

Grass Pollen Hyposensitization versus Placebo Therapy

II. Immunotherapy-Induced Changes in Serum IgE and IgG Levels

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This study presents the pre- and post-treatment levels of specific IgE and IgG and of total IgE in the sera of two groups of grass pollen-sensitive patients, one receiving placebo injection (16 patients), the other being treated by repeated doses of a four-grass pollen extract (17 patients).

Total and specific IgE were evaluated by the radioimmunological methods based on the IgE-anti-IgE interaction. IgG specific to the pollen extract was determined by a radioimmunoassay measuring protein-A bound pollen-specific antibodies (IgG). The whole immunoglobulin content specific to the allergen was quantified by a haemagglutination technique.

During the treatment period, the relative variations of total IgE, and specific IgE and IgG levels are significantly different in placebo- and pollen-treated groups: 1) for total and specific IgE, were observed a relative decrease in the placebo group, and stable levels in the treated group; 2) for IgG, the level was stable in the placebo group and a relative increase was seen in the treated group.

For most of the patients the specific serum IgE and IgG levels were determined also after the pollen season consecutive to the treatment. During the pollen season, the placebo group is characterized by an increase in specific IgE concentrations and stable IgG levels. The hyposensitized patients showed stable levels of specific IgE and a decrease in specific IgG during the same period.

There is a significant correlation between the levels of specific haemagglutination titres and the IgG levels. After the treatment period the haemagglutination titre is significantly lower in the placebo-treated patients than in the pollen-treated ones.

A significant positive correlation exists between the levels of specific IgE and IgG before and after treatment, for the 33 patients. Moreover, during hyposensitization, the relative variations of specific IgE and IgG are highly correlated in the treated group only.

No significant correlation at the $P \leq 0.05$ level is observed between the clinical scores and the levels of the different biological parameters.

Key words: allergen extracts; grass pollen; hay fever; total IgE; haemagglutination titre; immunotherapy; specific IgE; specific IgG.

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CLINICAL ASPECTS

During a clinical trial designed to evaluate the efficacy of one pre-seasonal immunotherapy course with a crude four-grass pollen extract, serum samples were collected from the placebo- and from the pollen-treated patients. Pre-treatment, post-treatment and post-seasonal samples were analysed for their levels of total and specific IgE and for their concentration of specific IgG. The whole immunoglobulin content specific to the allergen was quantified by a haemagglutination technique in the post-treatment samples only.

During the treatment, a lowering of total IgE, specific IgE and IgG is observed in the control group. In the treated group IgE levels are stable, while a net increase of specific IgG is noticed. The haemagglutination titre is significantly lower in the placebo-treated patients than in the pollen-treated ones.

During the pollen season, the placebo group is characterized by an increase of the specific IgE concentrations and stable IgG levels. For the hyposensitized patients stable levels of specific IgE and a diminution of the specific IgG are observed.

No significant correlation at the $P \leq 0.05$ level is observed between the clinical scores and the levels of the different biological parameters.

In order to check the changes in specific serum immunoglobulin levels due to the effects of immunotherapy, comparative studies have already been performed on blood samples collected in patients not treated or placebo-treated, and allergen-treated patients (2, 8, 18, 23). Such studies were mainly effectuated on pollen sensitive patients. One of the most relevant data reported in literature is the reduction of the seasonal rise of specific IgE in the treated group compared with the non-treated group (8, 16, 18).

On the correlation between the levels of specific IgE and IgG in treated patients some controversial data exist: Gleich et al. (8) mention a positive correlation between these immunoglobulin levels, whereas Evans et al. (5) observed an inverse relationship. Consequently, these authors try to explain their different findings by proposing various models for the regulation of the biosynthesis of the IgE and IgG antibody classes in man. New information on the relationship between these immunoglobulin levels could help to clarify these working hypotheses.

