

Specific IgE response toward allergenic molecules during perennial hyposensitization: a three-year prospective double-blind study

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Allergenic extracts are multicomponent systems containing a number of potentially allergenic molecules. In a 3 yr prospective double-blind study, sera from 40 patients hyposensitized with either a two-component timothy extract or a 20-component timothy extract were evaluated by means of crossed radioimmuno-electrophoresis. The patients exhibited specific IgE response toward individual allergenic molecules, and IgE with new specificities did not develop after hyposensitization. A high degree of clinical protection could generally be obtained on treatment with the 20-component timothy extract, but this was true only with the two-component timothy extract containing the allergenic molecules that matched the individual IgE response. (J ALLERGY CLIN IMMUNOL 71:40, 1983.)

Allergic patients show specific reactions toward various allergenic materials such as grass pollen and animal dander. It is well established that allergenic extracts are multicomponent systems containing a number of antigenic molecules (15 to 80).¹ Depending on the genetic capability, the allergic patient's specific IgE can be raised against various combinations of the allergenic molecules from a particular allergenic extract.^{1, 2} Patients' specific IgE toward individual antigens can be revealed by means of CRIE, and studies performed on groups of patients have shown that some antigens (major allergens) are more likely to give rise to IgE than other antigenic molecules (minor allergens).¹

Recent studies³⁻⁵ indicate that hyposensitization with purified timothy pollen extracts may provide better clinical protection than that with crude extracts. The present study compared a commercially available partially purified timothy pollen extract (WPA) containing approximately 20 components, including all

potentially allergenic molecules,⁶ with an available two-component timothy extract (PPA) containing two major allergens.¹¹ The study included in vivo tests (symptom scores, medicine consumption, skin prick test, and nasal provocation test)^{7, 8} and in vitro tests (total IgE, RAST, CRIE, and allergen-specific IgG).^{9, 10}

In the present study, CRIE has been employed to evaluate three main points. (1) Is the patient's IgE response specific against individual allergenic molecules? (2) If so, do "new" IgE responses develop after hyposensitization of patients who were previously not sensitive to particular allergenic components in antigenic mixtures? (3) Is the IgE response toward the individual allergenic molecules correlated to the degree of clinical protection during hyposensitization?

MATERIALS AND METHODS

Experimental design is described in detail elsewhere.¹²⁻¹⁴ In brief, 40 patients (ages 15 to 43 yr) with typical grass pollen hay fever during at least two seasons in spite of

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This study was supported by grants from the Danish Medical Research Council (Nos. 512-20532 and 12-2183), and the Danish Asthma-Allergy Association.

Received for publication April 20, 1982.

Accepted for publication July 26, 1982.

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Abbreviations used

WPA:	Whole pollen allergen
PPA:	Purified pollen allergen
CRIE:	Crossed radioimmuno-electrophoresis
SRRID:	Single radio radial immunodiffusion
RAST:	Radioallergosorbent test
BU:	Biological units

TABLE I. Specific IgE response toward individual timothy allergens*

Sample Nos.	Individual timothy allergens													
	Group PPA							Group WPA						
	1	15	19	25	27	30	41	1	15	19	25	27	30	41
1	2	32	14	14	32	2	5	12	27	10	12	32	1	7
2	2	33	41	40	18	5	11	17	45	86	28	35	5	8
3	1	34	28	26	25	5	12	5	25	73	20	34	5	11
4	1	26	18	31	26	5	7	7	28	33	14	32	5	7
5	0	10	7	15	15	0	3	2	15	16	14	25	4	6
6	1	23	10	20	28	5	4	5	22	31	19	32	8	3
7	3	20	10	17	23	4	4	5	24	28	18	21	8	6
8	2	21	8	11	34	4	3	7	13	15	10	9	4	3
9	1	7	5	6	16	1	2	3	13	6	5	9	5	3

*The mean CRIE scores ($\sum \bar{N}_{xi}$) for patient groups PPA and WPA during hyposensitization are listed for the individual allergens.

