24)	7 (3–55)	17 (5–33)
season –				
82	31 (8–46)	12 (4–25)	19 (10–63)	19 (5–33)
116	15 (2–51)	3 (1–6)	3 (1–14)	1 (0–6)
season –				
134	5 (1–13)	4 (2–13)	6 (2–12)	4 (2–12)
168	14 (4–42)	13 (5–24)	9 (5–29)	9 (4–18)

Statistics: * $P \leq 0.05$ or ** $P \leq 0.01$.

Intergroup differences: SSiGE-WPA: week 12*, 30*, 64*. SSiGE-PPA: week 8*.

Intragroup changes: Group WPA: SSiGE-WPA, weeks 0–12**, 0–134**, 64–82**, 116–134**, 134–168*; SSiGE-PPA, weeks 0–12**, 0–134**. Group PPA: SSiGE-WPA, weeks 0–12**, 0–134**, 134–168**; SSiGE-PPA, weeks 0–12**, 116–134*.

duced compared with the first response and subsequently became depressed through the remaining treatment period to preseason 3, but only six patients developed negative RAST (< 2 SU). The third pollen season induced no (group WPA) or slight (group PPA) IgE response, but 1 year after the end of treatment (preseason 4) SSiGE increased in both groups. Total IgE response partly reflected SSiGE but decreased after the third pollen season.

The SSiGG response reached a maximum after the first – but before the second pollen season and decreased during the second pollen season to a level similar to that of the first season. One year after the end of treatment (preseason 4) SSiGG had fallen by approximately 25 % (SSiGG-WPA)–50 % (SSiGG-PPA).

The controls from the second pollen season showed some seasonal IgE stimulation but a depression of SSiGG (Table 4).

Cellular immune response

As illustrated in Fig. 2, no intergroup difference was observed in basophil cell sensitivity. Seasonal reduction in cell sensitivity was recorded in groups WPA and PPA, in contrast to controls who showed a tendency to increased cell sensitivity. Cell sensitivity (groups WPA + PPA) decreased significantly ($P < 0.05$) from the second to the third pollen season (weeks 64–116 and 82–134) without intergroup difference. More females than males showed low cell sensitivity but the difference was not statistically significant.

Secretory immune response

The results for group WPA and group PPA are shown in Tables 5 and 6. NSiGE-PPA for

Table 3

Serum-specific IgG (SSIgG) (%).

Medians of SSiG-WPA and SSiG-PPA (25–75 % percentiles) before (week 0), during (weeks 8–116), and up to 1 year after the end of perennial hyposensitization.

Week No.	Group WPA		Group PPA	
	SSiG-WPA	SSiG-PPA	SSiG-WPA	SSiG-PPA
0	6 (2–10)	9 (5–13)	1 (0–11)	4 (1–12)
8	53 (42–75)	88 (50–126)	31 (9–44)	70 (17–98)
12	77 (55–91)	130 (81–148)	46 (32–58)	112 (44–143)
season —				
30	83 (65–105)	134 (95–162)	49 (33–63)	110 (53–142)
64	107 (91–169)	246 (178–267)	70 (62–89)	174 (112–221)
season —				
82	81 (65–110)	138 (120–151)	45 (36–51)	96 (82–119)
116	90 (69–112)	152 (112–161)	45 (32–56)	105 (84–136)
season —				
134	79 (58–101)	128 (103–153)	40 (29–54)	96 (69–112)
168	61 (44–74)	63 (53–75)	32 (23–40)	42 (20–53)

Statistics: * $P \leq 0.05$ or ** $P \leq 0.01$.

Intergroup differences: SSiG-WPA: weeks 8**, 12**, 30**, 64*, 82**, 116**, 134**, 168**, SSiG-PPA: weeks 30*, 64*, 82*, 116**, 134**, 168**.