During a double-blind trial comparing the effectiveness of placebo versus four-grass pollen extract injections (19), changes in grass pollen-specific immunoglobulin levels after treatment were looked for. The hyposensitization procedure was performed with an $\text{Al}(\text{OH})_3$ -adsorbed extract and the IgE adjuvant property (15) of this compound made it necessary to pay particular attention to the development of the IgE level after treatment.

The results of the variations of serum immunoglobulin levels over a 1-year observation period are compared with those observed in similar trials.

MATERIAL AND METHODS

Patients

Thirty-three patients with severe seasonal rhinitis between April and July and with positive skin tests to a four-grass pollen extract (*Dactylis glomerata*, *Lolium perenne*, *Secale cereale*, *Phleum pratense*) were recruited in May and June 1978. None of these patients had been desensitized with pollen extracts before. Clinical history, hyposensitization treatment and the clinical scores observed during the pollen season 1979 are presented in a preceding paper (19). The period of treatment was from November 1978 to April 1979 and daily symptom recording by the patients was carried out between May 15 and June 30 1979.

Serum sampling

Pretreatment sera were collected from each patient in October 1978 and the post-treatment samples in May 1979. Post-seasonal sera were obtained in October and November 1979 for 26 patients. The serum samples were stored at 4°C.

Allergen extract

A mixture of four crude extracts prepared from *Dactylis glomerata*, *Lolium perenne*, *Secale cereale* and *Phleum pratense* pollens has been utilized for all the biological determinations. The schedule of extraction and the conservation of the 4-grass pollen extract are described in a preceding paper (19). The stability of the extracts has been tested by the RAST inhibition method as described by Yman et al. (22).

Specific IgE evaluation

The coupling of the allergen extract on activated paper discs (Whatman No. 1) was based on the method described by Ceska (3). The paper discs were stocked in phosphate saline pH 7.4, in the presence of 1% Tween 20. The technique performed is a modification of the original method of Wide et al. (20): 50 μ l of 10 times diluted serum samples were incubated for 3 h with the paper discs, followed by three washings with saline; an additional incubation in the presence of 50 μ l of ^{125} Iodine labelled anti-IgE during 16 h followed by three washings were then done. All the sera collected before and after the treatment, and after the pollen season, were tested in the same experiment with the same reagents. The results are expressed as per cent of the total input counts.

Specific IgE determinations were carried out on serial 3-fold dilutions of pretreatment sera samples for a high-titre IgE serum, a mean-titre IgE serum, and serum corresponding to the lowest IgE level. To avoid in RAST results the plateau zone in high-titre undiluted sera as well as the background zone in low-titer 100-fold diluted sera, the 10-fold dilution was chosen for the current investigation of the variations of specific IgE levels during immunotherapy.

Total IgE determination

Total IgE levels were quantified by the method of Ceska et al. (4). The results are expressed in IU. All the serum samples from a single patient were analysed in the same experiment.

Specific IgG determination

The method performed was based on the technique described by Zola et al. (24).

Labelling of the pollen extract was performed by the chloramine T technique (10). 350 μ g pollen extract were mixed with 500 μ Ci ^{125} Iodine. Free ^{125} Iodine was separated from the fixed ^{125} -I on an IRA 400 column. The labelled pollen extract was dialysed. The specific activity of this preparation was 1 μ Ci/ μ g

protein. To determine the specific IgG level in the sera, 60 μ l of 2,5 and 10 times diluted samples were mixed with 150 ng of labelled pollen extract. All the dilutions were performed in phosphate saline buffer pH 7.2 in the presence of 1% Tween 20. After 1 h horizontal stirring at 22°C, 200 μ l of protein A-Sepharose, four times diluted, were added. Incubation of 2 h at 22°C was followed by four washings with saline. The results are expressed as per cent of the total input counts.

Five and 10 times diluted samples of some of the sera gave results near to the base-line values. For this reason data corresponding to the 2-fold dilutions were chosen for the serial comparisons.

All the serum samples from a single patient were analysed in the same experiment.