TABLE II. Number of patients with specific IgE response toward individual timothy allergens

Sample Nos.	Individual timothy allergens																					
	Group PPA							Group WPA														
	1	15	19	25	27	30	41	1	15	19	25	27	30	41								
No. of patients with positive CRIE																						
1	3	10	14	20	5	2	10	7	15	14	20	7	1	10								
2	4	9	15	20	4	2	10	7	15	14	20	7	1	10								
3	3	10	14	20	4	2	10	7	15	13	20	7	1	10								
4	4	11	14	20	5	2	10	7	15	13	20	7	1	10								
5	1	9	14	17	5	0	8	6	14	14	18	7	1	9								
6	4	10	15	19	5	2	9	7	15	15	20	7	1	10								
7	4	9	15	20	5	2	9	7	15	14	20	7	1	7								
8	4	9	15	18	4	2	7	6	14	15	20	7	1	6								
9	1	10	15	16	5	1	6	7	13	13	20	6	1	7								
Patients with positive CRIE before but negative after treatment																						
8,9	a			a			c			e												
8,9				b			d															
Patients with negative CRIE before but positive after treatment																						
8	f	g	g	h																		

conventional medication were included in the study. Only grass pollen allergy without associated type I allergy was allowed before treatment. Patients who had been previously hyposensitized were excluded from the study. The patients were divided randomly into two equal groups (PPA and WPA) according to age and sex and were treated either with the two-component extract (PPA), the two timothy major allergens 19 and 25, or with a partially purified 20-component timothy extract (WPA). The study covered three grass pollen seasons and the subsequent 12 mo after the end of treatment.

All patients received perennial conventional systemic hyposensitization and received one injection per week up to maintenance dose (maximal tolerated dose); thereafter patients received one injection per month. During the grass pollen season the dose was reduced to one third to one

fourth of the usual concentration. The mean single treatment dosages are shown in Figs. 1 and 2. The accumulated dosages showed no statistical intergroup differences. The medians of accumulated dosage (25% to 75% percentiles) were at the end of treatment 112,200 BU (76,600 to 148,000) in the WPA group and 117,200 BU (78,500 to 135,900) in the PPA group.⁸

Using a score system,⁷ patients recorded symptoms and concomitant medication during the pollen season. Scores for symptoms and concomitant medication were added up into total scores for comparison with the CRIE results in the present study.

Allergenic extracts

Two purified extracts, WPA and PPA, from timothy grass pollen were used for treatment and in analyses. Ex-

