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EVOLUTION OF REAGINIC ANTIBODIES IN SPECIFIC HYPOSENSITIZATION

By

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The specific, allergic property of serological components detected in transfer experiments by Prausnitz & Küstner (24) in 1921 is associated with a new class of immunoglobulin: IgE. Ishizaka et al. (9) working on patients allergic to ragweed, as well as Johansson et al. (14) working on an atypical myeloma protein have shown by different means, the existence of this immunoglobulin. The isolation and purification of those proteins associated with the discovery of two new IgE myeloma have permitted a deep knowledge of their biological and physicochemical properties (4, 11). Parallel research in animals has shown that some homocytropic antibodies belong to a type corresponding to human IgE.

The study of the IgE level (3) required the setting up of very sensitive techniques. Although IgE is present in all normal subjects at a very low concentration, Ishizaka & Johansson (10, 15) have shown that the serum concentration is higher in subjects presenting immediate allergic phenomena. The goal of our work is to study the level of total and specific IgE in pollen sensitive patients during the first year of their specific desensitization. Until recently, the actual mechanism of this desensitization was not very well known and the criterion of its efficiency badly defined. At present, we have objective criterion to study the efficiency of such a treatment.

Next to clinical criteria such as the use of placebo (32) and functional or pollinic scores (7), some biological facts such as the appearance of blocking antibodies and the changes of the total or specific, antigen-directed IgE level, give new objective methods to follow the course and effect of desensitization.

MATERIALS AND METHODS

Materials:

The human anti-IgE serum, the IgE at 10,000 U/ml reference as well as Seraphose 4B activated by cyanogene bromide were supplied by Pharmacia, Uppsala, Sweden. The ^{125}I at 200 mCi/ml in NaOH 0.1 N, without reducing agent and 'carrier-free' was supplied by Philips-Duphar Laboratories, Petten, Holland. For specific desensitization and the Radio-Allergosorbent-Test we used aqueous extracts of the following allergens:

- Grass pollen extracts (10,000 PNU) without phenol or glycerine, Institut Pasteur (Paris).
- Platan extracts (1/20°) phenol 5 %, glycerine 40 %: B. D. Mérieux, Lyon.
- Parietaria officinalis extracts (1/20°) phenol 5 %, glycerine 40 %: B. D. Mérieux, Lyon.

Methods:

a) *Specific desensitization*: we undertook specific desensitization in adult subjects showing clinical symptoms of pollen allergy, spasmodic coryza, and conjunctivitis with or without asthma. The desensitization started in december 1971 and was made with aqueous extracts of pollen with alumin hydroxide at 2/10° ml. The subjects received:

- One subcutaneous injection every week.

Dilution of aqueous extracts:

1/100,000	1/10° ml	2/10° ml	4/10° ml	8/10° ml
1/10,000	1/10° ml	2/10° ml	4/10° ml	8/10° ml
1/1,000	1/10° ml	2/10° ml	4/10° ml	8/10° ml

- One subcutaneous injection every 15 days.

Dilution of aqueous extracts:

7/100	1/10° ml	1,5/10° ml
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The rate of injections was sometimes changed because of individual reactions.

The blood samples were taken before the beginning of the treatment and subsequently once a month until the pollen season; then twice at the end of the season.

b) Assay of serum IgE: we used the radial radioactive immunodiffusion technique described by Rowe (27) that has already been mentioned (1).

- Preparation of rabbit anti-sheep IgG:

The anti-serum was obtained by injection in rabbits of purified sheep IgG solution at 1 mg/ml in 0.15 M NaCl in the presence of Freund's complete adjuvant. The rabbits were immunized by intramuscular injection of 1 mg/ml of this mixture at the rate of one injection each week for two months, after which were bled. The globulin fraction of the anti-serum was obtained by precipitation with 3.2 M ammonium sulphate.

- Labelling of rabbit IgG anti-sheep IgG:

The technique used here has been described by Mac Conahey & Dixon (21); 5 mg purified rabbit γ -globulin in 4 ml phosphate buffer 0.1 M (pH = 7.4) was labelled with 5 mCi ^{125}I in presence of 100 μl of a 1% chloramine T solution. When the mixture had been shaken the reaction was stopped by addition of 100 μl of 2% sodium metabisulphite solution.