Specific haemagglutinating antibody determination

Grass pollen extract was coated on to O human packed red cells with chromic chloride as coupling agent (9). Anti-grass pollen antibody detection and titration with grass pollen-coated cells were carried out on white slides by haemagglutination tests performed by mixing one drop of a 0.5% suspension of coated red cells or uncoated red cells as control, in physiological saline with one drop of undiluted or diluted serum.

The slides were then incubated at room temperature for 10 min under gentle shaking in a moist chamber. The agglutination was graded from + to ++++ under low magnification. The titre of a serum was expressed as the reciprocal of the highest dilution at which at least + agglutination was observed. Specificity of agglutination obtained with the patient's sera was checked by inhibition of haemagglutination tests using several rows of serially double diluted grass pollen extract. All antibodies detected were found to be specific, i.e. specifically inhibited by grass pollen extract in haemagglutination inhibition assay.

Statistical methods

The results are presented by the median and

range values. In order to compare the different immunoglobulin levels and their relative variations:

$$\left(\frac{\text{post-treatment level} - \text{pre-treatment level}}{\text{Pre-treatment level}} \right)$$

between the treated patients and the placebo controls, a two-sided Wilcoxon test has been used.

RESULTS

Table 1 presents the pre-treatment median and range values of the grass pollen specific IgE and IgG antibodies and of total IgE for the placebo and the treated groups. The data show that before treatment began, the levels were similar.

Fig. 1 indicates the development of the specific IgE level in the blood of individual patients. The variation has been recorded from the pre-treatment period to the post-seasonal period. The results are given separately for the placebo and for the treated patients. During the immunotherapy period the specific IgE level is more frequently decreasing in placebo patients than in the treated ones. During the pollination period significant seasonal increases are observed in the placebo group, but not in the treated group.

Table 1
Pre-treatment levels of specific IgE, specific IgG and of total IgE. Median values and range in the placebo and the treated group

	Pre-treatment values October 1978		Wilcoxon test
	Placebo n = 16	Treated n = 17	
Total IgE (IU/ml)	260 (24-2,075)	260 (75-4,125)	NS
Specific IgE (%)	19 (3-42)	15 (5-48)	NS
Specific IgG (%)	2.4 (0-9)	2.2 (0-10)	NS

Fig. 2 illustrates the IgG levels for each patient in the three consecutive blood samples. In all the treated patients an increase of the specific IgG level was observed during immunotherapy, followed by a lowering during the pollen season. In placebo patients the IgG levels remained constant throughout the period of the study. In the whole population, though none of the patients had been treated before, four high pretreatment values were observed for specific IgG, two of them corresponding to children of 7 and 11 years old.

After immunotherapy (Table 2A) the specific IgG level was higher in the treated group than in the placebo group ($P \leq 0.002$). No significant differences are observed between the two groups as regards total and specific IgE levels. However, when relative variations of these levels are calculated in each group of patients during the immunotherapy period (Table 2B), significant differences between the two groups of patients are revealed: a decrease of about 30% in total and specific IgE in placebo versus treated patients, confirming the tendency noticed in Fig. 1 for specific IgE levels during the immunotherapy period.

As there were some dropouts during the post-seasonal serum sampling, no valid comparisons between the two groups were possible because of the small sample numbers. Thus only the tendencies of the changes after the pollen season could be deduced: suppression of the post-seasonal peak of specific IgE in the treated group and decrease of the specific IgG after the season in the same patients (Figs. 1 and 2).

Table 3 presents IgE and IgG determinations on two individual sera, before and after absorption on anti-IgE coated paper discs. It is shown that partial IgE absorption diminishes the IgE level in the sera, but does not alter the IgG level.