Intragroup changes: Group WPA: SSiG-WPA, weeks 0–64**, 64–82**, 116–134*, 134–168**; SSiG-PPA, weeks 0–64**, 64–82**, 116–134**, 134–168**. Group PPA: SSiG-WPA, weeks 0–64**, 64–82**, 134–168**; SSiG-PPA, weeks 0–64**, 64–82**, 116–134**, 134–168**.

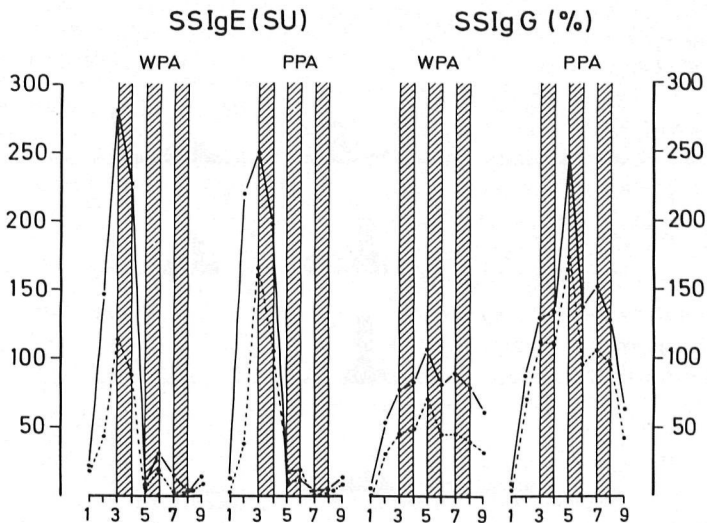


Fig. 1. Changes (medians) in serum-specific IgE (SSiGE) and allergen-specific IgG (SSiG) towards WPA and PPA during (until week 116) and after perennial hyposensitization with WPA (—) or PPA (---). Nine serum samples were investigated: weeks 0 [1], 8 [2], 12 [3], 30 [4], 64 [5], 82 [6], 116 [7], 134 [8], and 168 [9]. For statistics, cf. Tables 2 and 3. The grass pollen seasons are indicated by the hatched columns.

Table 4

Antibodies in controls.

Medians of serum and nasal antibodies (25–75 % percentiles) in non-hyposensitized grass pollen allergic patients (controls) just before (week 64) and just after (week 82) the second pollen season.

	Week 64	Week 82
Serum total IgE (U/ml)	63 (26–362)	88 (32–304)
SSIgE-PPA (SU)	5 (4–18)	5 (1–23)
SSIgE-WPA (SU)	1 (1–14)	5 (1–32)
SSIgG-PPA (%)	8 (7–12)	0 (0–7)
SSIgG-WPA (%)	4 (0–11)	0 (0–3)
Nasal total IgE (U/ml)	1 (1–17)	10 (1–30)
NSIgE-PPA (SU)	16 (11–22)	ND
NSIgE-WPA (SU)	18 (13–23)	20 (20–38)

Statistics: * $P \leq 0.05$ or ** $P \leq 0.01$.
Intragroup changes weeks 64–82: SSiGE-WPA and SSiG-PPA**.
ND = not done.

samples from week 82 was missed due to technical reasons.

No significant intergroup difference was observed in pretreatment nasal IgE values. For both groups week 64 was followed by seasonal and interseasonal increase in NSiGE and marked decrease during the third pollen season

Table 5

Nasal total IgE (U/ml).

Medians of nasal total IgE (25–75 % percentiles) before (week 0), during (weeks 8–116), and after (week 134) perennial hyposensitization

Week No.	Group WPA	Group PPA
0	19 (2–35)	10 (1–25)
8	15 (1–37)	3 (1–24)
12	10 (1–33)	3 (1–22)
season -----		
30	19 (1–33)	12 (1–17)
64	8 (1–14)	1 (1–1)
season -----		
82	12 (1–23)	1 (1–27)
116	7 (1–13)	5 (1–9)
season -----		
134	8 (1–25)	9 (1–12)

Statistics: * $P \leq 0.05$ or ** $P \leq 0.01$.
Intergroup differences: Week 64*.
Intragroup changes: Not statistically significant.

to below pretreatment values. No significant changes were detected in nasal total IgE but minimal initial changes reflected changes in NSiGE.