The separation of the labelled proteins and iodide that had not reacted was done on a Dowex column Ag 1×1 (50 Mesh) (1×10 cm) equilibrated with 0.1 M phosphate buffer (pH = 7.4). The radioactivity of collected fractions was measured in a γ meter. The final product was diluted in 8 ml of 0.005 M phosphate buffer (pH = 7.2) and bovine serum albumin 0.5 %.

- Assay of total serum IgE: The sheep serum anti human IgE was diluted to 1/800 in 0.005 M phosphate buffer, 0.15 M NaCl and bovine serum albumin 0.5 %; an aliquot of 10 ml was warmed at 56° C for an hour. At the same time, 0.2 g of agarose was warmly diluted in 10 ml 0.3 M phosphate buffer (pH = 8.0) containing 1% sodium azoture. The warmed anti-serum was mixed with the agarose solution and poured on a glass plate (9 x 10 cm) and put away at 4° C overnight. The following day several ranges of wells were cut in the agarose plate. In the first range the wells were filled with successive dilutions of reference serum at 10,000 U/ml in 0.005 M phosphate buffer (pH = 7.2), 0.15 M NaCl and bovine serum albumin 0.5 %; in other ranges they were filled with successive dilutions of serum to be studied. The agarose plate incubated for two days at 37° and washed afterwards for two days at 4° C in 0.005 M phosphate buffer and 0.15 M NaCl was exposed to the radioactive rabbit IgG solution for one hour. It was then left in a moist box for 24 hours and washed in several buffer baths and distilled water baths. The plate was then dried, put into contact with an autoradiography film Kodak Kodirex X-ray for 48 hours, and developed. The diameter of each precipitation circle varies according to the concentration in total serum IgE.

c) *Evaluation of specific IgE*: The Radio-Allergosorbent-Test (RAST) technique used here has been described by Wide, Bennich & Johansson (31).

- Coupling of allergens to Sepharose 4 B activated by cyanogene bromide: 1 ml of allergen aqueous extract was coupled with 150 mg of Seraphose 4 B activated by cyanogene bromide in the presence of 0.5 ml sodium bicarbonate 0.5 M. The mixture was incubated for 3 days at 4° C and washed according to the process described by Wide (30).

- Labelling of anti human IgE: The IgG fraction of the anti human IgE

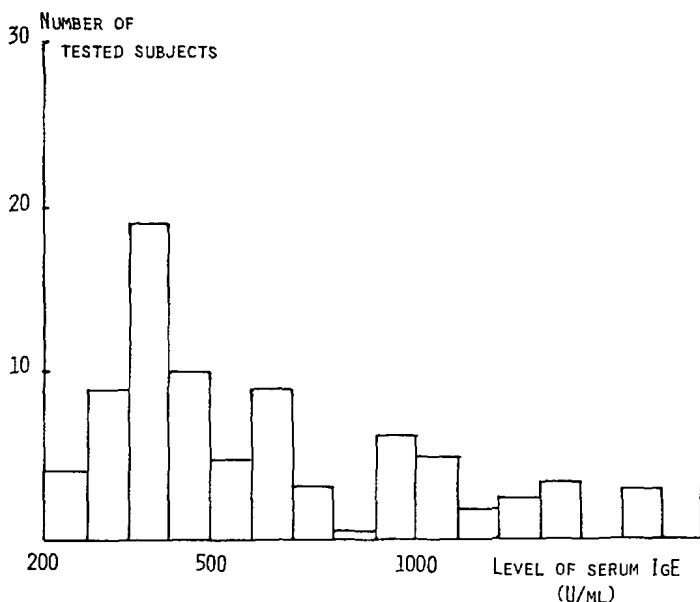


Fig. 1.

Concentration of total serum IgE in a population of pollen allergic subjects.

sheep serum was isolated by precipitation with ammonium sulphate 3.2 M. The technique used here again is the one described by Mac aonahey & Dixon (21): 40 μ g of IgG anti-IgE was labelled with 4 mCi of ^{125} I in the presence of 0.1 M Tris HCl (pH = 7.6) buffer. The labelled proteins and iodide are separated through a Dowex column Ag 1×1.50 (0.5 $\times$ 30 cm) equilibrated with 0.1 M Tris HCl (pH = 7.6) 0.2 M NaCl, EDTA 0.002 M, Tween 20 0.05 % in buffer solution. The radioactivity of the collected fractions (0.8 ml per tube) was measured in gamma-counter. The specific activity of labelled anti-IgE was 100 μ Ci per μ g protein.