Table 4 presents correlation values between the specific IgE and IgG levels. For the whole population, in the pre-treatment as well as in the post-treatment sera, the titres of these two immunoglobulin classes were correlated at the $P \leq 0.001$ level. Moreover the parallel development of the specific IgE and IgG levels during immunotherapy in the treated group is well demonstrated in Table 5 by the high correlation

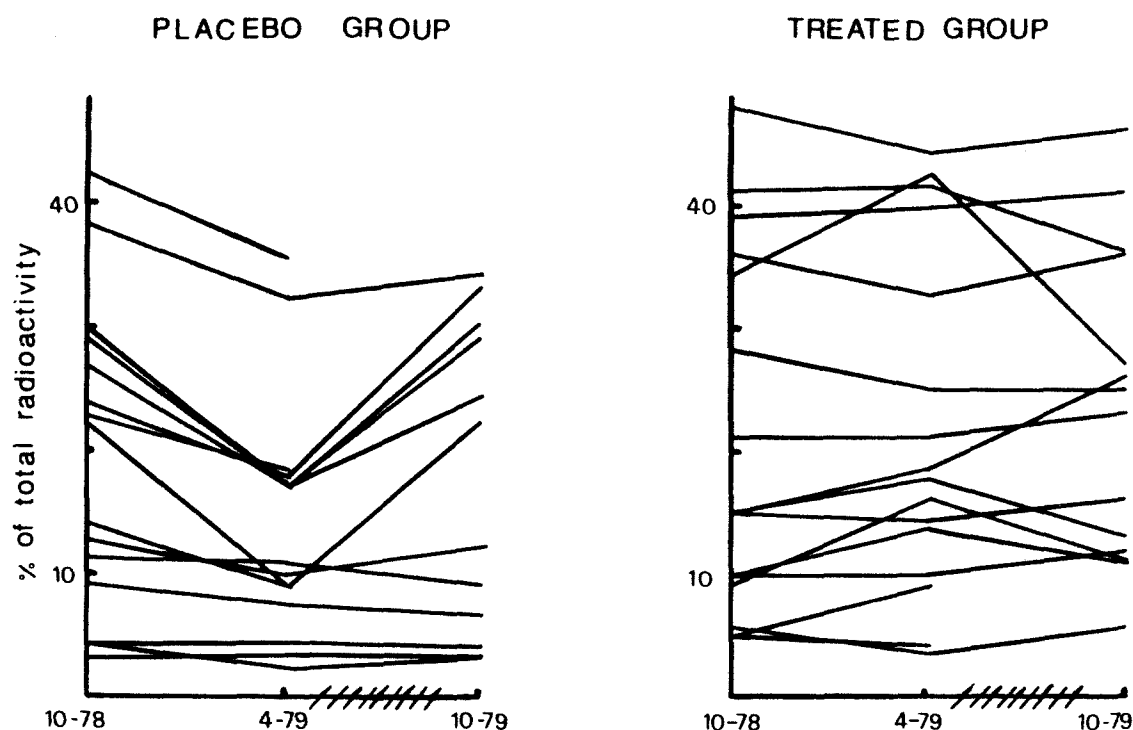


Fig. 1. Pre-, post-treatment and post-seasonal specific IgE values in placebo- and pollen-treated patients. *////*: pollen season.