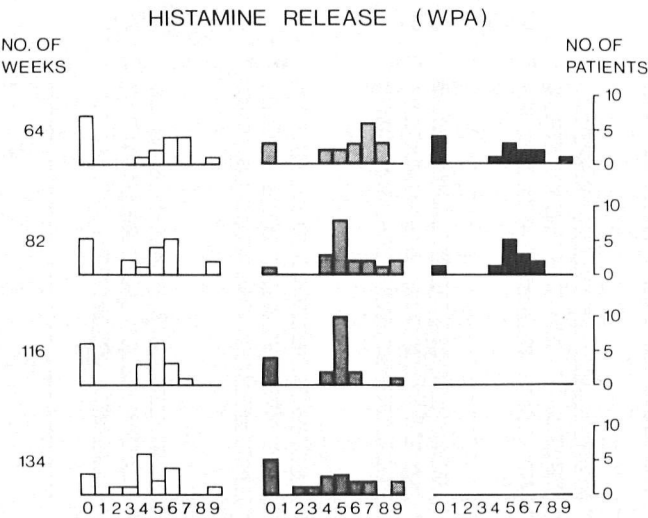


Fig. 2. No. of patients with different basophil cell sensitivity after 64, 82, 116, and 134 weeks of hyposensitization with extract WPA (group WPA □) or extract PPA (group PPA ▨) compared with non-hyposensitized grass pollen allergic patients (controls ■) for weeks 64 and 82. Ten fold threshold doses of 0 (more than 10^{-1}) or 1 (10^{-1}) to 9 (10^{-9}) w/v of partially purified timothy extract (WPA) releasing more than 25 % histamine are shown.

Table 6

Nasal-specific IgE (NSIgE) (SU).

Medians of NSIgE-WPA and NSIgE-PPA (25–75 % percentiles) before (week 0), during (weeks 8–116), and after (week 134) perennial hyposensitization

Week No.	Group WPA		Group PPA	
	NSIgE-WPA	NSIgE-PPA	NSIgE-WPA	NSIgE-PPA
0	20 (10–74)	15 (10–45)	20 (10–44)	18 (5–43)
8	25 (10–75)	18 (6–51)	20 (8–45)	18 (6–49)
12	25 (10–85)	25 (10–65)	18 (9–53)	18 (9–28)
season –				
30	50 (25–70)	40 (25–45)	28 (20–44)	25 (20–39)
64	17 (14–22)	17 (10–21)	15 (11–19)	16 (11–18)
season –				
82	25 (19–35)	ND	23 (15–40)	ND
116	43 (35–54)	48 (36–60)	45 (25–65)	50 (30–60)
season –				
134	10 (5–10)	10 (5–15)	5 (5–10)	10 (9–11)

Statistics: * $P \leq 0.05$ or ** $P \leq 0.01$.

Intergroup differences: NSIgE-WPA: week 30*.

Intragroup changes: Group WPA: NSIgE-WPA, weeks 0–134**, 64–82*, 82–116*, 116–134**; NSIgE-PPA, weeks 0–134*, 116–134**.

Group PPA: NSIgE-WPA, weeks 0–134**, 64–82**, 82–116*, 116–134**; NSIgE-PPA, weeks 116–134**.

ND = not done.

Table 7

Stability of the timothy stock reference preparation (WPA) stored as 1 mg/1 ml 0.5 % (w/v) sodium chloride, 0.25 % (w/v) sodium bicarbonate, at -20°C . ¹QIE-measurement of the area delimiting the major allergen-antigen 25 (Ag 25) and ²the per cent retained activity of the timothy stock reference RAST disc, employing uninhibited patient serum pool

Weeks of storage	Area delimited ¹ by Ag 25 (%)	RAST-activity ² (% retained)
0	100	100
2	89	117
4	112	105
8	93	–
16	102	81
32	74	81
47	45	84
52	67	78
78	77	–
156	70	82

The controls from the second pollen season showed a slight seasonal increase but only for nasal total IgE (Table 4).