- Assay of specific IgE: Each assay was done in duplicate. 100 ml of serum and 500 mg of the allergen immunoabsorbent complex were incubated overnight at room temperature with stirring. The suspension was centrifugated for 3 min at 2500 r.p.m. and washed 3 times with 2 ml of 0.1 M NaCl (pH = 7.2), 0.15 M Tween 20 in 1 % phosphate buffer, 0.3 % bovine serum albumin, and incubated overnight at room temperature with 200 μ l of an anti-IgE solution labelled with ^{125} I corresponding to 60,000 CPM. The complex was washed three times

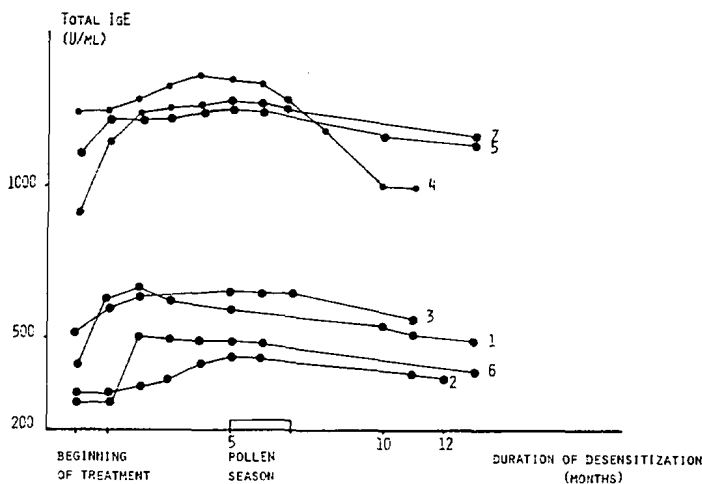


Fig. 2.

Variation of level of total IgE in subjects desensitized with aqueous extracts of grass pollen.

as before with an 0.5 M NaCl solution containing 1 % Tween 20. The radioactivity bound to particles of 4 B Sepharose was measured in a gamma-counter. The results obtained were compared with a blank made from 100 μ l distilled water.

RESULTS

a) Assay of total serum IgE in a pollen allergic population.

We undertook titrations outside all pollen seasons to determine the average value of serum IgE in a group of adult subjects allergic to pollens who had never gone through desensitization treatment. Figure 1 shows the levels of total IgE in sixty subjects. It appears that 53 per cent of the patients with symptoms of allergy to grass pollens have a lower concentration than 500 U/ml, 24 per cent have a level between 500 and 1000 U/ml, and in 23 per cent of the patients the concentration of serum IgE is higher than 1000 U/ml. The average value obtained is 634 U/ml and is higher than that

TABLE I
Evolution of level in total serum IgE in eight adult subjects during specific desensitization.

Subjects	Allergens	Age (years)	Sex	Before treatment	Total IgE (U/ml)			Value of increase U/ml in 5th month	Value of increase U/ml after 10 months
1 Abe.	Grass pollen	22	M	360	620	610	540	250	180
2 All.	Grass pollen	34	M	310	330	440	380	130	70
3 Car.	Grass pollen	52	M	520	600	650	570	130	50
4 Cop.	Grass pollen	17	M	1235	1240	1440	990	205	-
5 Fra.	Grass pollen	18	M	1105	1210	1250	1200	145	95
6 Moi.	Grass pollen	51	W	290	290	490	440	200	150
7 Lan.	Parietaria officinalis	22	W	900	1140	1270	1170	370	270
	Grass pollen								
8 Mal.	Platan	33	W	1550	1610	1730	1590	180	40

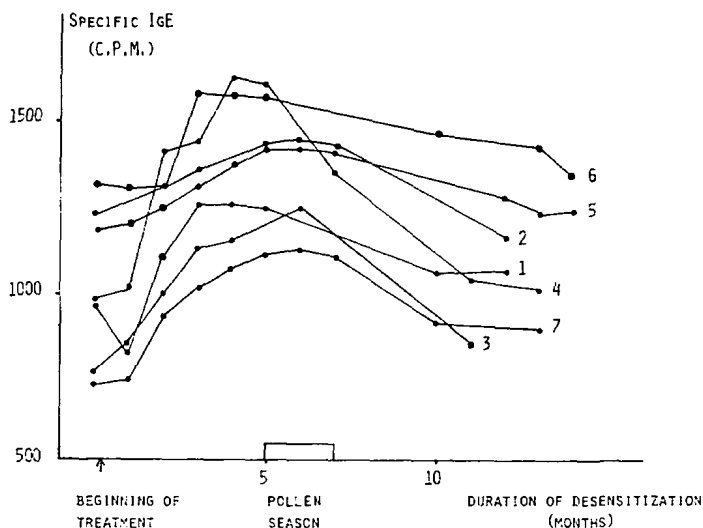


Fig. 3.