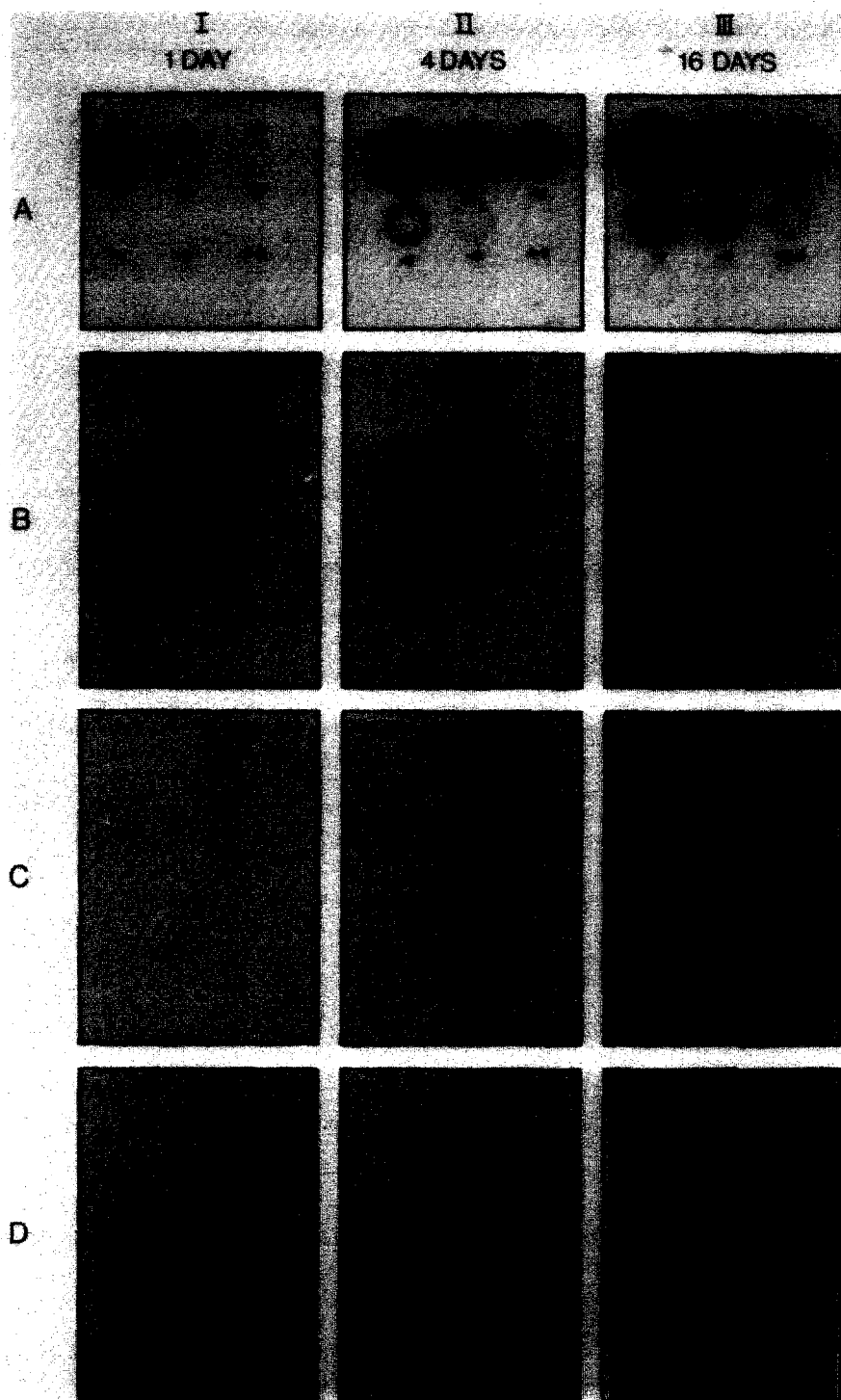


FIG. 1. Evaluation of radiostaining intensities on individual antigens in CRIE autoradiographs. Columns (I to III) indicate duration of autoradiography (1, 4, and 16 days, respectively). Rows (A to D) indicate: A, SRRID autoradiography, 1-, 4-, 16-; 1 to 9 are the SRRID samples of the twofold dilutions of ^{125}I -anti-IgE (7 to 0.03 nCi); B to D, examples of CRIE autoradiographs of serum samples from three different patients. The scores of radiostained antigens were assigned by visual comparison between the SRRID rings and the CRIE precipitate in question; for example, the radiostaining of allergens 15, 19, 25, and 27 in autoradiography (I,B) correspond to SRRID rings (I,A) 1-3, 1-3, 1-3, and 1-2, respectively, and the radiostaining of antigen 41 (II,D) corresponds to SRRID ring (II,A) 4-5.

tracts for treatment were adsorbed to aluminum hydroxide. The same allergen batch was used for test and treatment during the entire study. WPA was identical to the commercially available Alutard SQ timothy purchased from ALK, Copenhagen, Denmark, and PPA was prepared as described previously.¹¹

The extracts were used in 10-fold dilutions from 0.01 HEP (10 BU/ml) up to 10 HEP (10,000 BU/ml). The Nordic Committee for Allergen Standardization has defined 1 HEP extract as 1000 BU/ml.¹² The amounts of allergens 19 and 25 were equal in extracts WPA and PPA. The amounts of protein and of allergens 19 and 25 in extract WPA, 1 HEP, were approximately 40, 3, and 6 µg/ml, respectively, whereas the amounts of protein and of allergens 19 and 25 in extract PPA, 1 HEP, were approximately 9, 3, and 6 µg/ml, respectively.

Serum samples

Blood samples were collected and identified as follows: 1, week 0; 2, week 8; 3, week 12 (preseason 1, 1978); 4, week 30 (postseason 1); 5, week 64 (preseason 2); 6, week 82 (postseason 2); 7, week 116 (preseason 3); 8, week 134 (postseason 3); and 9, week 168 (preseason 4, 1 yr after the end of hyposensitization). Serum samples were stored at -20° C until analyzed. Nine serum samples were obtained from all patients except one, who dropped out of the investigation after season 1 (Table III, subgroup PPA I, patient 6).⁷

CRIE

The CRIE experiments were carried out essentially as described elsewhere,¹ with the first-dimension electrophoresis performed for 30 min at 10 V/cm (agarose: Litex, type HSA, Glostrup, Denmark; antigen applied: 20 µg WPA; 17 µl of rabbit antibodies/cm² gel).