Clinical parameters vs. immune response

The group of patients treated with partially purified timothy extract showed a statistically significantly higher degree of clinical protection (fewer symptom scores and lower nasal sensitivity) and greater increase in SSiG and SSiGE than the group treated with the two timothy major allergens 19, 25. However, within the groups the individual SSiG and SSiGE levels and their ratio did not correlate with the individual symptom scores or nasal sensitivity.

Perennial hyposensitization depressed the stimulated IgE response and basophil cell sensitivity to below pretreatment levels in both groups.

Stability of extracts

As shown in Table 7, a small decrease in the amount of major allergen in the stock reference preparation could be observed after week 16. However, no further decrease was observed

Table 8

Per cent preserved activity/concentration of PPA⁵, WPA⁵, and FGM⁵ relative to a reference⁶

Type of extract	Storage temp. (°C)	Method used	Time period	PPA			WPA			FGM		
				PPA	Ag19	Ag25	WPA	Ag19	Ag25	FGM	Ag19	Ag25
Al(OH) ₃ adsorbed	5	Al-RAST ³	1978–1979	95			78			–		
Al(OH) ₃ adsorbed	5	Al-RAST ³	1979–1980	100			70			–		
Glycerinized	5	QIE ¹	1978–1979		–	–		88	–		77	–
Glycerinized	5	QIE ¹	1979–1980		92	–		100	–		–	–
Glycerinized	5	QIE ²	1979–1980		84	73		89	75		86	78
Glycerinized	5	RAST INH ⁴	1979–1980	82			100			78		
Aqueous	5	QIE ¹	1978–1979		–	–		59	–		64	–
Aqueous	5	QIE ¹	1979–1980		44	–		58	–		81	–
Aqueous	5	QIE ²	1979–1980		51	66		40	52		–	–
Aqueous	5	RAST INH ⁴	1979–1980	44			–			76		

¹ Quantification by means of crossed immunoelectrophoresis. Coefficient of variation $\pm 10\%$.² Quantification by means of rocket immunoelectrophoresis. Coefficient of variation $\pm 10\%$.³ Allergosorbent: The actual samples. Mean of double determinations performed on four concentrations (10^{-3} , 10^{-1} , 10^0 , and 10^1 HEP). Calculated as maximum IgE binding. Coefficient of variation $\pm 25\%$ ⁴ Pp 0375 (16), 2 mg/ml used as allergosorbent. Double determinations performed on eight concentrations.Calculated as allergenic activities = $\frac{C\ 50\ \% \text{ ref}}{C\ 50\ \% \text{ sample}} \times 100$. Coefficient of variation $\pm 25\%$.⁵ The analysed samples were those actually used and stored (in the 1 year time period given) in the clinic according to the manufacturer's instructions.⁶ Fresh preparations of frozen (-20°C) stock solution of WPA (cf. Table 7).

until week 156, the level stabilizing at a value of approximately 70% preserved native Ag 25.

Using stock reference preparation discs RAST activity showed a decline after week 4. However, no further decrease was observed, values stabilizing at a level corresponding to 82% preserved activity.

Table 8 illustrates the activity of the extracts stored for 1 year. However, all extracts except the treatment extracts were used within approximately 1 (aqueous) or 4 (Soluprick® SQ) months (cf. Material and Method). All activities were measured relative to the WPA-stock reference preparation and preserved activity between 44 and 100% obtained. The preserved amount of Ag 25 and Ag 19 was found to be between 40% and 100%.

Released antigenic material from Alutard SQ above 1% of total antigenic content was not observed for the time periods 1978–1979 and 1979–1980 (Table 8).