Variation of the titre of specific IgE in subjects desensitized with aqueous extracts of grass pollen.

determined in a population of normal subjects (1, 2, 15). The increase in the level of serum IgE among atopsics has also been observed by other authors (5, 16). During the assays we found no correlation between the intensities of the clinical manifestations of pollen allergy (asthma, spasmodic coryza, conjunctivitis) and the values of total serum IgE.

b) Evolution of the concentration of total IgE among subjects being hyposensitized.

Eight subjects previously tested were submitted to specific desensitization treatment. Among 4 of those patients the initial value of IgE was lower than 500 U/ml; among 4 others the concentration of serum IgE was higher than 900 U/ml. The first assay corresponds to the quantity of serum IgE before the beginning of immunotherapy treatment. The change of level is shown in Fig. 2 for seven patients de-

TABLE 2
Evolution of the titre of specific IgE in eight subjects being desensitized.

Subjects	Allergens	Variation of specific IgE (C.P.M.)				Value reached in % of increase at the end of 5th month	Value reached in % of increase at the end of 10th month
		Before treatment	1st month	5th month	10th month		
1 Abe.	Grass pollen	945	820	1340	1050	143	111
2 All.	Grass pollen	1180	1240	1547	1253	142	107
3 Car.	Grass pollen	760	820	1240	940	163	123
4 Cop.	Grass pollen	1300	1290	1560	1450	120	111
5 Fra.	Grass pollen	1220	1300	1430	1300	117	106
6 Mol.	Grass pollen	960	1000	1620	1230	168	128
7 Lan.	Parietaria officinalis	720	950	1560	1180	223	153
8 Mal.	Grass pollen	700	730	1100	910	142	130
	Platan	720		2200		304	158

sensitized with grass pollen. The evolution profile is the same for all patients. We observed during the course of treatment an increase of total IgE through the first five months. In some subjects the increase appeared as soon as the treatment had begun, in others, from the second month of desensitization. The answer to the antigenic stimulus thus seems to vary according to the individual. In response to the same quantity of injected material each subject produces a different quantity of antibodies. Table 1 shows the increase of IgE in U/ml in each individual through one year of treatment. The maximum values are reached during the fifth month and are constantly maintained for two months. This period corresponds to the pollen season in other respects. The level of total IgE diminished slightly afterwards. It must be noticed that after twelve months of desensitization the IgE concentration is still higher than initial concentration, except in one subject.

Specific desensitization induces a change of total IgE, but it is of essential interest to know the evolution of specific IgE which is directly concerned by this therapy.

c) Change of the titre of specific IgE.

The RAST technique allowed us to test the specific IgE in eight of the previous subjects. The evolution of the titre of serum reagins is shown in Fig. 3. During immunotherapy an increase of the titre of specific IgE occurs through the first months of treatment. The variation of reagins is shown in Table 2. The increase of the synthesis of specific IgE apparently does not depend upon the initial concentration of total IgE. Each individual does not react in the same way to the same quantity of antigen, the reagin synthesis being more important in some subjects. In this figure, we also see that the different allergens do not give the same response. The *parietaria officinalis* and *platan* pollens have induced a variation of specific IgE faster than the grass pollens.

Berg & Johansson (6) have shown that the increase level of specific IgE was superior to that of total IgE. The results obtained with our subjects are presented in Table 3. Except in subject no. 1 for whom the variation of total IgE is higher

TABLE 3
Effect of specific desensitization on variations of total IgE and reagins.