The rabbit antibodies used were a mixture (1:1:2:4:8) consisting of antibodies raised against extracts of false oat (one part), orchard grass (one part), PPA (two parts), and two different crude extracts of timothy (four and eight parts). Rabbit antibodies against the extracts were raised, using three rabbits for each extract. These antibodies revealed the presence of an allergen (antigen 41), which had not been detected previously. The antibody titer against antigen 41 originated from the antibodies raised against one of the crude timothy extracts. A minute amount of antigen 41 was present in the PPA preparation used both for treatment of the patients and for immunization of rabbits.

The degree of radiostaining of individual precipitates was evaluated by visual comparison of CRIE autoradiographs (140 nCi ¹²⁵I-anti-IgE) with autoradiographs of ¹²⁵I-anti-IgE Mancini rings (SRRID) (standard internal reference for CRIE studies was performed at the Protein Laboratory). SRRID was performed as follows: 100 µl aliquots of twofold dilutions (nine in all) of ¹²⁵I-anti-IgE were each mixed with 5 µl of rabbit IgG (normal rabbit serum immunoglobulin fraction, DAKO X903, lot 109B; DAKO A/S, Denmark). Immunodiffusion was performed with 5 µl of each dilution (7 to 0.03 nCi) for 48 hr in 5 by 5 cm

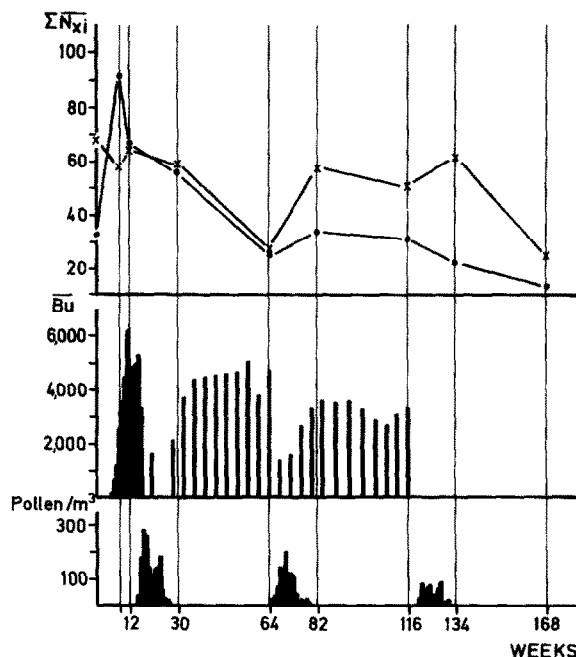


FIG. 2. Group PPA IgE response toward individual timothy allergens during the course of treatment. Mean CRIE scores (ΣN_{xi}) for allergens 1, 15, 27, and 30 not present in PPA (x) and for allergens 19, 25, and 41 present in PPA (•) are illustrated. Corresponding mean single treatment dosages (Bu) as well as accumulated seasonal pollen counts (pollen/m³) and weeks of treatment are shown. Statistical analysis: •, weeks 0 to 8, $p \leq 0.001$; weeks 64 to 82, $p \leq 0.05$; weeks 116 to 134, $p \leq 0.05$; and weeks 0 to 168, $p \leq 0.01$. x, weeks 0 to 8, $p > 0.05$ (NS); weeks 64 to 82, $p \leq 0.001$; weeks 116 to 134, $p \leq 0.05$; and weeks 0 to 168, $p > 0.05$ (NS).

agarose-coated (type HSA, 3.75 ml) glass plates containing 3 µl/cm² of swine anti-rabbit IgG (DAKO Z196, lot 011B, titer 800; DAKO A/S, Denmark). One SRRID plate was autoradiographed together with 15 CRIE plates on Kodak XL-1 X-ray film for 1, 4, and 16 days. For each of the nine SRRID rings a score number, N_x , was calculated from the following equation:

$$N_x = \frac{1000}{D_1 \times \int_{t_1}^{t_2} e^{-kt}}$$

where D_1 is the dilution factor for ¹²⁵I-anti-IgE, t_1 and t_2 are the start and end, respectively, of the exposure, and k is the isotope decay-rate constant.