DISCUSSION

Changes in the serological variables during hyposensitization confirm the results from most other studies on SSiGE (1, 4, 5, 7, 8, 10, 11, 12, 25) and SSiGG (1, 5, 7, 8, 9, 10, 12, 14, 15, 17, 27, 29). The intercorrelation of SSiGE and SSiGG corresponds with only two other studies (8, 33).

SSiGE and SSiGG response showed an inter-correlated increase before the first pollen season and then diverged before the second pollen season. This may indicate that the SSiGE depression was not decisively influenced by SSiGG but by other mechanisms, e.g. stimulation of T-suppressor cells (2, 26). Though the maintenance dose was unchanged, SSiGG continued to increase before the second pollen season. However, the subsequent decrease in SSiGG corresponds to reduced dosage due to increase in delayed local side effects in both

groups (21). Preseasonal treatment stimulated an increase in SSiG and IgE response but perennial treatment stimulated a further increase in SSiG response and depressed the IgE response, corresponding to a higher degree of clinical protection from the second pollen season (21, 22).

One year after the end of hyposensitization the suppression of IgE response seems partly eliminated as evaluated by RAST but not by total IgE or CRiE response (23) and SSiG was only reduced by 25–50%.

Seasonal SSiG depression during treatment was partly due to reduced seasonal dosage but decrease in SSiG of controls speaks in favour of consumption of IgG antibodies during seasonal pollen exposure. Other trials have not demonstrated such seasonal SSiG variations (5, 6, 8, 14, 17, 27, 29, 31).

The significant intergroup difference in serological variables was not reflected in basophil cell sensitivity, but histamine release was included in the study only from week 64 without knowing the pretreatment cell sensitivity. In accordance with IgE, the cell sensitivity decreased during treatment. A lower cell sensitivity was shown in females than in males – this agrees with females being SSiGE low-responders (20). Only one out of the 14 females used contraceptives during the study.

Secretory values did not include NSiGE, and investigators disagree on the importance of NSiGE. In non-hyposensitized patients nasal total IgE (34) and NSiGE (5, 18) may show seasonal increase and no changes, respectively. During systemic hyposensitization NSiGE ranged from no change (31) to minimal change (5). Before week 64 the present study demonstrated no significant NSiGE response but after interseasonal stimulation the production of NSiGE was depressed during the third pollen season. Symptom and medicine scores were considerably lower during season 3 compared with seasons 1 and 2, which may be due to low pollen counts rather than seasonal NSiGE depression. On the other hand, the delayed NSiGE stimulation may reflect other local immune responses, in accordance with the delayed protection on nasal sensitivity (22).

As no seasonal NSiGE response was shown in the non-treated controls, the NSiGE response from the beginning of the second season seems induced by treatment. Compared with the serological results the NSiGE response might be followed by NSiG response, as demonstrated in a study on local intranasal immunotherapy (18).

Changes in total IgE may partly be related to the incidence of infections and not to allergy, in agreement with discordance to specific IgE after seasonal pollen exposure in allergic patients (25) and seasonal increase of total IgE in non-allergic subjects (30).

Changes in skin and nasal sensitivity predicted clinical high-response after 12 and 64 weeks of hyposensitization but did not correlate to changes in SSiG. The explanation may be that females were immunologically low-responders although clinical response and dosage were comparable to those of males (20). Even if the patients were divided according to sex, the pretreatment or changes in nasal and skin sensitivity did not correlate to changes in SSiG. Nor did dosage correlate to SSiG changes in females or males, even in subgroups with pretreatment nasal or skin threshold doses of 1 HEP or 10 HEP. As in other trials (12, 13, 14, 27), some patients showed reduced cell sensitivity during hyposensitization but the changes did not correlate to individual symptoms.

According to the stability measurements the changes in allergenic activities have no essential influence on the *in vivo* and *in vitro* results.

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Address:

Ole Østerballe

Paediatric Department G 4064
University Hospital (Rigshospitalet)
Blegdamsvej 9
DK-2100 Copenhagen Ø
Denmark

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