Subjects	Allergens	% of increase of total IgE; 5th month compared to initial value	% of increase of specific IgE; 5th month compared to initial value	% of total IgE diminution; 10th month compared to 5th month	% of specific IgE diminution; 10th month compared to 5th month
1 Abe.	Grass pollen	+ 66 %	+ 43 %	- 11 %	- 28 %
2 All.	Grass pollen	+ 41 %	+ 42 %	- 15 %	- 32 %
3 Car.	Grass pollen	+ 25 %	+ 63 %	- 14 %	- 32 %
4 Cop.	Grass pollen	+ 16 %	+ 20 %	- 45 %	- 8 %
5 Fra.	Grass pollen	+ 13 %	+ 17 %	- 4 %	- 10 %
6 Moi.	Grass pollen	+ 68 %	+ 68 %	- 11 %	- 32 %
7 Lan.	Grass pollen	+ 41 %	+ 57 %	- 8 %	- 22 %
	Parietaria officinalis	+ 41 %	+ 123 %	- 8 %	- 37 %
8 Mal.	Platan	+ 11 %	+ 165 %	- 9 %	- 55 %

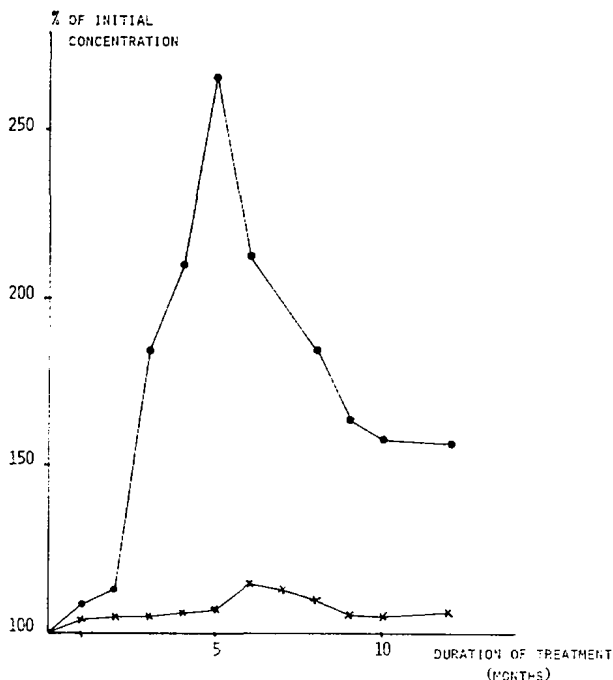


Fig. 4.

Variation of total serum IgE (* — *) and specific IgE (● — ●) in a subject desensitized with an aqueous extract of platane pollen.

than that of specific IgE, and for patient no. 8, who shows the same variation titre for both IgE categories, we notice larger variations of specific IgE in proportion to total IgE.

In Fig. 4, we compare those variations in a subject desensitized with platane pollen. The increase of specific IgE is much larger with the pollen than with aqueous grass extracts. In Fig. 5, we show the immune response of a subject desensitized to two allergens at the same time: there is an important difference in the answer to both pollen extracts; the immunocompetent cells synthesizing IgE antibodies against parietaria

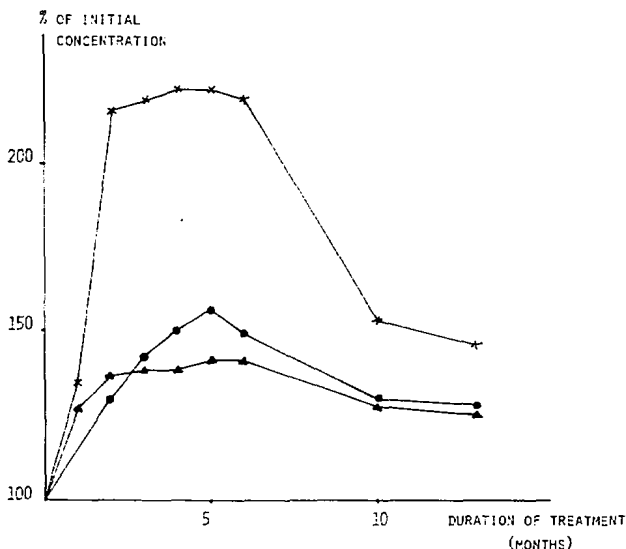


Fig. 5.

Variation of total IgE ($\blacktriangle$ — $\blacktriangle$) and anti-parietaria officinalis (*—*) and anti-grass pollen ($\bullet$ — $\bullet$) in a subject desensitized at the same time with aqueous extracts of parietaria officinalis pollen and grass pollen.

officinalis anti-pollen and platan anti-pollen do not produce the same quantity of antibodies. This suggests that both pollens do not have the same antigenic power. Identical cases have been described by Berg & Johansson (6).

The decreasing amount of specific IgE, after the first five months of treatment is faster than that of total IgE. Here the problem is to know whether there has not been an effect of specific blocking antibody that would competitively inhibit the binding between IgE and allergen.