The intensity of the "radiostaining" of individual precipitates in the CRIE autoradiographs was estimated by visual comparison with the SRRID rings and was assigned the score N_x corresponding to the SRRID ring (dilution of ¹²⁵I-anti-IgE) with equal intensity of radiostaining. The CRIE results were evaluated independently by at least two persons. Examples of CRIE evaluation are shown in Fig. 1.

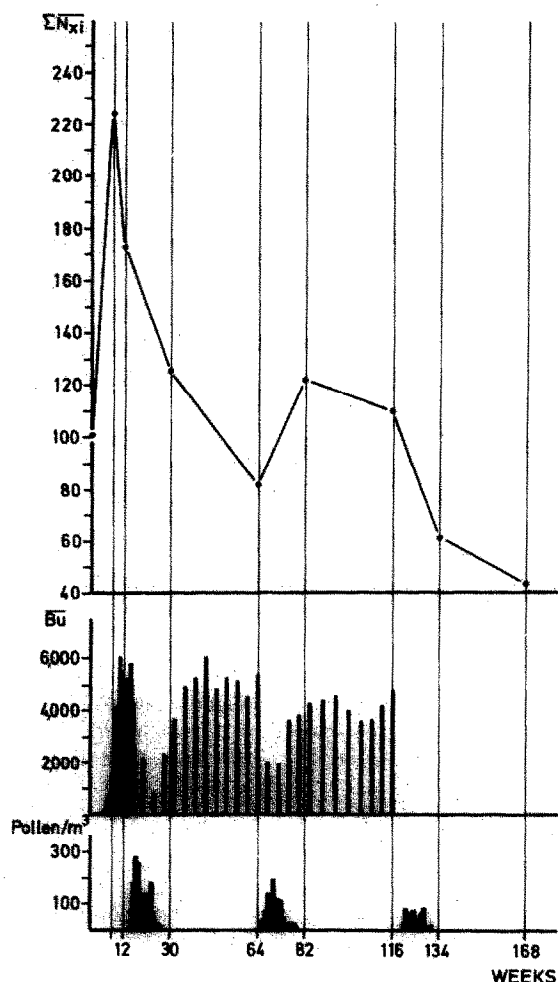


FIG. 3. Group WPA IgE response toward individual timothy allergens during course of treatment. Mean CRIE scores (ΣN_{xi}) for the individual allergens 1, 15, 19, 25, 27, 30, and 41 present in WPA are illustrated. Corresponding mean single treatment dosages ($\bar{BU}$) as well as accumulated seasonal pollen counts (pollen/m³) and weeks of treatment are shown. Statistical analysis: weeks 0 to 8, $p \leq 0.001$; weeks 64 to 82, $p \leq 0.05$; weeks 116 to 134, $p \leq 0.01$; and weeks 0 to 168, $p \leq 0.001$.

Statistics

Paired and unpaired rank sum tests were employed using two-tailed tests with a 5% significance level.

RESULTS

As shown in Table I and Fig. 2, patients in the PPA group seem to exhibit specific IgE response toward only the allergens included in the treatment extract PPA (allergens 19, 25, and 41), whereas the IgE response in the WPA group shown in Table I and Fig. 3 seems to involve all the allergenic molecules studied (allergens 1, 15, 19, 25, 27, 30, and 41). In general

(Figs. 2 and 3), the stimulated IgE response appeared to reach a maximum before the first pollen season, followed by a depression lasting through season 1 to preseason 2. The second IgE response induced by treatment and/or the second pollen season was lower compared with that of the first response; it decreased during the remaining treatment period and the third pollen season to below pretreatment level.

The IgE response in the PPA group (Table I and Fig. 2) toward the allergens not included in the treatment extract PPA (allergens 1, 15, 27, and 30) seems to reflect only the seasonal pollen stimulation, with the possible exception of the first pollen season.

The sera of five patients (a to e, Table II) exhibited positive CRIE results before and negative CRIE results after treatment against three timothy components (allergens 15, 25, and 41); only the case of the specific IgE against allergen 15 in group PPA was not suppressed by the treatment.