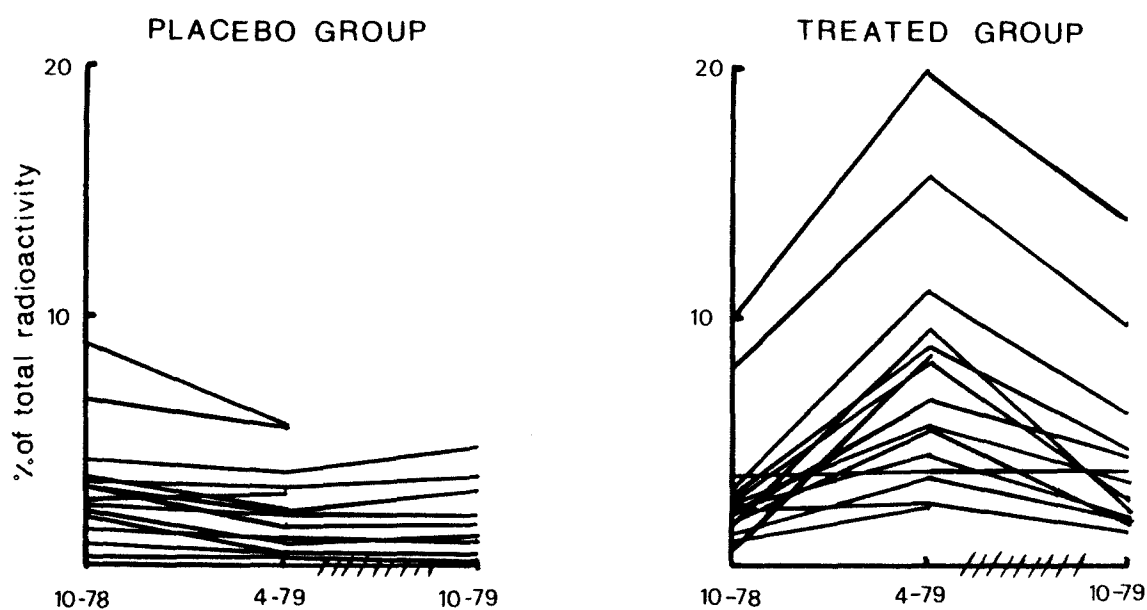


Fig. 2. Pre-, post-treatment and post-seasonal specific IgG values in placebo- and pollen-treated patients. *////*: pollen season.

coefficient observed for these variables ($r = 0.84$).

The haemagglutination titre was only studied in the post-treatment sera. The median and range values of the highest dilution factor giving a positive haemagglutination are 4 (1-16) and 8 (1-256) for the control and treated group

respectively ($P \leq 0.01$). The correlation coefficient ($r = 0.38$) between these haemagglutination titres and the IgG titres for the 33 post-treatment sera is significant at the $P \leq 0.05$ level.

Table 6 summarizes the results for the whole population of the correlation studies between

Table 2

A. Post-treatment levels of specific IgE, specific IgG and total IgE. Median values and range in placebo and treated groups

B. Percentual variations $\left(\frac{\text{Post-treatment value} - \text{Pre-treatment value}}{\text{Pre-treatment value}} \times 100 \right)$ of specific IgE, specific IgG in the placebo and the treated group

	A			B		
	Placebo n = 16	Treated n = 17	Wilcoxon test	Placebo	Treated	Wilcoxon test
Total IgE (IU/ml)	175 (17-1,000)	295 (68-4,700)	NS	- 35	+ 3	S $P \leq 0.003$
Specific IgE (%)	10 (2-35)	16 (3-41)	NS	- 29	- 10	S $P \leq 0.005$
Specific IgG (%)	1.8 (0-5.5)	5.6 (0-20)	S $P \leq 0.002$	- 25	+ 160	S $P \leq 0.002$

Table 3

Specific IgE and IgG values on unabsorbed and absorbed sera.

Sera		IgE (%)		IgG (%)	
		Unabsorbed	Absorbed*	Unabsorbed	Absorbed*
A	1/2	41	21	18.9	19
	1/10	22	5.8	7	7.9
B	1/2	46.7	30	5.2	6.6
	1/10	35	7	1.1	1.4

* Partial absorption of IgE was performed by incubating 50 µl of the diluted serum in the presence of one anti-human ε disc for 1 h at 22°C.

Table 4

Relationship between specific IgE and IgG levels in pre- and post-treatment sera (n = 33)

	Pre-treatment sera	Post-treatment sera
Correlation between specific IgE and specific IgG levels	$r = 0.65$ $P \leq 0.001$	$r = 0.62$ $P \leq 0.001$

Table 5

Relationship between percentual variations $\left(\frac{\text{Post-treatment value} - \text{Pre-treatment value}}{\text{Pre-treatment value}} \times 100 \right)$ of the specific IgE and IgG levels	
Correlation between relative variation of specific IgE and relative variation of specific IgG during the treatment period	
Placebo (n = 16):	NS
Treated (n = 17):	$r = 0.84$ $P \leq 0.001$