DISCUSSION

We have determined in this work the average of total IgE of an adult hay fever population not submitted to desensitization

treatment and outside all pollen seasons. In seventy-four subjects some have been submitted to specific immunotherapy treatment, and we studied the associated evolution of specific and total IgE.

The average value found for subjects suffering from hay fever is higher than the normal concentration of serum IgE but the levels (195 U/ml and 1550 U/ml) are extremely separated in subjects all presenting clinical symptoms of allergy; there is no correlation between the intensity of manifestation and concentration of serum IgE. The IgE level must consequently not be used to consider or reject an indication for desensitization.

During the first year of specific desensitization in these patients, we observed an increase in the concentration of specific and total IgE during the first five months. At the time that coincides with the pollen season, the maximum values are reached. The values then decrease. After one year of treatment the concentrations are still higher than the initial values. The increase of IgE is due to a continuous stimulation induced by the antigen; if the evolution profile is the same for all subjects, the answer still varies according to the individual. This reaginic variation during immunotherapy treatment confirms the works of Shermann which appeared before IgE had been characterized, and agrees with those of Ishizaka et al. (13) and Reisman et al. (25).

The mechanism which causes a decrease of specific IgE is still not very well known. The apparent suppression could be the result of the diminution of immunocompetent cells (17) capable of answering the antigenic stimulus. In the course of treatment, blocking IgG would block the antigen and prevent it stimulating the immunocompetent cells. In fact, Ishizaka has shown that the IgG antibody levels are constantly maintained during treatment and thus do not seem responsible of the diminution of specific IgE. Allergen complexes, allergen-IgE could induce a tolerance of the cells producing IgE (23).

In non desensitized subjects, the antigen causes an increase of specific IgE due to a booster effect (5, 25). The immuno-

therapy treatment will favour the production of circulating antibodies: the blocking antibodies that belong essentially to the IgG type (20). These IgG will combine with the allergen in the fluid phase and therapy prevent formation of IgE-allergen complex fixed to the cell membranes.

To support this evidence, the most employed technique is the release of histamine described by Lichtenstein et al. (18). The immediate hypersensitivity reactions determine the release of vasoactive substances such as histamine (29) induced by the formation of a bridge between the IgE fixed on mast cells or basophilic leukocytes and the allergen. Ishizaka et al. (12) have shown that all the released histamine comes from those leukocytes. During specific desensitization one can evaluate the defined cell sensitivity as being the minimum allergen concentration necessary to give a definite release of histamine (19). Ishizaka et al. (13) have shown that blocking IgG increased during the first four months after the beginning of the treatment; they will suppress the booster effect due to antigen. The suppression of the synthesis has been demonstrated by Malley et al. too (22) and Romagnani et al. (26).

Since those reactions introduce the same component: the allergen molecule, it is necessary to use purified extracts with a well-defined allergenic power. Until now, allergen standardization methods, because of the heterogeneity of the allergenic components and of the reaction of the subjects to those components has not yet been satisfactory. Foucard et al. (8) tested three extracts by a modification of the RAST technique. They found that their efficiency compared to given allergic subjects varied from 1 to 1000.

SUMMARY

The level and changes in total serum IgE in seventy-four non desensitized subjects treated for pollinosis has been studied. The average value found is equal to 635 U/ml. Among eight of those subjects, we have followed for one year the production of total serum IgE, and of reagins in response to specific

desensitization. During the first five immunotherapy months, the level of specific and total IgE increases, then it gradually diminishes. The final concentrations after ten months of treatment are still higher than the initial value. The comparison between the production of specific IgE and total IgE show that the reaginic variations are most important.

RESUME

Nous avons étudié le taux et la répartition des IgE sériques totales de soixante quatorze sujets atteints de pollinose, non désensibilisés, en dehors de toute saison pollinique. La valeur moyenne trouvée est égale à 635 U/ml, chez huit de ces sujets nous avons suivi pendant un an l'évolution des IgE totales sériques et des réagines en réponse aux désensibilisations spécifiques. Pendant les cinq premiers mois de l'immunothérapie les IgE spécifiques et totales augmentent, puis diminuent graduellement. Les concentrations finales après dix mois de traitement sont encore supérieures aux valeurs initiales. La comparaison entre les évolutions des IgE spécifiques et des IgE totales, montre que les variations des réagines sont les plus importantes.

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