The sera of only three patients (f to h, Table II) exhibited negative CRIE results before and positive CRIE results after treatment against three timothy components (allergens 1, 15, and 19), but the formation of specific IgE against allergens 1 and 15 in the PPA group was not induced by treatment and probably occurred as a result of natural pollen exposure.

Prior to treatment (Table III), nine of 20 patients in the PPA group (subgroup PPA I) reacted only toward the allergenic molecules present in extract PPA (allergens 19, 25, and 41). These patients showed statistically significantly higher clinical protection with treatment than did the patients (subgroup PPA II) with pretreatment IgE against allergenic molecules besides those included in PPA. The two subgroups (Table III) were comparable with respect to age, sex, dosage, and pretreatment duration of symptoms.

DISCUSSION

Hyposensitization did not induce IgE with new antigenic specificities, since none of the 40 patients involved showed or developed a statistically significant IgE response toward timothy antigens against which an IgE response has not been detected previously.^{1, 9} A possible treatment-induced IgE response toward allergenic molecules to which the patients did not react prior to treatment was observed in only two out of 82 (2.4%) possible cases (Table II, sample 1, group PPA, allergens 19, 25, and 41; and group WPA, allergens 1, 15, 19, 25, 27, 30, and 41), whereas new IgE specificities due to natural pollen exposure were observed in two out of 60 (3.3%) possible cases (Table II, group PPA, sample 1, allergens 1, 15, 27, and 30). In all the cases of development of IgE with

TABLE III. Total scores (symptoms and medication) obtained for patients in group PPA, divided into subgroup PPA I and PPA II according to specific IgE response prior to treatment

Patients	1978	1979	1980	Difference between 1978 and 1980
Subgroup PPA I*				
1	246	455	220	26
2	525	359	130	395
3	3	69	24	-21
4	192	350	0	192
5	119	178	38	81
6	713	—	—	—
7	109	129	32	77
8	298	257	55	243
9	304	177	91	213
Subgroup PPA II†				
10	171	903	185	-14
11	103	124	0	103
12	163	92	180	-17
13	54	157	0	54
14	232	472	409	-177
15	614	491	526	88
16	338	488	646	-308
17	202	401	271	-69
18	395	657	229	166
19	11	63	106	-95
20	248	188	145	103
p values	>0.05	>0.05	>0.05 <0.10	<0.05

*Patients with IgE response only toward allergens present in PPA.

†Patients with IgE response toward allergens not present in PPA.

new specificities, the observed radiostaining in CRIE was close to the detection limit, indicating a possible pretreatment IgE response below this limit.

Previous communications^{1, 9} concerning the first preseasonal treatment did not consider antigen 41 as an allergen, and the significance of antigen 30 in the present study seems to be considerably less than in the previous report. This apparent discrepancy can be explained by the use of different rabbit antibodies in the two CRIE experiments. The antibodies used in our present study exhibit a much lower titer toward antigen 41 than the antibodies used previously, and it seems likely that the precipitates for antigens 41 and 30 coincide in the previous CRIE experiments, leading to overestimation of antigen 30. In the experiments described here a separate evaluation of the two allergens was possible. Furthermore, PPA contains minute amounts of antigen 41, which gives rise to the observed IgE response toward this allergen in patient group PPA.

Hyposensitization with WPA was generally superior to that with PPA,⁷⁻⁹ but the symptom scores

also indicate difference ($p < 0.05$) between subgroup PPA I and subgroup PPA II. This indicates that the specific IgE response correlates to the degree of clinical protection. Since CRIE showed no statistically significant development of IgE with new specificities, hyposensitization should be performed with extracts containing all the potential allergens (e.g., WPA). However, it is possible that stronger clinical response may be obtained with extracts containing only those allergenic molecules that exactly match the patients' pretreatment IgE response.

The mean CRIE scores (Figs. 2 and 3) generally parallel the RAST values,^{9, 10} but evaluation of CRIE scores for the individual patient (27 CRIE analyses) reveals a very individual response, with high, medium, low, and/or non-responders. As described previously,¹³ a high response in CRIE was predictive of clinical high response, since low degree of symptoms was positively correlated with an increase in specific IgE binding to allergen 19 after 8 to 12 wk of treatment.

Chief technician Kirsten Billesbølle is thanked for her skillful technical performance of the analyses.

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