Table 6

Relationship between the post-treatment immunoglobulin levels and the clinical scores corresponding to the consecutive pollen season

Correlation coefficients between total scores and post-treatment levels ($n = 33$) of:	
Specific IgE	$r = 0.20^*$
Total IgE	$r = -0.06^*$
Specific IgG	$r = -0.11^*$
Specific haemagglutination titer	$r = -0.13^*$
Specific IgG	$r = -0.21^*$
Specific IgE	
Specific haemagglutination titer	$r = -0.20^*$
Specific IgE	

* NS at the $P \leq 0.05$ level.

the total clinical scores (19) and the different immunoglobulin levels after the immunotherapy period. In this search for a relationship only the post-treatment values were taken into consideration, the latter characteristics being the closest to the pollen season revealing the clinical status. No correlation was found at the $P \leq 0.05$ level, for any of the relationships questioned.

DISCUSSION

This study reports the immunotherapy-induced and seasonal variations of the IgE and IgG levels in the sera of 33 grass pollen-sensitive patients divided into two groups: one receiving hyposensitization treatment, the other placebo injections. Details concerning recruitment of the patients, the hyposensitization schedule and the clinical scores are fully described in a preceding paper (19). The main purpose of this trial was to evaluate the effectiveness of the allergen treatment during the period when pollination was most intense. Throughout the clinical trial (cutaneous tests, hyposensitization) and the biological determinations (radioimmunological methods, haemagglutination technique) the same four-grass extract was used in order to look for relationships between clinical and biological data.

The effects of immunotherapy on specific immunoglobulin levels have been reported by various authors (2, 5, 6, 8, 11, 17, 18, 23), with different conclusions concerning the significance and extent of the variations observed. The nature of the allergen and of the adjuvant used, the total dose of allergen administered, the treatment schedule and times of serum sampling are factors contributing to the diversity of the reported results. Moreover, biological measurements are based on a large variety of techniques, most of them leading to semi-quantitative data. Finally, the different ways of presenting the data – absolute values (5), absolute variations (18), log transformed values (11), and relative variations (2) – play an important role in the diversity of the conclusions.

For the methodology of this trial, we have estimated that the variations relative to the pre-treatment values constitute the most sensitive way to express the observed changes. Moreover, non-parametrical statistical tests on the median values are presented here as the most appropriate, since this trial comprised small numbers of patients and because no information exists on the statistical distribution of the parameters studied within the population of patients.

Quantitations of immunoglobulin levels in sera call for the following remarks:

The quantitations of the specific IgE and IgG were performed on a relative scale, taking care, however, to work under the conditions where changes in the serum levels would be detected with maximal sensitivity.

Earlier work detailed the features on the protein A-immunoglobulin interactions in IgG level determinations: Zola et al. (24) reported that insolubilized protein-A could retain IgG subclasses, 1, 2 and 4, though the fixation of other immunoglobulin classes on protein-A has been noted (12). For this reason the possible interference of IgE in the IgG determinations was queried. No differences were observed in the results regarding specific IgG determinations, whether the sera were depleted in IgE or not (Table 3), and thus the IgG determinations are considered not to be influenced by the IgE titre in the sera.

The interference of IgG with the IgE determinations has been analysed by Yunginger & Gleich (23) and Fujita et al. (7). From their studies, we conclude that only the specific IgE values obtained on sera which have very high levels of non-IgE pollen-specific immunoglobulins may be slightly underestimated.

In the present work, the variations relative to the pre-treatment values of total and specific IgE, and of IgG, are significantly different in the treated and in the control patients. The levels of the three parameters studied were lowered in the placebo group (Table 2B), whereas stable IgE levels and an increased serum concentration for specific IgG were seen in the treated group.

As earlier observed by Foucard & Johansson (6), the present study shows a very close pattern of development for the total and the specific IgE levels (Table 2B). Some of the patients in this trial were sensitive to allergens other than the four-grass pollen extract, and the fact that their total IgE consisted of multi-specific IgE may contribute to the small differences nevertheless observed between the development of total and specific IgE.

No significant changes of the pollen-specific IgE are seen for the treated patients in this study, or in other reports on hyposensitization performed during a 1-year period (17, 21). Some authors (14), however, mention a diminution of ragweed-specific IgE in treated patients, but only after intense immunotherapy during 2 consecutive years. The decrease of specific IgE in the placebo group after the treatment period is similar to the post-seasonal variation observed in ragweed-sensitive non-treated patients (23). It is worth noticing that in our study, on the average, the post-seasonal specific IgE level of each patient is similar to the pretreatment value (Fig. 1).

As far as the development of the non-IgE-immunoglobulin level is concerned, we must note that after the treatment period the higher IgG level in the treated group compared with the placebo may lead to a significantly higher value of the haemagglutination titre in the treated patients versus the placebo controls. A significant increase of haemagglutination titres in ragweed-treated patients compared with pla-

cebo controls has also been reported by other investigators (2). The weak relationship observed between the haemagglutination titre and the IgG level after immunotherapy may be due to various factors. On one hand, the antigen specificity of the immunoglobulins measured by the two methods is probably not identical; the iodination of the pollen extract and the pollen coating on the red blood cells not selecting exactly the same pollen components to the same extent. On the other hand, the participation of immunoglobulin classes other than just the IgG class, e.g. IgM, may play a role in the haemagglutination results.

During our study a close relationship has been found between the specific IgE and IgG in both pre- and post-treatment sera (Table 4). The value of the correlation coefficient observed for all the post-treatment samples (0.62, $P \leq 0.001$) is close to that reported by Gleich et al. (8) for ragweed-sensitive treated patients (0.73, $P \leq 0.001$). Another interesting finding is the strong correlation between the relative increases of these two immunoglobulin levels in the treated patients after immunotherapy (Table 5).

Our results lead to the following conclusions:

- 1) Treatment using the crude four-grass pollen extract has effects on the specific immunoglobulin levels similar to those observed after immunotherapy with ragweed pollen extracts: an increase of the IgG titres and a suppression of the post-seasonal peak of IgE.

- 2) Though performed partly in the presence of $\text{Al}(\text{OH})_3$, an adjuvant known for its IgE-inducing capacity in animals (15), the hyposensitization described in the present study has not favoured the synthesis of the IgE class above the IgG class.

- 3) The strong relationship observed between specific IgE and IgG increases during immunotherapy indicates that the ratio between these relative variations is similar in the treated patients (Table 5). This observation supports the hypothesis that during the biosynthesis of these immunoglobulins belonging to two distinct classes, a regulatory mechanism maintains this constant proportion between their relative increases. Further experiments are needed to

identify at which level this regulatory phenomenon operates.

Several investigators have already questioned the existence of relationships between the allergen specific immunoglobulin levels and the clinical scores (1, 5, 13, 14). Except for the study of Lichtenstein et al. (14), who noticed a positive correlation in 20 placebo-treated patients between the specific IgE level and the symptom index corresponding to the consecutive pollen season, no other data are available indicating a significant correlation between immunoglobulin levels and clinical scores. In spite of the fact that the same extract was used throughout this study, no significant correlation value was found (Table 6) between the clinical scores and the biological serum characteristics. The IgG/IgE and the haemagglutination antibodies/IgE ratios do not seem to reflect the clinical status better than the levels of the different immunoglobulins alone. In fact, the lack of significant correlations between the serum immunoglobulin levels and the severity of the clinical manifestations leads to the conclusion that post-treatment biological characteristics have no prognostic value with regard to clinical features during the consecutive pollen season.

The mechanisms leading to the different allergic manifestations are numerous and multiple pathways may interfere by enhancing or inhibiting the severity of the clinical status. Such a complex situation can probably not be reflected by the serum immunoglobulin levels alone.

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